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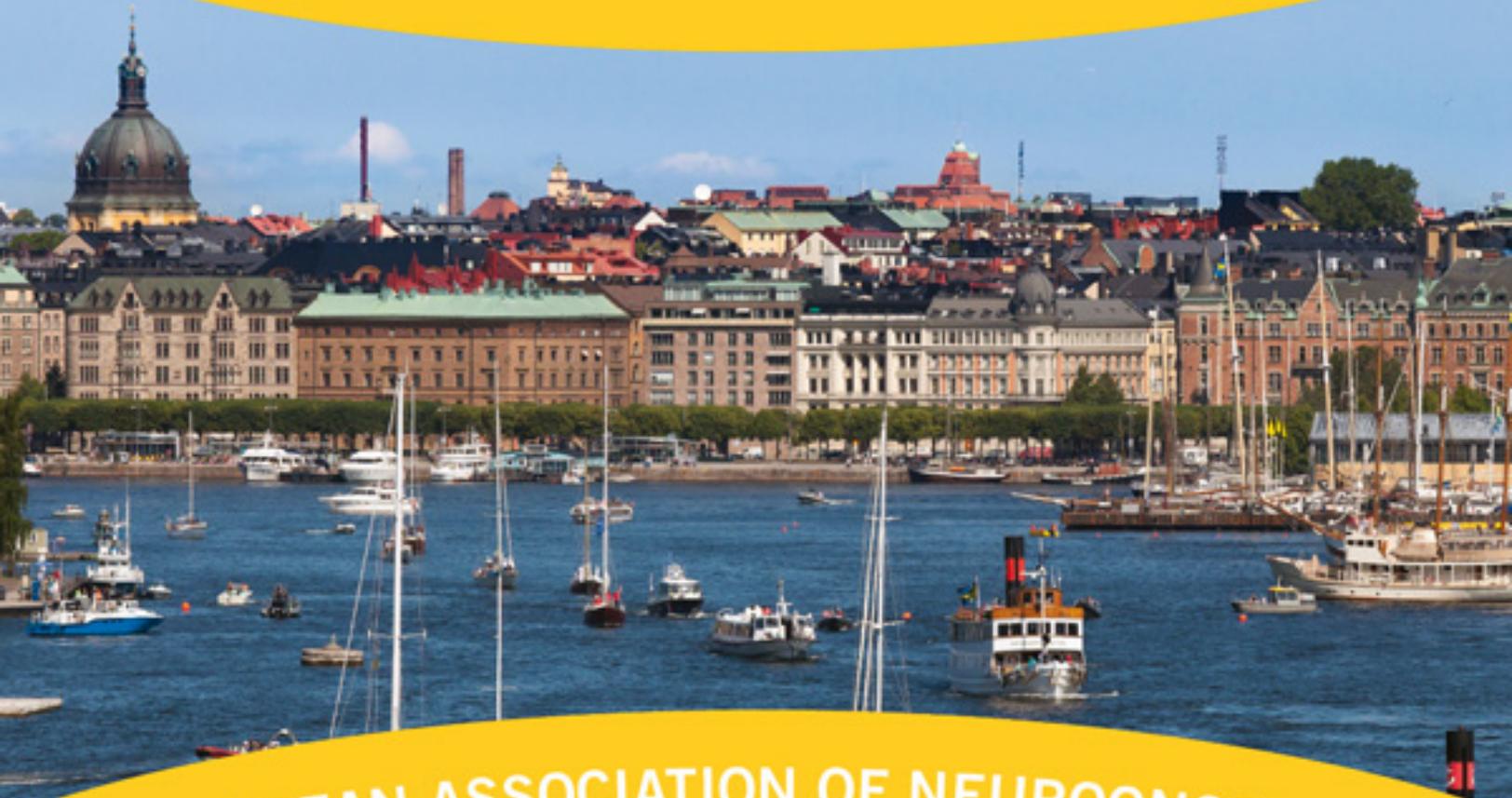
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# Influence of Natural Lung Surfactant Inhalations on Clinical Symptoms and Pulmonary Function Parameters in Patients with Bronchial Asthma. Communication 2

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## Abstract

**Background:** Damage to lung surfactant (LS) enabling the lung local immunity may contribute to the development of bronchial inflammation in patients with bronchial asthma (BA).

**Methods and Results:** A 70-day course of 21 LS (Surfactant-BL) inhalations at the dose of 25 mg was added to inhaled corticosteroids (ICS) and short/long-acting bronchodilators or combined inhalers in 13 BA patients. After 21 inhalations, many patients reported lower frequency of cough and profuse expectoration, fewer night and day attacks, resolution of wheezing, resolution or lower frequency of bronchospasm episodes under moderate exercise, and termination (1 patient) or double reduction (8 patients out of 13) of the ICS dose. The values of pulmonary function parameters in patients at Days 250 and 340 did not differ significantly from the values achieved at Day 41. The mean values at Days 250 and 340 were as follows: FVC = 86.4±6.43%, FEV1 = 61.4±8.41%, FEV1/FVC = 66.5±8.87%.

**Conclusion:** LS inhalations improve the condition of patients with BA, allow ICS dose reduction by 2 times, and improve pulmonary function parameters. A total of 70% of patients had quite a long remission of BA symptoms following a 21-inhalation therapy course of the formulation. (**International Journal of Biomedicine. 2017;7(3):167-170.**)

**Key Words:** lung surfactant • bronchial asthma • inhaled corticosteroids • dose • pulmonary function

## Abbreviations

BA, bronchial asthma; ICS, inhaled corticosteroids; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; LS, lung surfactant; PFPs, pulmonary function parameters.

## Introduction

Deficiency or qualitative changes in lung surfactant (LS) are found in many lung conditions, including bronchial asthma (BA).<sup>(1,2)</sup> Apart from enabling the breathing mechanism, LS

is known to provide molecular mechanisms of innate and adaptive lung tissue immunity and to have anti-inflammatory properties.<sup>(3,4)</sup> Aside from the review by Hohlfeld<sup>(5)</sup> discussing in detail the role of LS system impairment and the possible use of LS formulations for BA, little research has been carried out so far to investigate the possibility of restoring pulmonary function and achieving stable improvement of clinical manifestations in BA patients by inhaled LS formulations.

In our earlier work, we showed that long-term inhalations of prednisolone hemisuccinate significantly decrease lung

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surfactant levels in rats, and we hypothesized that inhalations of natural formulations of lung surfactant will stimulate the synthesis of endogenous surfactant and weaken dependency on ICS in BA patients.<sup>(6)</sup>

Our previous article demonstrated that a course of natural LS inhalations, given as part of combined therapy for patients with BA, leads to significant improvement of clinical manifestations and PFPs. The 41-day surfactant therapy course consisting of 16 inhalations has allowed ICS dose reduction by 2 times.<sup>(7)</sup>

The objective of this study was to evaluate the effectiveness of surfactant treatment based on the evaluation of clinical manifestations in BA patients who have received a course of surfactant therapy comprising 21 inhalations of Surfactant-BL (Biosurf, Russia) over 70 days and who have been followed up for 340 days.

## Materials and Methods

We examined 13 patients (one was withdrawn from the study having moved to another city) with partly controlled and uncontrolled persistent BA at the Pulmonary Center of Chita Road Clinical Hospital. The clinical trial was carried out based on the decision of the Local Ethical Committee under the Chita State Medical Academy and according to the approved Protocol. The enrolled patients were diagnosed according to the 2016 GINA guidelines<sup>(8)</sup> and had had a history of asthma for a period of time varying from 6 months to 24 years. The study was conducted from January 20, 2016 to December 30, 2016. The treatment they had been receiving prior to their entry into the study included antibiotics during exacerbations, with 8 out of 13 patients receiving short courses of systemic per-oral and/or parenteral glucocorticoids. After their condition became stable, they had received either inhaled ICS and short/long-acting bronchodilators or combined inhalers for 12 months to 12 years prior to enrollment. Upon enrollment, the patients started a course of inhalations with Surfactant-BL (OOO Biosurf, Saint Petersburg, Russia), a natural LS formulation, at the dose of 25 mg per inhalation. The surfactant was administered using the compressor nebulizer Boreal (Italy). The patients were instructed how to use it at the first visit (V1) and then continued to use it on their own for inhalations at home. The surfactant was taken daily for the first 7 days of the study and then at Days 10, 13, 16, 19, 22, 26, 30, 35, 41, 47, 54, 61, 68 and 70 (a total of 21 inhalations). Patients were examined over the whole study period at 9 visits on Days 1(V1), 8(V2), 15(V3), 29(V4), 41(V5), 70(V6), 160(V7), 250(V8) and 340(V9). At each visit, the clinical condition of the patient was evaluated, including frequency of bronchospasm episodes, cough, wheezing, profuse expectoration, shortness of breath with exercise and speaking, and frequency of attacks while sleeping.

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). The mean (M) and standard error of the mean (SEM) were calculated. Student's unpaired and paired t-tests were used to compare mean values for data with normal distribution. Regression analysis was performed by the least squares method. The statistical

significance of the b coefficients of the linear regression equations was evaluated using Student's t-test. A probability value of  $P < 0.05$  was considered statistically significant.

## Results

Earlier we demonstrated that after 7 inhalations (V2) wheezing stopped and frequency of attacks decreased in most patients, as did shortness of breath and bronchospasm episodes under strenuous exertion.<sup>(7)</sup>

Follow-up according to the Protocol allowed us to evaluate the dynamics of BA clinical manifestations after 21 inhalations (Day 70) and at the following 9 visits, in comparison with the baseline. The results of the evaluation are presented in Table 1. Data show a significant decrease in patients complaining of nocturnal attacks, profuse expectoration, cough, and wheezing after the 21 surfactant inhalations of a 70-day therapy course. Patient condition continued to improve over the complete follow-up period, and by Day 340 the number of patients complaining of the above symptoms and of attacks under exertion and day attacks as compared to baseline had significantly decreased. A double reduction of the ICS dose was achieved for 8 patients out of 13, while 2 other patients stopped taking ICS and only 3 patients out of 13 had to continue receiving the same ICS dose. The above data show that the decrease or resolution of BA symptoms holds steadily over 10 months after termination of a surfactant therapy course.

Figure 1 demonstrates the dynamics of the percentage of patients showing resolution or decreased severity of BA symptoms as represented by the solid line ("Improvement") from baseline to Day 70. The dynamics of ICS dose decrease was evaluated over the same time period for the same patients and is represented by the dotted line ("ICS") in the figure. Both parameters change exponentially, the "Improvement" line equation being  $\lg y = 1.45 + 0.012 \cdot x$  ( $P < 0.05$ ), and the "ICS" line equation being  $\lg y = 1.98 + 0.003 \cdot x$  ( $P < 0.01$ ).

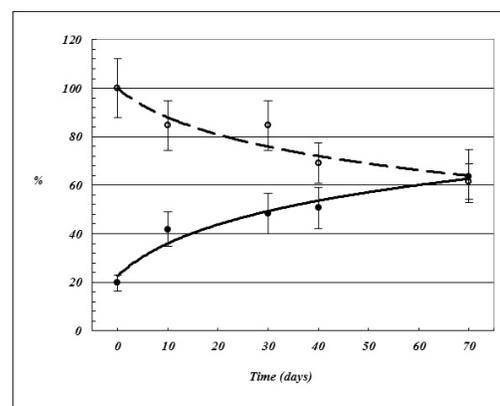


Fig. 1. The dynamics of the percentage of patients showing improvement of their clinical condition (solid line) and of the decrease of the ICS dose taken by the patients (dotted line) during the 70-day surfactant therapy course accompanied by combined treatment with ICS and bronchodilators.

Table 1.

**BA symptom frequency dynamics after the termination of Surfactant-BL inhalations (Day 70) and at the end of the follow-up period (340 days)**

Symptom (complaint)	Number of patients having the symptom at baseline (%)*	Number of patients having the symptom after Visit 6 (Day 70) (%)*	<i>P</i>	Number of patients having the symptom at the end of the follow-up period (Day 340) (%)*	<i>P</i>
Bronchospasm under exertion	46.2±14.39	15.4±10.42	>0.05	0.0±0.00	<0.01
Nocturnal attacks	61.5±14.05	7.7±7.70	<0.01	0.0±0.00	<0.001
Expectoration	100.0±0.00	23.1±12.17	<0.001	30.8±13.33	<0.001
Cough	100.0±0.00	46.2±14.39	<0.01	30.8±13.33	<0.001
Wheezing	100.0±0.00	30.8±13.33	<0.001	15.4±10.42	<0.001
Day attacks	84.6±10.42	53.8±14.39	>0.05	38.5±14.05	<0.05
Shortness of breath under exertion	100.0±0.00	61.5±14.05	<0.05	53.8±14.39	<0.01
All parameters (mean values of the mean values)	84.6±3.24	34.0±4.75	<0.001	24.2±4.21	<0.001
Decrease of the ICS dose	100.0±0.00	61.5±14.05	<0.05	23.1±12.17	<0.001

Note: \* - 100% is the total number of patients (n=13); *P* - significance of differences compared to baseline.

The data in Table 1 and Figure 1 provide evidence that surfactant-BL given as part of combined therapy with ICS and bronchodilators to BA patients results not only in a significant decrease in the frequency and severity of many BA symptoms, but also in a continual decrease in the ICS dose.

Thus, clinical data of patients with partly controlled and uncontrolled persistent BA who had received 21 inhalations of natural LS at the dose of 25 mg as add-on therapy to ICS and short/long-acting bronchodilators suggests a positive effect of surfactant therapy as part of combined therapy. As a result of this therapy, many patients reported lower frequency of cough and profuse expectoration, fewer night and day attacks, resolution of wheezing, resolution or lower frequency of bronchospasm episodes under moderate exercise, and termination (1 patient) or double reduction (8 patients out of 13) of the ICS dose.

It is also important that the objective data showing pulmonary function improvement already after 16 inhalations demonstrate significant improvement of the functional state of the bronchi.<sup>(7)</sup> This improvement was confirmed during the follow-up. The values of PFPs in patients at Days 250 and 340 (V8 and V9) did not differ significantly from the values achieved at Day 41 (V5). The mean values at Days 250 and 340 (V8 and V9) were as follows: FVC = 86.4±6.43%, FEV1 = 61.4±8.41%, FEV1/FVC = 66.5±8.87%.

The data obtained from the clinical study of Surfactant-BL used for treatment of BA patients leads to a preliminary conclusion that 70% of patients had quite a long remission of BA symptoms following a 21-inhalation therapy course of the formulation.

## Discussion

Earlier preclinical and clinical studies involving a limited number of patients have shown that LS formulations prevent the development of a bronchospasm when provoked by an antigen, improve PFPs, and normalize the LS phospholipid composition in BA patients.<sup>(9-12)</sup> It has been found that multiple administration of phosphatidylcholine and cholesterol liposomes to rats<sup>(13)</sup> and multiple administration of lung surfactant to newborn humans<sup>(14)</sup> enhances the synthesis of endogenous surfactant by its reutilization by the alveolocytes-II. The use of surfactant in therapy and prevention of acute respiratory distress syndrome in adults<sup>(15-18)</sup> and in lung tuberculosis<sup>(19-21)</sup> has been shown to be highly effective.

It is important to further investigate why 30% of patients with partly controlled and uncontrolled persistent BA did not respond to the surfactant therapy course. The ongoing evaluation of the immunological status of the enrolled patients may be helpful in finding an answer to this question.

## Competing interests

The authors declare that they have no competing interests.

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## Pulmonary Rehabilitation in Patients with Chronic Obstructive Pulmonary Disease and Metabolic Syndrome

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### Abstract

**Background:** Chronic obstructive pulmonary disease (COPD) is widespread and often combined with other diseases, including metabolic syndrome (MS), which are closely related. The purpose of this study was to assess the clinical efficacy of a pulmonary rehabilitation (PR) program for patients with COPD and MS, in addition to standard therapy.

**Materials and Methods:** We examined 70 patients with COPD and MS. The patients were divided into 2 groups. Group I included 35 patients who received standard pharmacologic therapy in combination with PR. Group II included 35 patients who received only standard pharmacologic therapy.

**Results:** Group I patients demonstrated a decrease in exacerbation events from  $3.96 \pm 0.43$  to  $2.24 \pm 0.10$  ( $P=0.0002$ ), in emergency service calls from  $3.80 \pm 0.37$  to  $1.59 \pm 0.25$  ( $P=0.0000$ ), and in hospital admissions from  $2.93 \pm 0.11$  to  $1.41 \pm 0.24$  ( $P=0.0004$ ) per year, as well as a significant decrease in the severity of COLD symptoms, improvements in exercise tolerance, MS components and QL physical and psychological domains, compared to Group II.

**Conclusion:** The main benefits of a comprehensive PR program for patients with COPD and MS include a decrease in symptoms (dyspnea and fatigue), improvements in exercise tolerance, and reduction in health care utilization (particularly bed-days), as well as an increase in physical activity and an improvement in MS components. (*International Journal of Biomedicine*. 2017;7(3):171-174.)

**Key Words:** chronic obstructive pulmonary disease • metabolic syndrome • pulmonary rehabilitation • quality of life

### Abbreviations

**6MWT**, the 6-minute walk test; **COPD**, chronic obstructive pulmonary disease; **CAT**, the COPD assessment test; **DBP**, diastolic blood pressure; **FPG**, fasting plasma glucose; **HDL-C**, high-density lipoprotein cholesterol; **LDL-C**, low-density lipoprotein cholesterol; **OGTT**, oral glucose tolerance test; **PR**, pulmonary rehabilitation; **QL**, the quality of life; **SBP**, systolic blood pressure; **SGRQ**, St. George's Respiratory Questionnaire; **SF-6**, the Short-Form Health Survey; **TG**, triglycerides.

### Introduction

COPD belongs to the group of chronic inflammatory lung diseases group that are characterized by incomplete obstruction of reversible airways. It is supposed that COPD will become one of the leading causes of death in the world

by 2020, and the high social importance of this disease has been confirmed.<sup>(1,2)</sup> In patients 40 years of age and more, COPD is often combined with pathology of other organs and systems.<sup>(2-4)</sup> On the other hand, MS affects 20% to 25% of the adult population and prevalence is on the increase, owing to obesity and a sedentary lifestyle, affecting as many as 42% of individuals aged over 60 years.<sup>(5-7)</sup>

According to Global Strategy For The Diagnosis, Management, And Prevention Of Chronic Obstructive Pulmonary Disease,<sup>(1)</sup> COPD has substantial manifestations beyond the lungs—the so-called systemic effects, such as

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unintentional weight loss and skeletal muscle dysfunction. The chronic systemic inflammation that is linked to COPD may also initiate or exacerbate comorbid diseases, such as cardiovascular disease, osteoporosis, anemia, MS, type 2 diabetes, lung cancer, and depression, and is one of the key mechanisms underlying these extrapulmonary effects.<sup>(2-4, 8-13)</sup>

It is important that COPD is a disease that can be prevented and treated.<sup>(1,2)</sup> However, over the last two decades, growing evidence of systemic manifestations in COPD patients and their negative effects on the functioning of these patients has accelerated the development and use of nonpharmacological treatments, such as pulmonary rehabilitation (PR). According to GOLD Guidelines, PR and pharmacological therapy must work closely together for a more successful outcome.<sup>(1,14-18)</sup> PR is a broad program that helps improve the well-being of patients with COPD; it uses exercise, education, and support to help patients to breathe—and function—at the highest level possible. Thus, in cases of the COPD+MS combination, an integrated approach to treatment and prevention and the development of a PR program are necessary.

The purpose of this study was to assess the clinical efficacy of a PR program for patients with COPD and MS, in addition to standard therapy.

## Materials and Methods

We examined 70 patients aged from 18 to 60 years with COPD and MS. The patients were divided into 2 groups. Group I included 35 patients (12/34.3% women and 23/65.7% men; mean age, 47.11±0.44 years) who received standard pharmacologic therapy in combination with PR. Group II included 35 patients (10/28.6% women and 25/71.4% men; mean age, 47.08±0.22 years) who received only standard pharmacologic therapy. COPD diagnosis was based on the integral assessment of symptoms, medical history, health status, and spirometry values according to GOLD. MS was diagnosed according to the IDF consensus criteria.<sup>(5)</sup> The study was approved by local ethics committee, and written informed consent was obtained from all participants

A comprehensive clinical examination and laboratory tests included the following procedures:

- Anthropometrical Reference Data: body mass index (BMI) was calculated using Quetelet's formula (in kg/cm<sup>2</sup>). Waist circumference (WC) was measured using centimetric tape at the navel level on a horizontal line (in cm)
- Assessment of COPD severity included the number of exacerbations, calls to emergency service, and hospital admissions for the past 12 months.
- Quantity assessment of COPD symptoms (dyspnea, cough, sputum production, general weakness) by the 10-point Visual Analogue Scale (VAS).
- Quality assessment of COPD symptoms by a modified British Medical Research Council (mMRC) questionnaire.
- Assessment of QL by SGRQ, SF-36 and CAT.
- Functional tests: spirometry, 6MWT.
- Assessment of blood pressure by Korotkov's method.
- Assessment of FPG, a 2-hour OGTT, blood levels of TG, HDL-C and LDL-C.

## Pulmonary Rehabilitation

The length of the PR program was 5 weeks: 5 practical classes of 1h 30min each for groups of 4-5 people. Topics covered were etiology, pathogenesis, symptoms, management and prevention of COPD and MS. For the smoking patients there was additional information about nicotine dependence and its influence on COPD. All the participants were given books, brochures, information sheets and booklets. The PR program for patients with COPD and MS included:

- Education sessions discussing breathing techniques, disease processes, respiratory medications, oxygen therapy, and exercise techniques
- Exercise reconditioning sessions
- Oxygen dosing (when appropriate)
- Nutrition and healthy lifestyle seminar
- Energy conservation techniques.

The patients' education program was followed by a 4-week exercise training program.

## Results and Discussion

During the observation, Group I demonstrated credible changes in some assessment parameters, while Group II showed no statistically significant changes in those parameters.

In Group I patients, we found a decrease in exacerbation events from 3.96±0.43 to 2.24±0.10 ( $P=0.0002$ ), in emergency service calls from 3.80±0.37 to 1.59±0.25 ( $P=0.0000$ ), and in hospital admissions from 2.93±0.11 to 1.41±0.24 ( $P=0.0004$ ) per year. There were significant improvements in subjective assessment of COPD symptoms according to the 10-point VAS: dyspnea – from 6.03±0.81 to 2.66±0.29 ( $P=0.0001$ ), cough – from 5.87±0.36 to 2.04±0.23 ( $P=0.0002$ ), sputum – from 3.81±0.21 to 1.06±0.49 ( $P=0.0008$ ), general weakness – from 4.55±0.81 to 1.88±0.49 ( $P=0.0003$ ). This group indicated significant improvement in dyspnea severity measured by mMRC (from 1.74±0.56 to 0.94±0.64 ( $P=0.0000$ )). We found also reliable positive dynamics of CAT results (from 22.54±4.06 to 16.32±3.05 ( $P=0.0000$ )). Group I demonstrated an obvious improvement in average values of SGRQ results: Symptoms score – from 77.21±3.78 to 70.32±3.46 ( $P=0.0000$ ), Activity score – from 70.32±2.98 to 58.62±5.62 ( $P=0.0002$ ), Impacts score – from 60.65±4.81 to 53.38±4.37 ( $P=0.0000$ ), and total score – from 67.51±3.78 to 59.42±3.86 ( $P=0.0000$ ).

Spirometry results were not significantly different in Groups I and II, but Group I showed significant improvement in the 6MWT result (from 347±6.1 m to 402±7.9 m;  $P=0.0000$ ).

Patients in this group also demonstrated a reliable improvement in QL physical and psychological domains, according to SF-36 (Table 1).

Group I displayed significant improvement in WC (from 111.04±2.11 cm to 102.24±2.79 cm in women,  $P=0.0394$ , and from 100.41±1.56 cm to 95.16±1.62 cm in men,  $P=0.0401$ ) and BMI (from 31.93±0.25 kg/m<sup>2</sup> to 30.65±0.44 kg/m<sup>2</sup> for the whole group,  $P=0.0311$ ).

Group I demonstrated a reliable decrease in SBP (from 151.63±0.77 mmHg to 146.26±1.04 mmHg;  $P=0.0001$ ) and DBP (from 96.14±0.87 mmHg to 91.12±0.55 mmHg;  $P=0.0067$ ).

Table 1.

SF-36 parameters (mean±SEM) in two groups

Parameters	Group I		Group II	
	Baseline	After 12 months	Baseline	After 12 months
PF	62.67±1.02	74.72±1.93*	61.53±1.04	52.35±1.28
RP	41.05±0.93	53.62±1.85*	42.13±1.27	41.58±0.92
BP	46.35±1.29	59.22±2.09*	45.08±1.85	46.33±1.41
GH	44.01±2.12	56.82±1.69*	45.82±2.05	42.72±1.34
VT	54.23±1.31	65.51±1.83*	53.67±1.03	54.14±1.22
SF	50.32±1.38	61.23±1.79*	49.21±2.05	47.54±1.38
RE	52.83±1.88	63.27±2.19*	51.36±1.95	48.78±1.69
MH	61.23±1.83	73.21±2.45*	63.04±1.59	60.45±2.01

PF -Physical Functioning, RP - Role-physical, BP - Bodily Pain, GH -General Health, VT -Vitality, SF - Social Functioning, RE - Role-emotional, MH -Mental Health; \* -  $P < 0.05$ .

We did not reveal significant differences in the level of serum lipids, FPG and OGTT between Group I and Group II. However, it should be noted that Group I indicated a tendency to decrease in the levels of TG (from  $2.30 \pm 0.24$  mmol/L to  $2.20 \pm 0.37$  mmol/L,  $P = 0.059$ ), LDL-C (from  $3.74 \pm 0.82$  mmol/L to  $3.64 \pm 0.66$  mmol/L,  $P = 0.053$ ), and to increase in HDL-C level (from  $0.86 \pm 0.31$  mmol/L to  $0.95 \pm 0.42$  mmol/L,  $P = 0.057$ ), as well as a tendency to decrease in the levels of FPG (from  $6.63 \pm 0.46$  mmol/L to  $6.25 \pm 0.54$  mmol/L,  $P = 0.053$ ) and postprandial glucose (from  $10.50 \pm 0.74$  mmol/L to  $10.01 \pm 0.66$  mmol/L,  $P = 0.058$ ).

Group I demonstrated a significant change of attitude towards smoking: 9(34.6%) patients out of 26 smokers quit smoking. Patients in this group also demonstrated a reliable improvement in QL physical and psychological domains, according to SF-36.

The main purposes of PR are symptom reduction, reduction in the expense of treatment, recovery of patient functional capacity, and improved QL. The most important aspect of PR is the individual approach, taking into account the degree of respiratory failure manifestation, associated diseases, and systemic COPD symptoms.<sup>(19)</sup>

According to our data, applying a PR program resulted in a significant decrease in the frequencies of COPD exacerbations, emergency service calls, and hospital admissions. In the complex of treatment, PR resulted in a degree of decrease in the manifestation of clinical COPD symptoms, in the severity of dyspnea and its influence on physical activity and patients' health condition, and in the impact of COPD on physical and emotional health and psychosocial adaptation of patients; PR also significantly increased the exercise tolerance and improved QL in the physical and psychological domains. Additionally, a positive influence on MS components was noted, which has added positive effects on the psycho-emotional state of patients.

## Conclusion

Thus, a PR program can be considered an important tool for the treatment of patients with COPD and MS. Along with a decrease in the symptoms of the disease, PR promotes respiratory muscle training, training of upper and lower extremity muscle groups, and improvement of the function of the immune system and metabolic processes with positive influence on the cardiovascular system and psychological status.<sup>(19)</sup> Indeed, the main benefits of a comprehensive PR program for patients with COPD and MS include a decrease in symptoms (dyspnea and fatigue), improvements in exercise tolerance, and reduction in health care utilization (particularly bed-days), as well as an increase in physical activity and an improvement in MS components.

## Competing interests

The authors declare that they have no competing interests.

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## Effects of the Presence of Left Main Coronary Artery Disease on the Results of Off-pump Coronary Artery Bypass Grafting Surgery

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### Abstract

**Background:** Left main coronary artery (LMCA) disease is one of the risk factors that affect the outcomes of coronary artery bypass grafting surgery. In particular, this risk factor pertains to the conduct of conventional on-pump CABG. Very little is known about the effects of the presence of LMCA disease on the results of off-pump CABG (OPCABG) surgery.

**Material and Methods:** In the Department of Cardiac Surgery of the Republican Specialized Center of Cardiology, during the period between April 2015 and April 2017, 270 consecutive OPCABG procedures were performed. Patients were divided into 2 groups depending on the presence or absence of LMCA disease. Group 1 consisted of 124(44.9%) patients with LMCA disease, and Group 2 consisted of 146(55.1%) patients without LMCA lesions (non-LMCA group).

**Results:** The average number of distal anastomoses in both groups was more than 3 anastomoses/patient. The incidence of nonfatal intraoperative complications was 8.9% in Group 1 and 15.1% in Group 2 ( $P=0.1212$ ). The conversion rate to on-pump CABG amounted to 3.2% (4 patients) in Group 1 and to 4.8% (7 patients) in Group 2. In the immediate postoperative period, 40(32.2%) patients of Group 1 and 45(30.8%) of Group 2 needed inotropic support until full restoration of normal hemodynamics with duration between 3.0 and 2.6 hours. The average duration of ventilation support was 6.4 hours in Group 1 and 5.6 hours in Group 2. Hospital mortality was 0.8% (1 patient) in Group 1 and 0.7% (1 patient) in Group 2.

**Conclusion:** Thus, the presence of left main stem lesion of LCA is not an additional risk factor that would complicate the performance of OPCABG surgery. The OPCABG operation in this group of patients is a safe method and can be performed without compromising the completeness of myocardial revascularization with the same low mortality as in low-risk patients. (International Journal of Biomedicine. 2017;7(3):175-179.)

**Key Words:** off-pump CABG • left main coronary artery • surgical technique • myocardial revascularization

### Abbreviations

AF, atrial fibrillation; ACS, acute coronary syndrome; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus LAD, left anterior descending artery; LCA, left coronary artery; LMCA, left main coronary artery; LIMA, left internal mammary artery; LVEF, left ventricular ejection fraction; LVEDD, LV end-diastolic diameter; LVEDV, LV end-diastolic volume; MI, myocardial infarction; OPCABG, off-pump CABG; PDA, posterior descending artery; PCI, percutaneous coronary intervention; PVCs, premature ventricular contractions; RIMA, right internal mammary artery; RCA, right coronary artery; VT, ventricular tachycardia; VF, ventricular fibrillation.

### Introduction

Left main coronary artery (LMCA) disease is one of the risk factors that affect the outcomes of coronary artery bypass

grafting surgery. In particular, this risk factor pertains to the conduct of these operations with cardio-pulmonary bypass and cardioplegia - conventional on-pump CABG. Very little is known about the effects of the presence of LMCA disease on the results of OPCABG surgery. Given all of the above, it seemed to us beneficial to conduct additional research in this area. In this regard, we would like to present our results from performing OPCABG surgery on patients with LMCA disease.

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## Material and Methods

In the Department of Cardiac Surgery of the Republican Specialized Center of Cardiology, during the period between April 2015 and April 2017, 270 consecutive OPCABG procedures were performed (this accounted for 90% of the total number of 300 patients who underwent isolated CABG operations).

The study protocol was reviewed and approved by the Ethics Committee of the Republican Specialized Centre of Cardiology. All participants provided the written informed consent. Patients were divided into 2 groups depending on the presence or absence of LMCA disease. Group 1 consisted of 124(44.9%) patients with LMCA disease, and Group 2 consisted of 146(55.1%) patients without LMCA lesions (non-LMCA group). Examination of clinical and demographic indicators in the groups (Table 1) showed that the distribution of patient characteristics in both groups was relatively homogeneous.

**Table 1.**

### Preoperative demographics and clinical data

Variable	Group 1 (n=124)	Group 2 (n=146)	P-value
Sex, M/F	M - 102 (82.2%) F - 22 (17.7%)	M-122 (83.6%) F- 24 (16.4%)	0.7759
Age, years	60.2±9.3	59.3±6.3	0.3469
Obesity	40 (32.2%)	59 (40.4%)	0.1659
COPD	24 (19.4%)	54 (37.0%)	0.0014
DM	38 (30.6%)	52 (35.6%)	0.3877
Chronic renal failure	7 (5.6%)	15 (10.3%)	0.1658
History of MI	95 (76.6%)	107 (73.3%)	0.5307
ACS	31 (25.0%)	13 (8.9%)	0.0003
History of PCI	9 (7.2%)	9 (6.2%)	0.7195
LV EDD, mm	56.7±7.7	57.3±7.9	0.5298
LV EDV, ml	165.4±60.3	166.3±58.3	0.9011
LV EF, %	54.5±10.1	54.9±10.1	0.7460
LV EF<45%	24 (19.4%)	24 (16.4%)	0.5323
NYHA III-IV	119 (96.0%)	126 (86.3%)	0.0063
Angina class CCA III	60 (48.4%)	89 (61.0%)	0.0384
Angina class CCA IV	64 (51.6%)	57 (39.0%)	0.0384
EuroScore add	4.32±2.6	3.43±2.7	0.0065
EuroScore log	4.63±4.8%	3.83±4.0%	0.1364

The majority of patients were male - 82.2% in Group 1 and 83.6% in Group 2. The mean age of patients was 60.2 and 59.3 years, respectively. More than two-thirds of patients in each group had a previous history of MI. DM was recorded in 30.6% of patients in Group 1 and in 35.6% of patients in Group 2. Chronic kidney disease stages 1 to 3 was identified in 5.6% and 10.3% of patients in Groups 1 and 2, respectively. Coronary artery stenting (PCI) in the anamnesis was performed in 7.3% and 6.2% of patients in Groups 1 and 2, respectively.

COPD was found in 36.9% of cases in Group 2 vs. 19.3% of cases in Group 1 ( $P=0.0014$ ). ACS was found in 25% of cases in Group 1 vs. 8.9% of cases in Group 2 ( $P=0.003$ ). Left ventricular measurements from echocardiography and LVEF were similar in both groups. LVEF was less than 45% in 19.4% and 16.4% of patients in Groups 1 and 2, respectively. The majority of patients in both groups were in NYHA functional classes III and IV (95.9% and 86.3%, respectively). Group 1 included more patients with unstable and severe angina (51.6% compared to 39.1% in Group 2, respectively,  $P=0.0384$ ). The risk of surgical intervention, calculated with a EuroScore risk calculator, was greater in patients of Group 1 -  $4.32\pm 2.6$  while in Group 2 it was  $3.43\pm 2.7$  ( $P=0.0065$ ). Nevertheless, for the majority of parameters, both groups were homogeneous.

### Surgical Technique

We used standard anesthesia monitoring and, in addition to conventional general anesthesia, the technique of high thoracic epidural analgesia. All operations were performed from the median sternotomy. In all patients, the left internal thoracic (or mammary) artery (LIMA) was harvested. When it was not possible to use the LIMA, we harvested the RIMA. In parallel, the great saphenous vein was harvested. Heparin was administered at a dose of 1 to 1.5 mg/kg of patient weight (until ACT value  $>300$  sec). The pericardium was opened in a standard inverted "T" fashion. In all cases, a deep pericardial suture (LIMA stitch) was used, by manipulation of which we could access various surfaces of the heart. If necessary (in case of problems with visualization of the lateral surface of the heart for access to the branches of the circumflex artery (Cx)), we applied additional deep pericardial sutures near the orifices of the left upper and lower pulmonary veins. We also used commercially available heart positioners (Starfish, Medtronic Minneapolis, MN). To stabilize the myocardium of the heart in the field of anastomosis, we used commercially available tissue stabilizers (Octopus 4 Tissue Stabilizer, Medtronic, Minneapolis, MN). Temporal occlusion of the coronary artery was performed by applying a silicone vessel loop to the proximal part of artery. After performing an arteriotomy, always, if possible, intra-coronary shunts were used (ClearView Intracoronary Shunt; Medtronic). Revascularization was initiated from an occluded recipient artery. Usually it was either LAD or RCA (or PDA). In cases where it was LAD, first LIMA-LAD anastomosis was constructed and then continued in the usual manner. If it was an RCA (or PDA), then at first a distal anastomosis with the vein was constructed. Then all proximal anastomoses were constructed on the ascending aorta using a side-bite clamp, and blood supply was restored to the RCA region. This was followed by revascularization of the LAD region. Arteries of the lateral side of the heart (Cx artery region) were revascularized only after the arteries of the anterior surface (LAD and RCA regions) had been revascularized and received a blood supply. If there were no occluded arteries, revascularization was always initiated with the LAD artery. After completion of the construction of all grafts, flowmetry (Transit-time flow measurement) was performed to assess the quality of anastomoses (MiraQ, Medistim, Norway). If there was no excess bleeding, we neutralized only half the dose of heparin.

Statistical analysis was performed using the statistical software SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. For data with normal distribution, inter-group comparisons were performed using Student's t-test. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. Two-tailed *P* values <0.05 were considered statistically significant.

## Results

Intraoperative data for groups are presented in Table 2.

**Table 2.**

### Intraoperative data

Variable	Group 1 (n=124)	Group 2 (n=146)	<i>P</i>
Operation times. min	255.14±55.0	264.8±56.1	0.1560
Usage of the left internal thoracic artery (or the right)	124 (100%)	146 (100%)	1
Distal anastomoses, per patient	3.07±0.7	3.02±0.8	0.5884
3 grafts	69 (55.6%)	81 (55.5%)	0.9748
4 grafts	29 (23.4%)	26 (17.8%)	0.2566
Sequential technique	9 (7.3%)	22 (15.1%)	0.0448
Composite grafts ("T", "Y"-grafts etc.)	18 (14.5%)	9 (6.2%)	0.0226
"LIMA first" technique	86 (69.4%)	121 (82.9%)	0.0088
"non LIMA first" technique	38 (30.6%)	25 (17.1%)	0.0088
Blood loss. ml	596.4±228.5	554.6±197.6	0.1082
Intraoperative complications:			
Frequent PVC	11 (8.9%) 0	22 (15.1%) 2 (1.4%)	0.1212
AF. VT. VF	7 (5.6%)	13 (8.9%)	
Need for inotropic support	4 (3.3%)	7 (4.8%)	
Conversion to on-pump	4 (3.2%)	7 (4.8%)	0.5159

The mean duration of the procedure was about 255 minutes in Group 1 and 264 minutes in Group 2. In both groups, LIMA (or RIMA) was used in all patients. The average number of distal anastomoses in both groups was more than 3 anastomoses/patient. In about 75%-80% of all patients, 3-4 grafts were constructed. In approximately 21%-22% of patients in both groups, we used a complex surgical technique in the form of sequential and composite grafts. In Group 2, the sequential grafting technique was used more frequently (15.1% vs. 7.3% in Group 1), while in Group 1 we constructed more composite grafts (14.5% vs. 6.2% in group 2). As we indicated earlier, the sequence of grafting varied depending on the presence or absence of occluded arteries. In accordance with this, initiation of revascularization with the LAD artery accounted for 69.4% in Group 1 and 82.9% in Group 2 (*P*=0.0088). In the remaining cases, grafting began from other territories (as a rule, it was the RCA region). Mean blood loss

in both groups did not differ and amounted to 596 ml and 554 ml, respectively. The incidence of nonfatal intraoperative complications was 8.9% in Group 1 and 15.1% in Group 2. As a rule, frequent PVCs, paroxysm of AF during intraoperative period, and hemodynamic collapse, which required the initiation of vasopressors, were the prevailing complications. The conversion rate to on-pump CABG amounted to 3.2% (4 patients) in Group 1 and to 4.8% (7 patients) in Group 2 (*P*=0.5159).

In the immediate postoperative period (Table 3), 40(32.2%) patients of Group 1 and 45(30.8%) of Group 2 (*P*=0.8002) needed inotropic support until full restoration of normal hemodynamics with duration between 3.0 and 2.6 hours. The average duration of ventilation support was 6.4 hours in Group 1 and 5.6 hours in Group 2. One patient in Group 1 required prolonged ventilation for more than 24 hours, while there were no such complications among the patients of Group 2.

**Table 3.**

### Postoperative data

Parameter	Group I (n=124)	Group II (n=146)	<i>P</i>
Need for inotropic support	40 (32.2%)	45 (30.8%)	0.8002
Inotropic support time, hours	3.0±6.57	2.6±6.15	0.6062
Ventilation time, hours	6.4±4.1	5.6 ± 3.1	0.0694
Prolonged ventilation, >24 hours	1(0.8%)	0	0.4592
RBC transfusion, ml	347.1±182.0	338.7±162.5	0.6891
FFP transfusion, ml	432.2±135.5	422.2±132.7	0.5412
AF	7 (5.6%)	9 (6.2%)	0.8580
Chest re-open for hemostasis	2 (1.6%)	1 (0.7%)	0.5954
Superficial wound infection	1 (0.8%)	1 (0.7%)	1
Perioperative MI	1 (0.8%)	1 (0.7%)	1
Neurologic complications	0	1 (0.7%)	1
Length of stay in ICU, hours	53.6±19.4	52.1±20.8	0.5431
Length of hospital stay after surgery, days	6.2±1.4	6.9±3.4	0.0330
Mortality	1 (0.8%)	1 (0.7%)	1

*RBC* = red blood cells; *FFP* = fresh frozen plasma.

Patients of both groups received hemo- and plasmatransfusions when necessary, intraoperatively and/or in the postoperative period. Thus, patients of Groups 1 and 2 received on average 347.1 ml and 338.7 ml of erythrocyte mass, respectively (*P*=0.6891), as well as on average 432.2 ml and 422.2 ml per patient of fresh frozen plasma, respectively (*P*=0.5412). AF, a complication of the postoperative period, was noted in 5.6% of patients in Group 1 and in 6.2% in Group 2 (*P*=0.8580). All paroxysms of AF in the postoperative period passed without serious hemodynamic compromise and were treated medically.

In one patient (0.7%) of Group 2, there was an episode of transitory ischemic attack without neurologic deficit, while

in Group 1 such complications were not noted. Perioperative MI was noted for one patient in each group (0.8% and 0.7%, respectively). In the postoperative period, two patients (1.6%) in Group 1 and one patient (0.7%) in Group 2 underwent re-exploration of the mediastinum for hemostasis. In Groups 1 and 2, superficial wound infection was noted in 0.8% and 0.7% of cases, respectively ( $P=1$ ).

The average length of stay in the surgical ICU was 53.6 hours in Group 1 and 52.1 hours in Group 2 ( $P=0.5431$ ). The average length of the hospital stay after the procedure was 6.2 days for patients in Group 1 and 6.9 days in Group 2 ( $P=0.0330$ ). Hospital mortality was 0.8% in Group 1 and 0.7% in Group 2 ( $P=1$ ).

## Discussion

Since the beginning of the era of coronary surgery, surgeons have had a special attitude towards the patients with LMCA disease, which was expressed in special requirements for preoperative tactics, anesthesia, surgical techniques and postoperative management. And this attitude became more marked in the early 21st century after surgeons began to perform mass OPCABG operations.<sup>(1)</sup> This attitude was also reflected in the creation of special instruments and techniques for performing OPCABG operations in patients with LMCA disease.<sup>(2)</sup>

In general, the initial period of performing OPCABG operations is characterized by a low proportion of high-risk patients (LMCA disease, low LVEF, dilated left ventricle, etc.), as well as a low number of patients with ACS, combined pathology of the lungs and kidneys, etc.<sup>(3-5)</sup> For example, E. Buffalo et al.<sup>(4)</sup> report a mean number of grafts per patient of 1.9, and the proportion of OPCABGs in the total number of isolated CABG was 46%. In general, the initial experience of performing OPCABG operations (performed generally in low-risk patients), based on numerous studies, could not convincingly demonstrate the advantages over performing such procedures on-pump.<sup>(5-8)</sup> During the same period, the first reports on the results of OPCABG in high-risk patients appeared.<sup>(9)</sup> The milestone work was published by J. Puskas et al.,<sup>(10)</sup> which convincingly demonstrated the advantages of OPCABG over on-pump CABG in high-risk patients. For the first time, the advantages of OPCABG operations in a high-risk group were demonstrated when comparison of patients by preoperative risk index was attempted.

We present a consecutive series of patients subjected to isolated OPCABG, which reflects the daily practice of our group. According to the main clinical and demographic parameters, patient characteristics in both groups were relatively homogeneous. As in most other series, a significant proportion of patients were males, although the average age of our patients (about 60 years in both groups) was lower than those of most other authors. More than 70% of patients in both groups had a history of MI, and there were no differences with respect to this factor between the groups. The vast majority of patients in both groups were in NYHA functional classes III and IV, as well as in the III-IV functional classes of angina pectoris by CCA. However, there were significantly more

patients with ACS (25% vs. 8.9%) in Group 1, which in our opinion was due to the specificity of coronary artery disease in patients of this group.

We detected some difference in the coronary region from which the myocardial revascularization was started. In 69% of cases in Group 1 and 83% in Group 2, revascularization started with the LAD artery. This index was lower in Group 1, because they had more occlusive lesions of the RCA region, from which we had to start revascularization.

One of the criteria for the effectiveness of performing OPCABG is the proportion of such operations in the total number of all isolated CABG operations. This proportion varies from 49% to 99%, according to the reports from different authors.<sup>(3-7,10,11)</sup> Leading groups in this area, such as J. Puskas' group, perform OPCABG operations in 99% of all cases. In our series of 300 isolated CABG operations, 270(90%) were performed off-pump. Of these, some of the patients were initially distributed to have the operation on-pump, and other patients, who were initially planned for OPCABG, were intraoperatively converted to on-pump CABG. On average, the rate of conversion varies from 1% to 15%.<sup>(3,9-12)</sup> In our series of operations, this indicator was 3.2% and 4.8% in Groups I and II, respectively. Our results are also within the limits of the values achieved by other groups.

The main argument against performing OPCABG operations that opponents of this method put forward is incomplete revascularization. In some studies, the average number of distal anastomoses per patient in OPCABG procedures was less than in similar patients subjected to conventional on-pump CABG. Such data may indicate whether the results were due to selection bias or true incomplete revascularization of patients. So, in the reports published by W. Turner et al.,<sup>(3)</sup> in a series of 100 operations the average number of distal anastomoses per patient was 1.9, and in E. Buffalo's series<sup>(4)</sup> of 3,866 operations, this indicator was also 1.9. M. Emmert et al.<sup>(11)</sup> report 3.62 distal anastomoses per patient in their series of OPCABG operations. The mean number of distal anastomoses per patient in our series was 3.07 and 3.02 in Groups 1 and 2, respectively, which may indicate the absence of a selection bias and a sufficiently high rate of complete revascularization cases (considering the number of patients with two- and three-vessel coronary lesions).

As can be seen in Table 3, according to the main parameters of the postoperative period, the results of operations in both groups were similar. According to the frequency of need for inotropic support, the time of respiratory support and the frequency and volume of plasma- and hemotransfusion, the parameters in the two groups did not differ. The AF frequency was 5.6% and 6.2% in Groups 1 and 2, respectively, a statistically insignificant difference. As is known, the rate of AF in the postoperative period after conventional on-pump CABG operations varies within the range of 30%-45%. According to various authors, after OPCABG surgery, AF occurs in 4%-26% of cases. Our results were also at the lower boundary of the given spectrum.

In addition, we did not find a significant difference in the incidence of non-fatal complications (perioperative MI, neurological disorders, bleeding, etc.) in the groups. Hospital

mortality was 0.8% and 0.7% in Groups 1 and 2, respectively. At the same time, the risk of surgical intervention calculated by the risk-calculator EuroScore was 4.63% for the patients of Group 1 and 3.83% for the patients of Group 2. J. Puskas et al.,<sup>(10)</sup> as well as A. Parolari et al.<sup>(13)</sup> and YN Youn et al.,<sup>(18)</sup> gave the same difference of the risk of operative intervention predicted by the EuroScore calculator and actual mortality after OPCABG surgery. Our results of hospital mortality are within the limits of data given by other authors.<sup>(3-5,7,10)</sup>

## Conclusion

Thus, the presence of left main stem lesion of LCA is not an additional risk factor that would complicate the performance of OPCABG surgery. The OPCABG operation in this group of patients is a safe method and can be performed without compromising the completeness of myocardial revascularization with the same low mortality as in low-risk patients.

## Study limitations

The retrospective nature of this study makes it difficult for the authors to draw any definite conclusions. In addition, the number of patients in both groups is relatively small, which may reduce the statistical reliability of the results obtained, or a possible difference is not apparent due to the small number of cases. Nevertheless, the results we obtained clearly demonstrate the safety of performing OPCABG surgery in patients with LMCA disease.

## Competing interests

The authors declare that they have no competing interests.

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# Electrical and Contractile Properties of the Heart Ventricle in Response to Ambient Temperature Changes in Frog *Rana temporaria*

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## Abstract

The aim of the study was to investigate the dynamics of change in the intraventricular pressure, depolarization and repolarization processes on the ventricular epicardium (VE) in *Rana temporaria* in response to a rise in ambient temperature. By methods of catheterization and electrophysiological mapping the dynamics of the intraventricular pressure change, the processes of depolarization and repolarization on the epicardium of the heart ventricle in adult frogs in a temperature range from 10°C to 20°C were studied. We found that the rise in body temperature by 10°C led to increase of the maximal systolic ventricular pressure (MSVP), maximal value of the MSVP derivative and maximal rate of MSVP decline but to a decrease in dispersion of depolarization time and durations of activation-recovery intervals on the ventral and dorsal sides of VE. The role of the electrical inhomogeneity of the myocardium was shown to be a modulator performing fine adjustment to factors in the external environment of the organism. (**International Journal of Biomedicine. 2017;7(3):180-184.**)

**Key Words:** intraventricular pressure • ventricular epicardium • depolarization • repolarization • temperature • *Rana temporaria*

## Abbreviations

AT, activation time; ARIs, activation-recovery intervals; ECG, electrocardiogram; EDP, end-diastolic pressure; HR, heart rate; MSVP, maximal systolic ventricular pressure; VE, ventricular epicardium.

## Introduction

The ambient temperature can limit or increase absorption of O<sub>2</sub> in animals; thus it has a drastic effect on the cardiovascular system.<sup>(1-4)</sup> Ectothermic animals inhabiting regions with a temperate climate have to compensate for temperature changes in order to cope with the seasonal thermal variations. It is known that ectothermic vertebrate hearts are highly sensitive to temperature effects. Therefore, ectothermic animals can be used to understand mechanisms controlling the electrical and contractile functions of the myocardium in response to changes in environmental temperature.<sup>(5-7)</sup>

At present there are only a few studies of the electrophysiological processes that occur in a frog's heart ventricle under changes in ambient temperature.<sup>(8,9)</sup> It has been shown that when the body temperature in the frogs is

decreased to 10°C, then HR is diminished and the durations of QRS and ST-T complexes of ECG are lengthened. At the same time, the repolarization sequence is changed on the ventricular epicardium (VE). There are no data about cardiohemodynamic in amphibia under temperature effects.

The aim of the study was to investigate the dynamics of change in the intraventricular pressure, depolarization and repolarization processes on the VE in *Rana temporaria* in response to a rise in ambient temperature.

## Materials and methods

### Animals and surgical procedure

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The Animal Care and Use Committee of Institute of Physiology of the Komi Science Center of the Russian Academy of Sciences approved the experimental protocol (approved number: 29).

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Data were obtained from 18 adult frogs *Rana temporaria* of both sexes (age, 2-3 years old). Two groups of animals were investigated: Group I consisted of 8 frogs with body temperature 10°C (body mass, 32±7 g), and Group II consisted of 10 frogs with body temperature 20°C (body mass, 40±5 g). The body temperature was measured rectally by means of a temperature sensor of the hemodynamic apparatus Prucka MacLab 2000 system. Measurements of rectal temperature did not show an essential difference between a frog's body temperature and the chamber of habitat. This is in agreement with the findings of other authors.<sup>(5,10)</sup> As in our previous studies on amphibians,<sup>(11,12)</sup> the animals were anaesthetized by placing them for 3 minutes in a jar containing 40% ethanol. After that, the ventral thoracic wall was removed and the pericardium was cut open. During the experiment, the heart was flushed with Ringer's solution. At the end of the experiment, the animals were euthanized by the intravenous injection of an overdose of alcoholic solution.

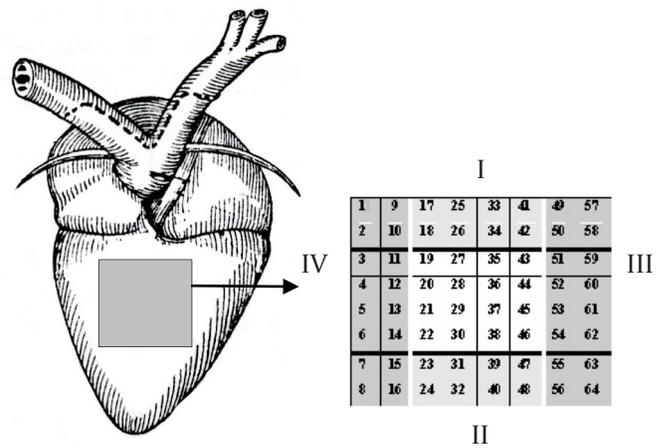
#### Hemodynamic recording

The hemodynamic variables were determined with the Prucka MacLab 2000 system (GE Medical System, GmbH). The pressure in the ventricle was measured with a catheter (internal diameter, 1 mm) filled with the heparinized 0.9 % saline inserted via the free wall into the ventricular cavity. Invasive monitoring of the pressure was carried out using transducers, transforming blood pressure inside of the vessels as the transducer registered mechanical changes. IP and ECG in standard bipolar limb leads were recorded synchronously. Hemodynamic parameters measured included MSVP (mmHg), EDP (mmHg), maximal value of the MSVP derivative ( $+dP/dt_{max}$ , mmHg/s), maximal rate of MSVP decline ( $-dP/dt_{min}$ , mmHg/s). The durations of QRS complex and QT interval were measured also.

#### Electrocardiographic recording and analysis

Unipolar electrograms and ECGs were recorded in reference to Wilson's central terminal. Simultaneous data acquisition was done by means of a custom-designed mapping system (16 bits; bandwidth 0.05 to 1000 Hz; sampling rate 4000 Hz). Standard bipolar limb lead ECGs were recorded with an application of subcutaneous steel needle electrodes. Registration of an epicardial electrogram was performed by using a matrix (5 mm x 5 mm) containing 64 electrodes at the sinoatrial rhythm. The matrix was alternately superimposed on the central portion of the ventral and dorsal sides of VE in such a way that the superior border of the matrix grasped the basal part and the inferior border – part of the ventricular apex (Fig.1).

In every epicardial lead, the activation time (AT), the repolarization time (RT) and the activation-recovery intervals (ARIs) were obtained. The latter was used to assess local repolarization durations. AT, RT and ARIs were determined as  $dV/dt_{min}$  during QRS complex,  $dV/dt_{max}$  during ST-T, and the difference between RT and AT, respectively. The values were determined automatically, inspected by the observer and corrected manually if necessary. In each set of simultaneously recorded electrograms, the beginning of the QRS complex in the II limb lead was chosen as a reference time point with respect to which ATs and RTs were measured in a given set of electrograms.



**Fig. 1.** Scheme of the position of the matrix (5 mm × 5 mm) with electrodes on the epicardial surface of the ventral side of the frog ventricle. Numbers from 1 to 64 show the location of the electrodes on the matrix. Black-and-white marking on the matrix shows the boundaries of the epicardial mapping zones.

I - the basal region of the matrix; II - the apical region of the matrix  
III - the right portion of the matrix; IV - the left portion of the matrix.

The dispersion of ATs, ARIs, and RTs of the ventricle were taken as the difference between the maximal and minimal AT, RT and ARIs values in a set of recorded electrograms, respectively.

In order to construct isochronal activation maps, the zero point was assigned to the timing of the epicardial activation breakthrough. Similarly, the zero points in the repolarization maps identify for the earliest repolarization on the epicardium.

Statistical examination was done using statistical package Primer of Biostatistics 4.03 and SPSS 11.5 using Wilcoxon test for paired comparisons and the Friedman test followed by the Wilcoxon test. Data are presented as mean±SD. A probability value of  $P<0.05$  was considered statistically significant.

## Results

At the body temperature of 10°C, HR in frogs was 24.3±2.3 bpm, and the duration of QRS complex and QT interval was 83.4±4.5 ms and 1375.6±363.2 ms, respectively. When body temperature was increased to 20°C, HR was raised to 38.8±3.1 bpm ( $P<0.05$ ), but the duration of QRS and QT interval was decreased to 68.7±7.2 ms and 783.3±102.2 ms, respectively.

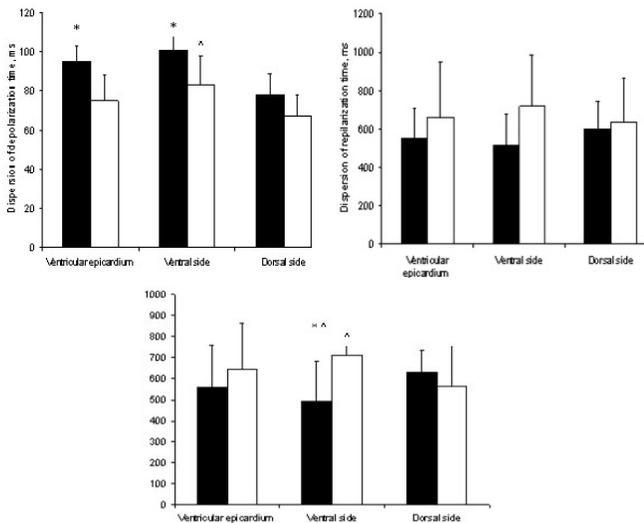
At 10°C, the earliest foci of depolarization were located in the basal part of the epicardial fragment on the dorsal surface ( $P<0.05$ ), and the latest foci in the left part of the epicardial fragment on the ventral surface. The sequence of activation on the dorsal surface of the epicardium is from the base to the apex ( $P<0.05$ ) (Table 1). For frogs in Group I, dispersion of AT in both epicardial surfaces was more than for those in Group II ( $P<0.05$ ) (Fig. 2). The duration of repolarization on the ventral surface of the epicardium was greater than on the dorsal one ( $P<0.05$ ) (Fig. 2). Dispersion of ARIs time in both epicardial surfaces of the ventricle was 559.0±200.8 ms in total (Fig. 2). At the same time, dispersion of ARIs time was less on the dorsal surface of the ventricle than on the ventral one ( $P<0.05$ ) (Fig. 2).

**Table 1.**

**Chronotopographic performance of different zones of VE of the frog heart at the body temperature of 10°C (Group I) and 20°C (Group II)**

Epicardial areas of the ventricle		Depolarization time		Repolarization time	
		Group I	Group II	Group I	Group II
VS	I	35.4±12.3	21.8±11.8#	961.5±342.2	855.4±250.7
	II	35.8±10.6	32.5±12.3	950.4±342.5	858.8±249.5
	III	32.7±10.2*	22.5±13.4*	972.2±334.6	828.1±245.6*
	IV	40.2±10.7	38.1±13.1	963.5±346.2	918.2±252.1
DS	I	26.6±9.4†	30.4±9.4†	1243.6±469.8^	813.5±220.1
	II	35.4±10.8	43.2±10.6	1250.0±450.4^	804.7±235.7
	III	29.6±12.7	44.1±14.3	1236.2±478.5^	814.4±221.1
	IV	31.4±14.7	37.2±17.5	1245.7±418.4^	805.7±240.7

\* $P < 0.05$  – in relation to the left part of the epicardial portion of the ventricle; # $P < 0.05$  – in relation to the apical part of the epicardial portion of the LV. ^ $P < 0.05$  – in relation to Group II. I – fragment of the basal region of the matrix; II – fragment of the apical region of the matrix; III – fragment of the right region of the matrix; IV – fragment of the left region of the matrix. VS – ventral side of the VE; DS – dorsal side of VE.



**Fig. 2.** Dispersion of depolarization, repolarization and ARIs times in the frog VE at the body temperature of 10°C and 20°C. \* $P < 0.05$  – in relation to the body temperature of 20°C. ^ $P < 0.05$  – in relation to the dorsal side of the VE. ■ Group I (10°C) □ Group II (20°C)

At 20°C, a wave of depolarization on the ventral and dorsal surfaces of the ventricle extends from the base to the apex ( $P < 0.05$ ). The earliest AT was found in the basal part of the epicardial fragment on the ventral side, and the latest in the right part on the dorsal surface (Table 1). Dispersion of AT on the ventral and dorsal surfaces of the epicardium was 75.4±13.0 ms, and AT on the ventral side of the ventricle was more than that on the dorsal side ( $P < 0.05$ ) (Table 1).

**Table 2.**

**ARIs duration different areas on the ventral and dorsal sides of the VE of the frog at the body temperature of 10°C (Group I) and 20°C (Group II)**

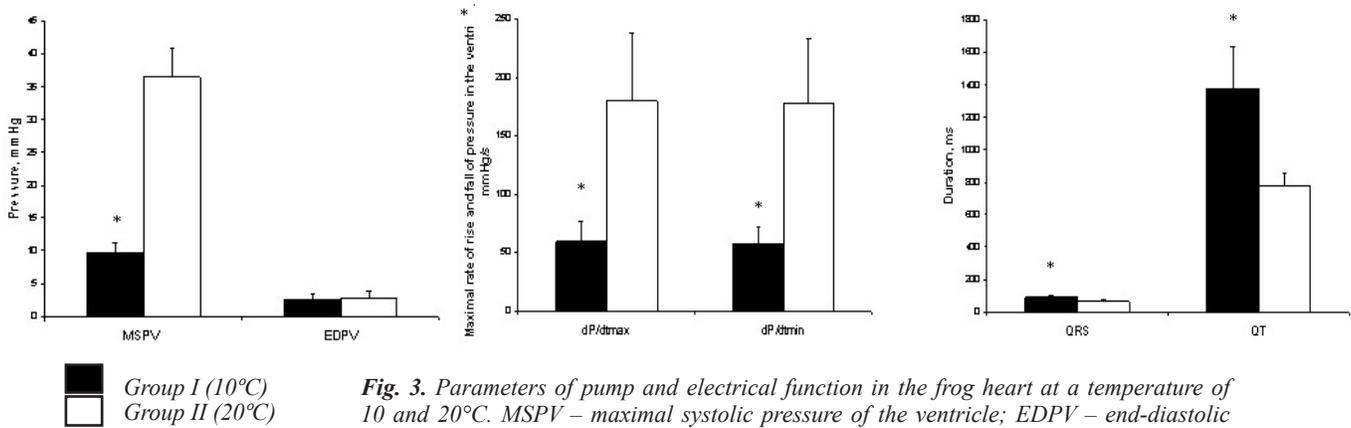
Epicardial areas of the ventricle		Group I	Group II
Total duration of ARIs both sides of the epicardium of the ventricle of the frog		1067.0±153.4*	800.8±33.5
VS	Duration of ARIs of VS	918.7±203.6##*	828.4±96.3#
	I	926.6±150.4*	837.1±252.7
	II	915.5±149.4*	816.3±270.2
	III	939.4±141.3*	806.6±207.2^
	IV	919.7±152.4	869.1±203.9
DS	Duration of ARIs of DS	1215.4±325.9*	772.1±101.0
	I	1220.5±360.2*	782.4±128.6
	II	1214.1±343.4*	762.4±137.3
	III	1235.0±294.3*	764.4±200.5
	IV	1217.7±318.2*	769.5±147.2

\* $P < 0.05$  – in relation to Group II; ^ $P < 0.05$  – in relation to the left side epicardial fragment of VS of the ventricle; # $P < 0.05$  – in relation to the duration of ARIs on DS of the epicardial portion of LV. I – fragment of the basal region of the matrix; II – fragment of the apical region of the matrix; III – fragment of the right pane of the matrix; IV – fragment of the left region of the matrix; VS – ventral side of the VE; DS – dorsal side of VE.

It should be noted that at this temperature the duration of ARIs on the ventral surface of the VE was increased as compared with the dorsal surface ( $P < 0.05$ ) (Table 2). On the ventral side of the epicardium the largest increase in repolarization duration was observed in the left part as compared with the right part ( $P < 0.05$ ) (Table 2). In Group II, the repolarization durations both on ventral and dorsal surfaces of the ventricle, as well as the total durations of ARIs, were less than in Group I ( $P < 0.05$ ) (Table 2). Dispersion of ARIs time in the ventral and dorsal surfaces of the ventricle was 645.4±219.8 ms; on the ventral side it was more than on the dorsal side ( $P < 0.05$ ) (Fig. 2). There was an increased dispersion of ARIs time on the ventral surface of the epicardium as compared with Group I ( $P < 0.05$ ).

The repolarization sequence was directed from right to left on both surfaces of the VE (Table 1). We did not find apicobasal differences in termination time of repolarization (Table 1). Dispersion of termination time of repolarization on the ventral and dorsal surfaces of the epicardium was 662.9±285.0 ms. There were no differences in dispersion of termination time of repolarization between the ventral and dorsal sides of the epicardium in both groups of animals (Fig. 2).

Thus when body temperature in frogs was increased to 20°C, then the dispersion of AT and ARIs on the ventral and dorsal sides of the VE were diminished.



**Fig. 3.** Parameters of pump and electrical function in the frog heart at a temperature of 10 and 20°C. MSPV – maximal systolic pressure of the ventricle; EDPV – end-diastolic pressure of ventricle; dP/dt<sub>max</sub> – maximal value of the MSVP derivative; dP/dt<sub>min</sub> – maximal rate of MSVP decline; QRS – duration of QRS complex in ECG; QT – duration of QT interval in ECG. \*P<0.05 – in relation to body temperature of 20°C.

### Dynamics of change of intraventricular pressure

When the body temperature in frogs was increased by 10°C, the indexes of the pump function of the ventricle were increased roughly by a factor of three: MSVP (9.0±3.1 vs. 36.2±6.8 mmHg;  $P<0.05$ ), dP/dt<sub>max</sub> (55.1±7.3 vs 182.8±62.3 mmHg/s;  $P<0.05$ ) and dP/dt<sub>min</sub> (49.5±6.7 vs 175.0±60.8 mmHg/s;  $P<0.05$ ). At the same time, EDP was not changed (Fig. 3).

## Discussion

The present investigation showed that in frogs, in response to the change in body temperature from 10°C to 20°C, the studied indexes of the pump function of the ventricle were increased, but dispersion of depolarization time and duration of ARIs on the ventral and dorsal sides of the VE were decreased.

The rise of body temperature in studied animals causes a decrease in the duration of ARIs on the both surfaces of the VE (the tissue level of the heart organization). And at the cellular level, the duration of the action potential of ventricular cardiomyocytes is shortened in ectothermic animals with an increase in ambient temperature,<sup>(13-15)</sup> that is, the same trend is observed.

Our study showed that in frogs, with the rise of ambient temperature there was an increase of HR and shortening of the duration of the ventricular complex of the ECG. These findings were in consistent agreement with previously obtained data.<sup>(8,9)</sup>

The regional (ventral-dorsal) changes in the repolarization process on the epicardium of frog ventricles at body temperature of 20°C are presented here for the first time. We assume that it is probably one of the mechanisms that ensure an adequate contractile act in response to the temperature effects.

We have found that the rise in body temperature in frogs from 10°C to 20°C causes an increase in heterogeneity of repolarization in the VE. The lack of significant changes in the excitation sequence of the VE, in dispersion of ARIs times and the termination of repolarization, possibly is due to heterogeneous change in the action potential duration of cardiomyocytes in different epicardial areas. According to the

contemporary paradigm,<sup>(16-18)</sup> the electrical and mechanical inhomogeneity of the myocardium is a modulator performing fine adjustments to the factors of the internal and external environments of the organism; that is, it performs an adaptive function.

At the same time, it is possible that there is a limiting effect of electrical heterogeneity of the myocardium to the optimal HR for a given species.<sup>(12)</sup> The obtained results point to the existence of adaptive potential or functional reserve in the heart of the studied animals that occurs in response to the change in the ambient temperature.

### Limitations and perspectives of the study

In our study, the frogs were anaesthetized by 40% ethanol. In experimental work with amphibians, authors have used different drugs for anaesthesia: methanesulfonate (MS-222),<sup>(19-20)</sup> ether<sup>(5,8)</sup> and urethane.<sup>(21)</sup> Therefore, further experiments are necessary to compare effects of these drugs on the amphibian cardiovascular system. The present experiments are limited in that they provide an assessment of the processes of depolarization and repolarization only on the fragments of VE. It would be interesting to broaden these studies using ultrasound methods and an increased temperature range to examine in detail the adaptive features of cardiohemodynamics in amphibia.

## Competing interests

The authors declare that they have no competing interests.

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## The Role of Integrated Gas Compounds in Regulation of Gas Homeostasis in the Norm

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### Abstract

In practically healthy people on the background of self-breathing, we used catheterization to obtain blood samples from Ao, PT, SC, VJI, SS, VH and VR. We believe that the standard tests of blood gases by volume ( $pO_2$  and  $pCO_2$ ) and their A-V gradients, quantitatively determined, are insufficient to fully assess the hypoxic states both in the whole organism and in individual organs. To estimate gas homeokinesis, we performed integral gas tests, including an additive criterion of blood gases—pressure in mmHg: 1) the summary pressure of the plasma gases, PS; 2) Gas functional, the gradient between the total indices of arterial and vein gases (Gradient D); and 3) the exchange gradient, Gradient DP [(arterial  $pO_2$  – venous ( $pO_2 + pCO_2$ ))]. Each test indicator at all studied points was determined in mmHg. Correlation analyses were carried out between the parameters of all tests.

We found that the processes forming PS limit the amplitude of the PS deviation under changing parameters of the constituent components ( $pO_2$  and  $pCO_2$ ) due to acts of mutual replacement between them, as well as the influence of integral gas complexes under shifts in  $pO_2$ ,  $pCO_2$ , A-V  $SO_2$ . Unlike the generally accepted tests that record quantitative differences between the points studied, the integral gas tests allow us to identify vectors and mechanisms of adaptive changes in gas homeostasis, to perform a qualitative comparison of the functioning of the studied organs by gas-dynamic tests in the norm and in pathology. (**International Journal of Biomedicine. 2017;7(3):185-191.**)

**Key Words:** integrated gas compounds • blood gases • gas homeokinesis • gas functional

### Abbreviations

**Ao**, aorta; **A-V**, arteriovenous; **BP**, blood pressure; **DP**, diastolic pressure; **Er**, erythrocyte; **MP**, mean pressure; **PT**, pulmonary trunk; **PI**, plasma; **PP**, pulse pressure; **PS**, summary gas pressure; **P-n**, total protein; **PSv** - PS of venous blood; **RV**, right ventricle; **SS**, sigmoid sinus; **SP**, systolic pressure; **SC**, coronary sinus; **SO<sub>2</sub>**, oxygen saturation; **VJI**, internal jugular vein; **VH**, right hepatic vein; **VR**, right renal vein.

**The aim** of the study was to evaluate the distribution of the PS values in the investigated points of the human body and their participation in the regulation of gas homeostasis in the norm.

### Methods and Results

With the patient in the supine position, we used catheterization to obtain blood samples from the outflow vessels of the three-chamber block of ventricles (Ao, PT, SC), VJI, VH, and VR.<sup>(1)</sup> To investigate general and organ gas exchange, in normal practice, we used data for temporal

dynamics arterial and venous blood and their A-V gradients. In our opinion, the separately taken values of  $pO_2$  and  $pCO_2$  are changed quantitatively (in absolute values) and are inadequate for evaluating polyorganic hypoxic states, since the use of the general average values does not reveal qualitative changes that characterize and explain the so-called mutual replacements of  $pO_2$  and  $pCO_2$  at varying ratios of the levels of  $pO_2$  and  $pCO_2$  (the biophysical equivalent of the Haldane effect) in the investigated areas of the human body.

To estimate the gas homeostasis, along with the generally accepted methods we used tests that take into account the additive trait for  $pO_2$  and  $pCO_2$ —a pressure in mmHg:

- 1)  $PS = pO_2 + pCO_2$  (at each investigated point)
- 2) Gas functional (Gradient D, GD) between PSs in arteries and veins ( $GD = (pO_2 + pCO_2)_A - (pO_2 + pCO_2)_V$ ).
- 3) Exchange gradient (Gradient DP, GDP) [(arterial  $pO_2$  –

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venous ( $pO_2 + pCO_2$ )]. GDP between Ao  $pO_2$  (which provides metabolic processes involving  $O_2$ , including the binding of  $O_2$  to Hb), and PSv (which is formed as a result of metabolism, including Hb- $O_2$  dissociation) that change conjugately and unidirectionally (+).

These tests can be changed quantitatively (absolute values) and qualitatively (by sign). Unlike gas volume tests, gradient DP is less dependent on the factors affecting  $O_2$  transport in the vascular bed, Hb concentration, or stroke and minute heart volumes. GDP helps to identify the qualitative differences in organ metabolism that are not determined by other methods. The complementary relationship between two test groups makes possible their joint application.

The following values and correlations between the proposed tests were calculated for Ao-TP (heart-lung block); Ao-CS (myocardium block); Ao-VJI (brain block); Ao-VH (liver block); and Ao-VR (kidney block). As a result, we obtained information on the distribution of integrated gas indicators at various points of the human cardiovascular system.<sup>(2-5)</sup> The vector and amplitude of the distribution of the indices studied were determined by the relationships with the mean hemodynamic pressure of each organ (Pmed, defined as the initial value of the perfusion pressure, which provides the metabolism). We present cases with the following parameters: Ao  $pCO_2$  – 30-45 mmHg, Ao  $pO_2$  – 70-100 mmHg, HR – 75.6±1.2 bpm, Hb – 13.1±1.4 g/l, Ht – 45.0±0.1%. The remaining data are presented in Table 2.

The quantitative, numerical results of gas homeostasis, which we have studied for a long time, were presented earlier in our works.<sup>(6-34)</sup> The results of the study of the qualitative relationships of the gas homeostasis in the norm are given in Table 1, which shows statistically significant ( $P < 0.05$ ) values (+, 0, -) of correlations for the studied parameters, without indicating their magnitudes.

It should be noted that the correlation analysis showed the absence of reliable links for all proposed tests with Hb in all investigated points (in contrast to volume-additive tests), gas functionals with  $pCO_2$  and  $pO_2$  at all the points studied, and Ao PS with  $pCO_2$  at all points.

Reliable correlations for PS levels in venous points with  $pO_2$  (positive) and  $pCO_2$  (positive) in Ao blood were revealed, which indicates a combined unidirectional dynamics of these processes. We believe that this is why PSv can remain constant at a significant amplitude of shifts in  $pO_2$ ,  $pCO_2$  and Hb $O_2$ (%), due to acts of mutual replacement of  $pO_2$  and  $pCO_2$ . Reflection of the general dependence on Ao  $pO_2$  is positively correlated with PS levels at the venous points. We believe that this is a reason for the stability of PSv with significant shifts in the parameters of blood gases and Hb $O_2$ . Thus, SC PS is positively correlated with VR  $pO_2$ , and gradient DP is positively correlated with Ao  $pO_2$ .

We estimated the distribution of PS in the outflow vessels of the three-chambered block of heart ventricles (Ao, TP, SC), including studying the reproducibility of this distribution in different states of the human body. According to data of the norm in the blood of Ao, TR, SC, VJI, VH, VR, we determined  $pO_2$ ,  $pCO_2$  in mmHg, their A-V gradients, PS, as well as special gradients: GD and GDP.

Thus, the following correlations between the proposed tests were calculated: Ao-TP (heart-lung block); AO - CS (myocardium block); Ao-VJI (brain block); Ao-VH (liver block), and Ao-VR (kidney block).

To determine the vector and amplitude of the distribution of the investigated indices, their relationships with Pmed were analyzed in the investigated areas. [bulbus Ao (86.5±1.7), SC (5.3±0.4), TP (14.0±0.22), SS (9.4±1.2), VJI (6.7±1.3), RA (4.1±0.72), LA (8.1±0.8); HR (75.6±1.2 bpm), Hb (13.1±1.4 g/l), Ht (45.0±0.1%)].

All data were obtained under self-breathing. The data presented were investigated in the same order as blood ejection from the three-chamber block of ventricles into the outflow vessels (Ao, TP, SC) during united systole.<sup>(14)</sup>

When analyzing the baseline data, we established the stable changes in PS, regardless of the sampling point of blood, conjugate to changes and equivalent to the magnitude of the changes of each gas by its total value (both an increase and a decrease). It is also noted that PS can vary in the following directions: 1) less than one of them, but more than the other; and 2) less than the value of each of them, minimizing PS deviations and compensating the shifts of individual parameters.

PS is greater in those areas where Pmed is also greater (Table 2): the maximum in Ao (for  $pO_2 > pCO_2$ ); the minimum in SC (for  $pO_2 < pCO_2$ ); intermediate in TP ( $pO_2 = pCO_2$ ). PS values in the outflow vessels are close, or correspond, to the values of systemic BP:

- Ao PS (for  $pO_2 > pCO_2$ ) is within SBP: maximum = 145 mmHg [ $pO_2$ (100 mmHg) +  $pCO_2$ (45 mmHg); minimum = 100 mmHg [ $pO_2$  (70 mmHg) +  $pCO_2$ (30 mmHg)].

- TP PS (with variable ratios of  $pO_2$  and  $pCO_2$ ) is mathematically close to the average BP in the norm (PS = 84.8±0.5 mmHg, BP=86.5±5.1 mmHg.)

- PS SC (for  $pO_2 < pCO_2$ ) is mathematically close (within the statistical error) to DBP (PS=71.3±0.6mmHg, DBP=71±0.38 mmHg)

The values of these parameters are formed in the chambers of the three-chambered block of ventricles at the end-diastolic pressure in the right and left ventricles and the average pressure in SC. All pressure values (PS and systemic BP) are created conjugately between each other and other parameters of the cardiocycle on a single basis (the impulse of a quantum generator): contraction of the myocardium of the three-chambered block of ventricles.

The data presented demonstrate the dynamics of the regulation of gas homeostasis in the norm in the investigated areas of the body:

#### SC:

- A) Levels of  $pO_2$  and PS are minimal in comparison with other investigated points.

- B) Gradients with respect to  $pO_2$ ,  $pCO_2$ , and gas functionals are maximal in comparison with the other investigated points.

- C) SC PS is less than arterial blood  $pO_2$ , and gradient DP for the heart is positive (20.66±0.44 mm.Hg).<sup>(2,10,36,37)</sup> PS of SC blood, outflowing from the spongy (venous) chamber of three-chambered block of ventricles, is minimal (71.0±0.6 mmHg) and close to the systolic pressure in the aorto-pulmonary block with placental and beginning pulmonary gas exchange (open

Botalli's duct), when it functions as a single chamber.<sup>(16)</sup>

SC  $PO_2$  corresponds to the values of the hypoxic limit found in the venous blood of other points: VJI of 20mmHg is a critical level, accompanied by loss of consciousness. Figuratively speaking, the blood flow from the spongy (venous) chamber of three-chambered block of ventricles includes the criteria of the hypoxic limit of the human body.<sup>(27)</sup>

#### **TP:**

PS in mixed venous blood, the flow of which is created at different ratios of  $pO_2$  and  $pCO_2$  flowing from RV ( $84.8 \pm 5.0$  mmHg), is lower than in Ao blood and close to arterial blood  $pO_2$  ( $91.7 \pm 0.5$  mmHg) and to Pmed in Ao bulb ( $86.5 \pm 1.7$  mmHg).

This venous flow is created by the mixing of blood flowing from the spongy chamber of three-chambered block of ventricles, mixing with the flow of venous blood from the lower and upper hollow veins. A measure of the adequacy of the combined and unidirectional shifts of TP PS and Ao  $pO_2$  is Gradient DP ( $91.91 \pm 0.5$  mm.Hg), varying both in magnitude and in sign (+, 0, -) within the values obtained for the kidneys and the heart.<sup>(11,15,16)</sup> TP PS is mathematically close (within the margin of error) to PS in VJI and VH, with a common proximity to Ao Pmed.

#### **Ao:**

PS of the blood flowing from VS, at  $pO_2$  greater than  $pCO_2$ , is maximal ( $128.3 \pm 0.6$  mmHg) and close to SBP in aortic bulb ( $107.6 \pm 2.6$  mmHg); it is created by the mixing of the blood of RV and Thebesian veins of LA and LV, having ability to direct and reverse the flow of blood (two-way communications of the left heart chambers with the myocardium by pressure).

#### **VJI:**

PS ( $84.89 \pm 0.54$  mmHg) is mathematically (within the margin of error) close to the following parameters of arterial and venous blood:

- Average Ao  $pO_2$  ( $85.72 \pm 0.65$  mmHg); the measure of adequacy of unidirectional shifts of Ao  $pO_2$  and VJI PS is Gradient DP ( $0.83 \pm 0.57$  mmHg);
- Average Ao Pmed ( $86.5 \pm 1.7$  mmHg);
- TS PS ( $84.8 \pm 0.5$  mmHg).

It should be noted that the blood of VJI is formed by mixing the venous streams of two zones:

- Tissue zone (removal of brain metabolites) with low  $pO_2$
- Cerebrospinal fluid (choroidal plexus) with high  $pO_2$

We consider a significant fact to be almost complete coincidence (within the margin of error) of quantitative indicators of  $pCO_2$  levels in the blood of VJI and SC, while noting the difference in  $pO_2$  levels. We assume that the minimum value of  $pO_2$  ( $23.77 \pm 0.3$  mmHg) among the recorded values during catheterization is the individual hypoxic limit of the organism.

#### **VH:**

PS ( $88.51 \pm 0.54$  mmHg) is mathematically close to IVC PS, Ao Pmed, and Ao  $pO_2$ .<sup>(20,29,35)</sup> A measure of the adequacy of the unidirectional shifts of Ao  $pO_2$  and VH PS is Gradient DP ( $1.93 \pm 0.54$  mmHg). VH Pmed is mathematically close to the pressure in the liver parenchyma ( $8.0 \pm 0.7$  mmHg) and PV ( $8.42 \pm 0.76$  mmHg) with a minimum of gradients between these three points.

#### **VR:**

Under superperfusion and the mixing of the blood inside the kidney (A-V shunts), including juxtglomerular blood fractions with differences in  $pO_2$  and  $pCO_2$  in countercurrent exchange (analogue of the mixing process), there are formed 1) the maximal  $pO_2$  and VR PS levels in comparison with those for brain block, heart-lungs block, myocardium block, and liver block, wherein the ratio  $VR pO_2 > VR pCO_2$  is similar to that in Ao blood; 2) the values of Gradient D for  $pO_2$ ,  $SO_2$  and PS for the studied organs and blocks (heart-lungs, splanchnic pool, brain) are minimal; 3) VR PS is greater than Ao  $pO_2$ , therefore, Gradient DP for the kidneys is negative ( $-11.9 \pm 0.57$ ).<sup>(20,25,27,28,31,32)</sup> We believe that the upper limit of the norm for oxygenation ( $pO_2$ ) of venous blood is VR  $pO_2$ . In the blood of TP, VJI and VH, the blood oxygenation levels can vary between the minimum limit (SC  $pO_2$ ) and the maximum limit (VR  $pO_2$ ). The values of gas parameters beyond the maximum and minimum values can be a sign of decompensation of gas homeostasis.

A detailed analysis of all the qualitative and quantitative indicators (Table 1 and 2) was presented by us in earlier works.<sup>(15,16,17-24,34)</sup>

The interaction of specific proteins with different affinities for  $O_2$  is a unifying factor in the gas exchange of the heart, brain and kidneys.

1) In the heart, myoglobin (Mb) has an affinity to  $O_2$  6 times greater than Hb, with a shorter dissociation time and association of Mb and  $O_2$ , with the possibility of forced dissociation of  $HbO_2$  at  $SO_2$  of 50% and lower.

2) In adults, the mixing of blood flows with differences in  $pO_2$  and  $SO_2$  is a common characteristic in the formation of the blood composition in VJI and VR, as in the fetus—the mixing of blood of v.umbilicalis with its own venous blood (intravascular gas exchange).

The dissociation and association of  $HbO_2$  in different sections of the S-shaped curve depends on temperature, pH, electrolytes, etc. in different ways, as well as the Hb affinity for  $O_2$ . When the flows are mixed, the processes proceed without the participation of membranes, with the creation of new levels of  $pO_2$  and  $SO_2$ . Blood with low  $SO_2$  carries out forced dissociation of  $HbO_2$ , as in the transmembrane interaction of Hb and Mb in the heart, as well as in arterial blood and VR blood in the counterflow system of the kidney. In other words, the described mechanism allows us to consider the venous system of the organs represented as an internal oxygenator, ensuring cooperative multi-organ regulation of homeostasis as a whole.

The proposed integral tests reveal qualitative differences in the functioning of the organ-blood systems, which are not detected by the generally accepted tests:

a) In the myocardium block, when Ao blood periodically enters the exchange zone, where the minimum levels of  $pO_2$ ,  $HbO_2$ (%), SC PS are created, Gradient DP is maintained positive, and maximum gas gradients are created, including Gas functional.

b) In the microcirculatory bed of the kidney block (with participation of countercurrent metabolism), the maximum (venous) levels of  $pO_2$ ,  $HbO_2$ (%), VR PS and the minimal Gas gradients, including Gas functional, are created during 1 cardiocycle.

**Table 1. Integrated gas indicators at various points of the human body**

Variable	PS Ao	PS PT	PS VJI	PS VH	PS SC	PS VR	GD	GDP
Ao	K pl			+				
	K er	+		+				
	Na pl	-	+	-	+			
	Na er	-	-		+			
	pH	-	-	-	+			
	pCO <sub>2</sub>		+	+	+			
	SB	-	-	-	-			
	pO <sub>2</sub>	+	+	+	+	+	+	
	HbO <sub>2</sub>	+	+	+				
	Hb							
pS Ao		+	+					
PT	K pl	+						
	K er		+		+			
	Na pl	+						
	Na er	-		+	+			
	pH		+		+			
	pCO <sub>2</sub>		+		+			
	SB	-	+					
	pO <sub>2</sub>	+	+	+	+			
	HbO <sub>2</sub>	+	+	+	-			
	Hb							
pS PT	+		+	+				
VJI	K pl		+	+				
	K er	-	-	-	+			
	Na pl	-	-	-				
	Na er		-		-			
	pH	-		-				
	pCO <sub>2</sub>		+	+	+			
	SB	-	-	-	-			
	pO <sub>2</sub>	+	+	+	+			
	HbO <sub>2</sub>	+		+	-			
	Hb							
PS VJI	+	+		-				

**Table 1. (Continued)**

Variable	PS AO	PS PT	PS VJI	PS VH	PS SC	PS VR	GD	GDP
VH	K pl	+		+				
	K er	-	+	-	+			
	Na pl	-	-	-				
	Na er		-		-			
	pH	-		-				
	pCO <sub>2</sub>		-	+	+			
	SB	-						
	pO <sub>2</sub>	+	+	+	+			
	HbO <sub>2</sub>	+		+	-			
	Hb							
pS VH		+	-					
SC	pCO <sub>2</sub>				+	+		
	pO <sub>2</sub>							
	Hb							
VR	pO <sub>2</sub>				+			
	pCO <sub>2</sub>					+		
	Hb							
Ao	sp	-	+	-		x	x	- -
	dp	-	-	-		x	x	-
	pp		+			x	x	
	mp	-	-	-		x	x	-
PT	sp	-	-	-		x	x	- -
	dp	-	-	-	+	x	x	-
	pp	-	-	-		x	x	- -
	mp	-	-	-		x	x	- -
VJI	sp	-	-	-	-	x	x	-
	dp	-	-	-	-	x	x	
	pp	-	-	-	-	x	x	-
	mp	-	-	-	-	x	x	
VH	sp		-	-	-	x	x	-
	dp	-	-	-	-	x	x	-
	pp	+	-	+	-	x	x	- +
	mp	-	-	-	-	x	x	-
PS PT		+		+	+	x	x	
PS VH			+	-		x	x	
GD		+	+				x	x
GDP		+	+				x	x

**Table 2. Blood gas parameters in the points studied**

Variable	pO <sub>2</sub>	PCO <sub>2</sub>	PS	pH	SO <sub>2</sub>	Pmed
SC	23.77±0.30	47.50±0.34	71.3±0.6	7.345±0.005	38.02±0.70	5.3 ±0.4
TP	42.96±0.40	41.86±0.30	84.80±0.50	7.372±0.003	76.80±0.42	14.0±0.2
Ao	91.70±0.54	36.62±0.21	128.3±0.6	7.396±0.003	96.74±0.07	86.5 ±1.7
VJ	37.95±0.57	47.95±0.24	84.89±0.54	7.353±0.003	68.21±0.74	6.7±1.3
VH	43.63±0.49	44.88±0.34	88.51±0.54	7.360±0.004	76.76±0.62	8.38±1.38
VR	59.4±1.20	41.35±0.24	100.7±0.80	7.375±0.003	87.75±0.46	8.97±1.74
D( Ao -V)	DpO <sub>2</sub>	DPCO <sub>2</sub>	DPS	DpH	DSO <sub>2</sub>	
D( Ao - SC)	67.93±0.43	-10.9±0.26	57.00±0.42	0.051±0.002	58.70±0.73	
D( Ao - TP)	43.77±0.47	-4.31±0.21	39.46±0.45	0.023±0.001	19.48±0.40	
D( Ao - VJI)	46.72±1.86	-8.79±0.97	37.93±2.27	0.030±0.006	24.35±2.53	
D( Ao -VH)	46.82±0.55	-5.51±0.25	41.31±0.60	0.025±0.002	20.00±0.60	
D( Ao - VR)	29.40±0.54	-2.88±0.16	26.51±0.50	0.013±0.001	8.76±0.40	

In this case,  $VR PS > Ao pO_2$ ; as a result, Gradient DP is negative and multidirectional with respect to the remaining gradients of the gas parameters in the heart-lung block, brain block and liver block with minimization of deviations from zero (destruction of differences between  $Ao pO_2$  and the venous PS), where the common mechanism is the formation (after mixing) of flows with different levels of  $pO_2$  and  $HbO_2(\%)$  in a zone of values between the maximum level of Gradient D (A-SC) created in the myocardium block and the minimum level of Gradient D (A-VR) created in the kidney block. (4,16-24,34-37) Normally, the possible deviations in Gradient DP of organs do not reach DP value in the myocardium block (with the + sign) or in the kidney block (with the sign -).

We consider the venous system as a special organ which, in addition to other functions, participates in the overall metabolism by mixing blood flows with differences in  $pO_2$ ,  $pCO_2$  and  $HbO_2\%$ , having the function of maintaining the gradients by integral gas parameters between different exchange zones. An important condition for the norm of gas exchange as a whole is the minimization of deviations from the average PS value.<sup>(26)</sup> Minimization of differences between organs by PS occurs while maintaining the differences between them, according to  $pO_2$ ,  $pCO_2$ , pH,  $SO_2$  and their ratios in the arterial and venous systems, being a reflection of polyorganic interactions.

Maintaining multidirectional deviations in Gradient DP from the zero in the kidney block and myocardium block indicates their conjugated functioning, the derivatives of which are gas-hemodynamic complexes that limit the amplitude of oscillations of the gas parameters in the brain, liver, and heart-lung blocks.

The stability of the homeostasis of the liquid medium of the body in interaction with the external gaseous environment is maintained through the interrelation of metabolic processes in the studied blocks, in which our tests, relatively independent from factors affecting  $O_2$  transport (Hb, stroke and minute heart volumes), allow us not only to detect changes, but also to create mechanisms influencing organ functioning, both in the normal and pathology states.

We believe that PS in the liquid media of the human body that depends on the combined processes (tissue, plasma, erythrocyte, external respiration, hemodynamics, etc.), as a derivative from the total pressure of gases in gas media (alveolar air, atmospheric air), is an evolutionary-deterministic basis for the formation of all types of pressure, including hemodynamic pressure. Tests for PS are an integral part of the analysis of a single cooperative system of the regulation of metabolism and other functions of the human body by pressure (arterial, venous, intracardiac, tissue, liquor, oncotic, amniotic, etc.).

## Conclusion

Summary gas pressure (PS) is a mechanism to maintain the stability of intrasystemic metabolic processes and gas homeostasis as a whole. The processes forming PS limit the amplitude of the PS deviation under changing the parameters of the constituent components ( $pO_2$  and  $pCO_2$ ) due to acts of

“mutual replacement” between them, as well as the influence of integral gas complexes under shifts in  $pO_2$ ,  $pCO_2$ , A-V  $SO_2$ . Processes that minimize the fluctuations in the sum of gases, in contrast to the volume tests ( $O_2v\%$ ,  $CO_2v\%$ ,  $HbO_2\%$ ), are relatively independent from factors affecting the  $O_2$  transport (Hb, stroke and minute heart volumes,  $SO_2$  dissociation regulation systems). This makes it possible to consider them as significant factors for stabilizing the homeostatic control of the gas-hemodynamic environment of the human body. GDP, as a measure of the adequacy of the combined changes in arterial  $pO_2$  and venous PS, is relevant for multi-organ monitoring of metabolic processes and inter-system relations in dynamics: norm - compensation - subcompensation – decompensation. Unlike the generally accepted tests that record quantitative differences between the points studied, the integral gas tests allow us to identify vectors and mechanisms of adaptive changes in gas homeostasis, to perform a qualitative comparison of the functioning of the studied organs by gas-dynamic tests in the norm and in pathology.

## Competing interests

The authors declare that they have no competing interests.

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## Differences Between Expressive Suppression and Cognitive Reappraisal Between Heart Disease and General Population

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### Abstract

**Aim:** Heart disease and emotional disorders often co-occur, but effective role in dysregulation of heart disease that is often overlooked. Evidence suggests that people with heart disease are more problems in regulating their emotions.

The study compared the re-evaluation of cognitive emotion regulation commonly used two strategies- and suppression-between heart disease and the general population.

**Methods:** Sixty men (30 with heart complaints and 30 without the condition) were selected by convenience sampling method and they responded to the Emotion Regulation Questionnaire (Gross and John) and a demographic questionnaire responded. To analyze the results and descriptive statistics such as frequency tables and inferential statistics, independent T-test was used SPSS software was used.

**Results:** The result shows that heart disease and general population re-evaluation strategies groups ( $P < 0.01$ ). This is not only different from the strategy reassessment, but in different repression, too. ( $P < 0.001$ ).

**Conclusion:** The results showed that heart disease and general population used different strategies to regulate their emotions. The key to finding the heart disease group prefer repression to regulate their emotions. (*International Journal of Biomedicine. 2017;7(3):192-195.*)

**Key Words:** emotional regulation • expressive suppression • reappraisal • heart diseases

### Background and Aim

In recent years evidence of the importance of psychological factors in the development and progression of cardiovascular disease.<sup>(1)</sup> Based on last report heart disease is a major cause of thirty eight percentages of death in the last year in Iran, and 15 million deaths in the world were based on this disease.<sup>(2)</sup>

Traditional risk factors (such as smoking, hypertension, hypercholesterolemia, obesity and diabetes) only about 40% incidence of coronary heart disease.<sup>(3-4)</sup> Although the relationship between emotions and heart has been hypothesized for many centuries, until recently, there was no convincing evidence for such relationships. Evidence that specific emotions such as depression, anxiety and stress as a major potential risk factors for coronary heart disease show appearance.<sup>(5-8)</sup>

Emotions on cardiovascular health through multiple pathways may influence the specific ways of activation of the sympathetic nervous system or the Hypothalamic-Pituitary-Adrenocortical (HPA) axis.<sup>(9-10)</sup> Evidence shows special association between heart disease and specific negative emotions: depression, anxiety and anger.

Empirical and anecdotal evidence indicated a relationship between negative affect and heart disease. This relationship is the concept that people experience higher levels of negative as at higher risk from the use of coping mechanisms such as drugs, food or alcohol to escape from the experience of these emotions.<sup>(11)</sup>

Theorists and researchers have been defined in a different way from concept to regulate emotions. Most definitions penetrated by Gross (1998), "The process by which individuals influence their emotions, when they are, and how they experience and express these emotions."<sup>(11)</sup> Thompson it is defined as internal and external processes responsible for monitoring, evaluating and modifying emotional reactions, especially intensive features and your time, to achieve one's goals."<sup>(12)</sup>

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People use different strategies to change their emotions. This strategy not only affect their current emotional experience, but also in interpersonal and cognitive process, too. Emotion regulation is regarded as a crucial factor in well-being and adaptive behavior and there are different strategies which individuals use for this purpose, but as Garefski (2002) argued some of these strategies are more adaptable than the others.<sup>(13)</sup> Two well-studied strategy to re-evaluate emotional regulation and suppression<sup>(14)</sup> to reduce (or increase) the tendency of emotional response or affective states.<sup>(15)</sup> Suppress emotionally-expressive behavior inhibition in emotional arousal decreases.<sup>(16)</sup> Re-evaluation reinterpretation of emotional stimuli valenced in and emotionless.<sup>(17)</sup> This includes the production of interpretation and perspective benign or positive in a stressful situation as a way to reduce distress.<sup>(18)</sup> Reassessment may be particularly important for psychopathology have good feelings and beliefs about who they are.<sup>(19)</sup> Both reappraisal and suppression of negative emotional stimuli can reduce the effect.

## Objectives

The aim of this study was to investigate differences in emotion regulation between heart disease patients and general population.

## Patients and Methods

### Participants

This population consisted of patients diagnosed with heart disease, Participants were 60 men, 30 inpatients with heart disease complain from Jamaran and Milad hospital in Tehran, Iran and control group include 30 men of patient's relatives but without having any heart disease history, these groups were selected by accessible sampling. The sample ranged in age from 42.16 years old (mean). Data collected between October and December of 2014. Demographic information be shown in Table 1.

Table 1.

Demographic characteristics of inpatient and control groups

	Inpatient No, (%) (n=30)	Control No. (%) (n=30)
Education		
Elementary	2(6.8)	4(13.3)
Guidance school	4(13.3)	2(6.7)
High school and more	24(80)	24(80)
AGE		
27-35	9(30)	7(23/3)
36-44	10(33.3)	9(30)
45-53	7(23.3)	11(36.7)
54-62	4(13.3)	3(10)
Marital Status		
Single	8(26.7)	6(20)
Married	16(53.3)	18(60)
Divorced	6(20)	6(20)

### Procedure

Sixty individual (Thirty heart disease inpatient men and 30 men without heart disease history) were selected by accessible sampling from Jamaran and Milad Hospital in Tehran, Iran. Eligible participants have said they are under no obligation to participate in the study, although they encourage them to do it. Initially, participants with a face to face interview using a demographic questionnaire was provided demographic information. This information included age, marital status and education level. They described their age, education and marital status. They were rested for 10 minutes, then they were asked to fill in Emotion Regulation Questionnaire (ERQ) by Gross and John with a series of 10 statements. The ERQ evaluation typical of suppressed emotions (4 items, eg., "I keep my emotions to myself) and reassessment (6 items, eg., "When I want to feel less *negative* emotion, I change the way I'm thinking about the situation) in person. They sitting separately in completing the questionnaire. The collected data were analyzed by SPSS 16 software and inferential statistics, independent T-test used to the analysis the data.

### Measures

**Emotion Regulation Questionnaire**<sup>(20)</sup> to assess individual differences in the habitual use of the style is designed in emotion regulation include cognitive reappraisal and suppression. Cognitive reappraisal (When I want to feel more positive emotion, I change the way I'm thinking about the situation) and suppression (I keep my emotions to myself). The questionnaire consisted of 10 items, of which four suppress evaluation and re-evaluation of the six appraisal strategy. Asked participants to rate how they regulate their emotions using a scale of 1 to 7, a higher score indicates more individual use strategies to regulate their emotions. Secondary points around the items for each scale were calculated in variable form of repression and re-evaluation. Gross and John (2003) reported Cronbach  $\alpha$  reliability coefficient value of 0.79 and 0.73 for re-evaluation and test-retest reliability for suppressing = 0.73 in 3 months = 0.69.<sup>(20-21)</sup> In this study the Persian translation of ERQ were used to estimate the internal consistency Cronbach's alpha coefficient was 0.83 for reassessment 0.68 for repression.

### Demographic questionnaire

Three factor age, education and marital status were asked by sample demographic questionnaire which was designed just for this study. This information was asked for norming these group by their age and educational levels.

### Data analysis

SPSS 16, descriptive statistics such as frequency tables and inferential statistics such as T-test was used. This study performed on 60 men (30 Heart disease inpatient, 30 as a control group). In terms of marital status, 14 subjects (23.3%) were single, 34(56.7%) were married and 3(12%) were divorced. Average level education was of about 11.45 years (M = 11.45; SD = 0.99). The level of education of the participants were as follows: 10% Max was an elementary school diploma, 10% of middle school diploma, 48% had a high school or higher education.

## Result

### Group comparisons

The result shows the two groups has no significant differences in age, education

The result indicates that the patient and control groups reassess the strategy is different. After re-evaluation in the control group showed significantly higher scores. Thus it appears that they usually use this strategy to adjust their emotions.

These groups are not only differs from the strategy of reassessment, but the suppression, too. As a result of that in Table 2, these groups is different in using your habitual the suppression. It appears inpatient group to use this strategy to adjust their emotions.

**Table 2.**

**Comparing age and education between inpatient heart disease and control population ( $p=0.01$ )**

		N	Mean	SD	Df	T	Sig (2. tailed)
AGE	Inpatient	30	42.16	8.87	58	-0.063	0.95
	Control	30	42.3	7.57			
EDUCATION	Inpatient	30	11.73	3.02	58	0/72	0.47
	Control	30	11.16	3.06			

**Table 3.**

**Description and the results of the t test in emotional regulation strategies between patients and control groups**

		N	Mean	SD	T	Df	Sig (2. tailed)
REAPPRAISAL	Inpatients	30	16	1.8	-20.06	58	0.00
	Control	30	27.9	2.6			
SUPPRESSION	Inpatient	30	18.46	2.33	19.25	58	0.000
	Control	30	11.63	2.8			

## Discussion

### Summary of Findings

The purpose of this study emotion regulation strategies (reassessment and suppression) in heart disease and general population. Results indicated that patients with heart disease and the control group used various strategies to adjust their emotions. This was a key finding, heart disease patients is prefer suppression and control group usually use reappraisal for this purpose.

Evidence shows that negative emotion such depression, anger and anxiety have different effect on heart disease. For instance, acute anxiety states may hyperventilation, which may cause to hyperventilation which then may cause coronary vasospasm.<sup>(23)</sup> It is assumed that the

acute hemodynamic stress caused severe emotional states of atherosclerotic plaque rupture after acute coronary events, including sudden cardiac death started.<sup>(24)</sup>

As to acute consequences of emotional regulation strategies, reappraisal successfully reduces the negative emotion and increased positive affect, but suppression seems to be ineffective, it may decreased positive affect, increased physiological arousal, or memory impairment.<sup>(25)</sup> In addition, signs of re-evaluation, but there is not suppression, optimal long-term consequences for the well-being and interpersonal functioning.<sup>(26)</sup> Memedovic and et al (2010) found that greater dispositional reliance on reappraisal and decreased blood pressure following an stimulating anger,<sup>(27)</sup> the findings is According to Mauss and colleague (2007) They found that people reassessment of trait anger were reported less frequently and cardiovascular responses more consistent for stimulating than those low in re-evaluation of property.<sup>(28)</sup> furthermore, reappraisal seemingly helps reducing negative affect.<sup>(29)</sup>

The study may not generalize to specific heart complication. The study relied on self-reported estimates of emotional regulation and the nature of the obstacles conclusions about the impact of the difference reassessment / suppression in this group. Another aspect of future research could examine dysregulation of emotion in this population.

## Conclusion

There is not any assurance that people would not experiencing negative affect in their life but their confrontation with this effect has different effects on their life. Based on evidence negative affect such depression associated with an increase risk of developing heart disease in a sample of healthy men.

Researchers believe that emotional symptoms of depression may cause emotional dysregulation. The suppression of emotion regulation strategies in the disorder. Patients with depression reported an increase suppression of negative affect not only repression but also for its positive affects, too is used.<sup>(30)</sup>

## Competing interests

The authors declare that they have no competing interests.

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## Association of SIRT-1, T helper 17-associated gene and Antimicrobial Peptides Genes in Inflammatory Bowel Disease Patients

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### Abstract

The aim of this study was to assess the expression of Th17-associated gene, genes encoding antimicrobial peptides (AMPs) and SIRT-1 protein in patients with inflammatory bowel disease (IBD).

**Patients and Methods:** We studied a group of 20 IBD patients, together with 20 subjects served as controls. Colonoscopy, terminal ileoscopy, and colonoscopic biopsy were performed for histopathology diagnosis, and quantitative gene expression of Th17-associated gene, CAMP, Elafin and SLPI by real-time PCR. SIRT-1 protein level expression was assessed by western blot.

**Results:** The expression of the four studied genes—elafin, SLPI, CAMP and Th17-associated gene—by relative quantification was higher in the patient group than in the control group. A statistically significant positive correlation was found between SLPI and elafin in the patient group ( $r=0.8325$ ,  $P<0.001$ ). A statistically significant positive correlation was reported as well between CAMP levels and elafin levels in the patient group ( $r=0.6842$ ,  $P<0.001$ ). In addition, CAMP levels had a positive correlation with SLPI levels in the patient group ( $r=0.6373$ ,  $P<0.001$ ). The highest expression of SIRT-1 was found in severe cases of IBD and the lowest expression was demonstrated in control subjects. A statistically significant positive correlation was detected between IL-17 levels and SIRT-1 levels in the patient group ( $r=0.7822$ ,  $P<0.001$ ).

**Conclusion:** A high expression of Th17-associated gene and AMPs gene has a significant impact on clinical assessment of patients with IBD. SIRT may participate in the progression of IBD. (*International Journal of Biomedicine*. 2017;7(3):196-203.)

**Key Words:** Th17-associated gene • inflammatory bowel disease • antimicrobial peptides • elafin • SIRT-1

### Abbreviations

AMPs, antimicrobial peptides; CD, Crohn's disease; CAMP, cathelicidin antimicrobial peptide; DEFBI, defensin beta 1; GWAS, genome-wide association studies; IBD, inflammatory bowel disease; LEAP2, liver expressed antimicrobial protein 2; SLPI, secretory leucocyte peptidase inhibitor; SIRT-1, silent information regulator-1; Th, T helper; UC, ulcerative colitis.

### Introduction

The term inflammatory bowel disease (IBD) mainly covers ulcerative colitis (UC) and Crohn's disease (CD). IBD is a global health problem, with a reported prevalence as high as 568 and 827 per 100,000 in the USA and Europe, respectively. <sup>(1)</sup>

CD can manifest itself anywhere in the gastrointestinal tract, while UC is only seen in the colon, with a varying length and degree of continuous inflammation extending proximally from the rectum. In UC, the inflammation is found in the mucosa while in CD, there is a deeper, often transmural inflammation with formation of strictures and fistula. Among complex diseases, GWAS have been successful in IBD, identifying 99 non-overlapping genetic risk loci, including 28 that are shared between CD and UC.<sup>(2,3)</sup> GWAS have generated insights into the mechanisms driving IBD and have implicated

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genes shared by multiple autoimmune and autoinflammatory diseases. Nevertheless, the challenge remains to determine how genetic variation can precipitate and sustain the inappropriate inflammatory response to commensals that is observed in IBD.<sup>(4)</sup> The coordinated effect of various Th subtypes is fundamental to gut homeostasis,<sup>(5)</sup> and UC and CD have been considered different with respect to Th cell activation.<sup>(6)</sup> The concept of Th17 cells has been introduced, and it was shown that inflammation previously attributed to Th1 could actually be Th17-driven and that these lymphocytes played an important role in CD.<sup>(7,8)</sup> The identification of Th17 as a unique subset of Th cells has challenged this view, and today some argue that the adaptive immune response in CD, and to some degree in UC, is dominated by Th1/Th17 cells.<sup>(9)</sup> Antimicrobial peptides (AMPs) have been identified as essential peptides in the maintenance of intestinal barrier function and immune homeostasis. Human peptides detectable in the gastrointestinal tract are divided into  $\alpha$ - and  $\beta$ -defensins (HD and HBD, respectively) and the cathelicidin LL-37, based on their amino-acid sequences.<sup>(10)</sup> There is increased expression of many AMPs in both inflamed UC and CD mucosa, with only a few interesting exceptions. DEFB1 is significantly down-regulated in both UC and CD, while the gene encoding LEAP2 showed a similar, but less prominent regulation. The loss of DEFB1 expression has previously been reported by Arijs et al., who suggested that alterations may be due to a loss of epithelial tissue in the inflamed colon.<sup>(11)</sup> A work by Peyrin-Biroulet et al. describes the role of DEFB1 in maintaining homeostasis, and explores the regulation of colonic DEFB1 expression.<sup>(12)</sup> In IBD, leukocyte infiltration, Paneth cell metaplasia, crypt hyperplasia and ulceration with loss of epithelial cells are factors that will influence measured levels of gene expression. As an example, the increase in  $\alpha$ -defensin expression in colonic IBD has been attributed to colonic Paneth cell metaplasia, while the decrease of  $\alpha$ -defensin expression in ileal CD has been linked to the loss of epithelial tissue, Paneth cell function and NOD2 status.<sup>(13-15)</sup> Another effect potentially interfering with IBD microarray analysis is the regional variation in gene expression, with both a dichotomous and a more gradually varying gene expression pattern along the colon.<sup>(16)</sup> Recently, numerous studies have demonstrated the impact of the NAD-dependent deacetylase sirtuin-1 (Sirt1) on human aging, age-related diseases, genome stability and inflammatory response.<sup>(17,18)</sup> We investigated the expression of Th17-associated gene and AMPs gene and evaluated their impact on clinical presentation of Egyptian patients with IBD. We also aimed to assess the expression level of SIRT-1 protein and its role in disease propagation.

## Patients and Methods

This is a case control, cross-sectional study that was conducted on 40 adult subjects referred to the Gastrointestinal Endoscopy and Liver Unit, Cairo University. Half of the patients had IBD, the other half were non-IBD subjects studied as case control. The study protocol was reviewed and approved by the Ethics Committee of the Cairo University Faculty of Medicine and Department of Endemic medicine.

All participants provided the written informed consent. The twenty IBD patients were selected out of a total of 80 patients who presented to our endoscopy unit from July 2013 to January 2015. The remaining 60 patients were excluded because they did not fit with our inclusion criteria (age, denial of consent, disagreement between endoscopic and histopathologic findings, dropout). The selection criteria included: (a) adult patients 18 years or older from both sexes who are willing to participate and to give an informed consent; (b) patients within the clinical spectrum consistent with IBD that was confirmed both endoscopically and histopathologically. The exclusion criteria included: (a) patients who have a discrepancy between endoscopic and histological assessments, making it difficult to establish and confirm a diagnosis of UC or CD; (b) patients who have an indeterminate colitis by histopathology.

All enrolled subjects were subjected to full history taking, stressing symptoms related to IBD, such as chronic diarrhea, weight loss, joint manifestations or other autoimmune associations. Subjects were given a full clinical examination, and we assessed and analyzed body mass index (BMI), complete blood count, CRP by ELISA, qualitative fecal calprotectin by ELISA and stool culture for Shigella on MacConkey agar supplemented with xylose and for E-coli on MacConkey agar containing sorbitol. Colonoscopy, terminal ileoscopy, and colonoscopic biopsy were performed for histopathology diagnosis, and quantitative gene expression of Th17-associated gene, CAMP, Elafin and SLPI by real-time PCR. SIRT-1 protein level expression was assessed by western blot.

### Real Time PCR

Colonic mucosal tissues of both studied groups were homogenized and RNA was extracted with an RNAeasy Mini Kit (Qiagen). Out of the total RNA from each sample, 1000 ng was used for PCR amplification using ViPrime One step RT-qPCR 2X SyberGreen Mix, HRoxin a 48-well plate using the Step One instrument (Applied Biosystem, USA) as follows: 15 minutes at 50°C for cDNA synthesis, 10 minutes at 95°C for enzyme activation followed by 40 cycles of 15 seconds at 95°C, 20 seconds at 55°C and 30 second at 72°C for the amplification step.. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of GAPDH as a housekeeping gene by the  $\Delta\Delta Ct$  method. We used 1  $\mu$ M of both primers specific for each target gene. Primers sequence specific for each gene demonstrated in Table (1).

**Table 1.**

**Primers sequence specific for each gene**

Target gene	Primer sequence: 5' - 3'	Gene bank accession number
IL-17A	F: CTGTCCCCATCCAGCAAGAG R: AGCCACATGGTGGACAATC	NG_033021.1
CAMP	F: TGCCCAGGTCCTCAGCTAC R: GTGACTGCTGTGTCGTCCT	NM_004345.4
Elafin	F: CGTGGTGGTGTTCCTCATC R: TTCAAGCAGCGGTTAGGG	S78387.1
SLPI	F: CCTGCCTTACCATGAAGT R: CCAAATGTCAGGAATCAGAC	NG_028137.1
GAPDH	F: CTCTACTGGCGCTGCCAAGGCT R: GTCCACCACTGCACGTTGG	NT_009759.16

### Western blotting

The antibody used was antigen affinity-purified polyclonal sheep IgG anti-human SIRT1 (R&D Systems, Cat N0: AF7714). Colonic mucosal tissue was subjected to a protein extraction procedure using ReadyPrep™ protein extraction kit (BIO-RAD, Catalog #163-2086). Protein was separated by SDS-PAGE on 4%-20% polyacrylamide gradient gels. After incubation in 5% non-fat dry milk, Tris-HCL, 0.1% Tween 20 for 1hr, the SIRT1 polyclonal monoclonal antibody was added to one of the membranes containing specimen samples and incubated at 4°C overnight. Appropriate secondary antibodies were incubated for 2hr at room temperature. After the antibodies were washed twice in 1xTBS-T, we performed densitometric analysis of the immunoblots to quantify the amounts of SIRT1 against a control sample by total protein normalization by image analysis software on the ChemiDoc MP imaging system (version 3) produced by Bio-Rad (Hercules, CA).

The statistical analysis was performed using SAS 9.2 for windows 7. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SD for continuous variables. Student's unpaired and paired t-tests were used to compare two groups for data with normal distribution. Differences of continuous variables departing from the normal distribution were tested by the Mann-Whitney U-test. Group comparisons with respect to categorical variables are performed using chi-square tests or, alternatively, Fisher's exact test when expected cell counts were less than 5. Prediction model was performed for calculating predicted probabilities. A probability value of  $P < 0.05$  was considered statistically significant.

## Results

A total of 40 study patients and controls who underwent complete colonoscopic examination and who complained of abdominal pain, bleeding, diarrhea and constipation were enrolled in this study. Demographic data of all subjects are shown in Table 2. There was no statistically significant difference in either age or sex distribution between the two groups. The control group showed a statistically significant higher mean BMI than did the patient group ( $P=0.001$ ). The assessment of clinical manifestations in the patient group revealed that all patients complained of diarrhea, 60% had 6 or more motions per day, and of that 60%, 8 patients complained of passage of blood in stools. While 75% of the control group complained of diarrhea, all of them had fewer than 6 motions per day and none of them complained of passing blood in stools.

**Table 2.**

#### Demographic data of both studied groups

Variable	Patient group	Control group	P
Age (years)	33.8±9.88	37.05±9.04	0.28
Sex(males/females)	16 / 4	11 / 9	0.17
BMI	25.0±3.3245	29.15±3.065	0.001

All patients complained of abdominal cramps, while only 18(90%) of the control group experienced this complaint. All patients and half of the control group complained of easy fatigability. Hip joint pain was reported in 80% of the patient group while it was present only in 2(10%) cases of the control group. Assessment of the distribution of joint pain showed that 87.5% of the patient group who experienced joint pain complained of pain in two or more big joints, while only 2 cases in the control group experienced pain in only the hip joint (Table 3).

**Table 3.**

#### Clinical features of both studied groups

Clinical parameter	Patient group	Control group
Diarrhea	20 (100%)	15 (75%)
Severity of diarrhea < 6 motions per day (mild to moderate disease)	8 (40%)	15 (75%)
6 or more motions per day (severe disease)	12 (60%)	0
Blood in stool	8 (40%)	0
Constipation	0	3 (15%)
Abdominal cramps	20 (100%)	18 (90%)
Easy fatiguability	20 (100%)	10 (50%)
Joint pain	16 (80%)	2 (10%)
Extent of arthralgia Single joint	2 (12.5%)	2 (100%)
2 or more joints	14 (87.5%)	0

All the study group cases were negative for ova and parasites by stool analysis or Shigella and E coli by stool culture. Fecal calprotectin was tested and it was positive in 70% of the study group and negative in the control group.

Both TLC and CRP were significantly higher in the patient group compared to the control group (Table 4). The colonoscopic findings and histopathological results of the patient group showed that 18 cases were diagnosed as UC and 2 cases were diagnosed as CD (Table 5).

**Table 4.**

#### Blood tests in both studied groups

Variable	Patient group	Control group	P
Hb (g/dL)	10.95±1.328	11.61±1.8	0.19
TLC ( $\times 10^3$ /cmm)	13.85±1.56	6.01±1.12	<0.001
CRP (mg/L)	51.9±9.60	6.7±1.94	<0.001

The expression of the four studied genes—elafin, SLPI, CAMP and Th17-associated gene—by relative quantification was higher in the patient group than in the control group (Table 6). In patients with severe disease, the levels of elafin, CAMP and SLPI were higher than those in patients with mild to moderate disease, and this difference is statistically significant ( $P < 0.001$  in all cases). No statistically significant difference was detected between two groups of patients regarding the IL17 level (Table 7, Fig. 1).

**Table 5.**

**Colonoscopic findings and histopathological results of the cases**

Colonoscopic findings	Patients
<b>UC</b>	
Erythema and erosions in rectum (proctitis)	15 (75%)
Erythema and erosions up to the splenic flexure (left sided colitis)	14 (70%)
Loss of the fine vascular pattern	18 (90%)
Pseudopolyps	10 (50%)
Ulcers	15 (75%)
<b>CD</b>	
Serpiginous ulcers with cobblestone appearance in left colon	1 (5%)
Small discrete aphthous ulcers with normal adjacent mucosa in left colon	1 (5%)
<b>Histological findings</b>	
<b>UC</b>	
Crypt abscesses	18 (90%)
Crypt branching and shortening	3 (15%)
Basal lymphoid aggregates	18 (90%)
<b>CD</b>	
Focal inflammation and granuloma	2 (10%)

**Table 6.**

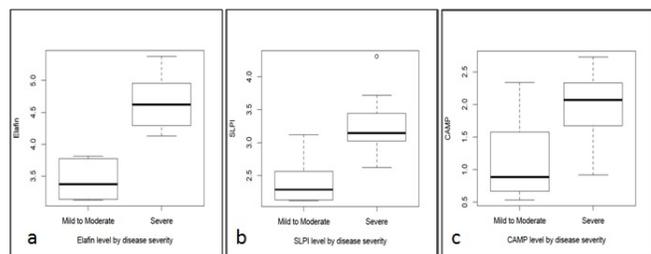
**Mean values of expressed genes in both studied groups**

Studied genes	Patient group	Control group	P
Elafin	4.157±0.7	0.95±0.4	<0.001
SLPI	2.917±0.588	0.476±0.264	<0.001
CAMP	1.63±0.70	0.018±0.017	<0.001
IL 17	0.357±0.26	0.11±0.04	<0.001

**Table 7.**

**Association between the studied genes and the disease severity**

Disease severity	Mild to moderate	Severe	P
Elafin	3.43 (0.317)	4.63 (0.406)	<0.001
CAMP	1.139 (0.66)	1.959 (0.53)	<0.001
SLPI	2.4 (0.35)	3.26 (0.44)	<0.001
IL 17	0.309 (0.21)	0.39 (0.29)	0.482



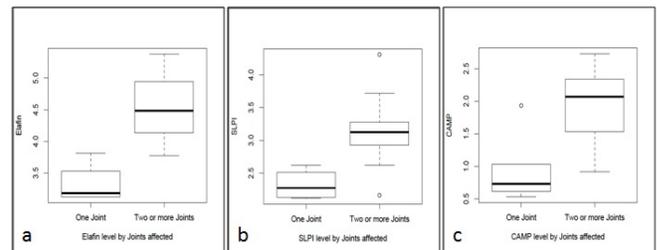
**Fig. 1.** The association between (a) elafin, (b) SLPI and (c) CAMP levels according to the disease severity in the studied group of patients.

We examined genes of patients in the study group who experienced pain in only one joint and those who experienced pain in two or more big joints, and the results showed higher levels of elafin, CAMP and SLPI in those who experienced pain in two or more big joints, and this difference was statistically significant ( $P<0.001$  in all cases). No statistically significant difference in the IL-17 level was detected between the 2 groups of patients (Table 8, Fig. 2). A positive correlation was found between SLPI and elafin in the patient group ( $r= 0.8325$   $P<0.001$ ; Fig. 3). A statistically significant positive correlation was reported as well between CAMP levels and elafin levels in the study group of IBD patients ( $r=0.6842$ ,  $P<0.001$ ; Fig.4). In addition, CAMP levels had a positive correlation with SLPI levels in the patient group ( $r=0.6373$ ,  $P<0.001$ ; Fig. 5). The highest expression of SIRT-1 was found in severe cases of IBD and the lowest expression was demonstrated in control subjects (Fig. 6). A statistically significant positive correlation was detected between IL-17 levels and SIRT-1 levels in the study group of IBD patients ( $r=0.7822$ ,  $P<0.001$ ).

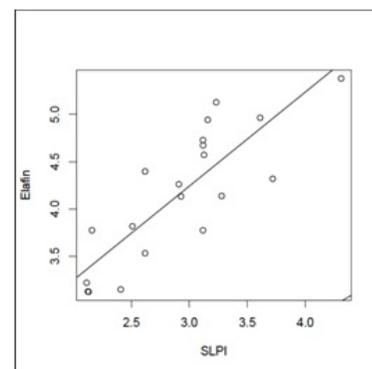
**Table 8.**

**Association between the studied genes and the number of painful joints**

Variable	One joint	2 or more joints	P
Elafin	3.327 (0.28)	4.51 (0.48)	<0.001
CAMP	0.9282 (0.52)	1.93 (0.53)	<0.001
SLPI	2.32 (0.22)	3.173 (0.501)	<0.001
IL 17	0.307 (0.22)	0.379 (0.28)	0.55



**Fig. 2.** The association between (a) elafin, (b) SLPI and (c) CAMP levels according to the number of painful joints in the studied group of patients.



**Fig. 3.** The correlation between elafin levels and SLPI levels in the studied group of patients.

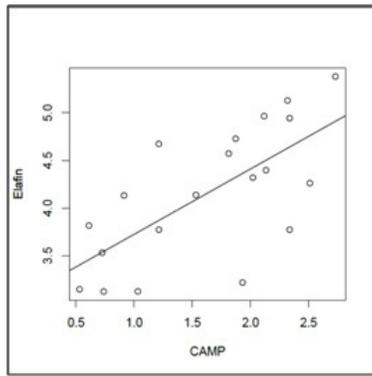


Fig. 4. The correlation between elafin levels and CAMP levels in the studied group of patients.

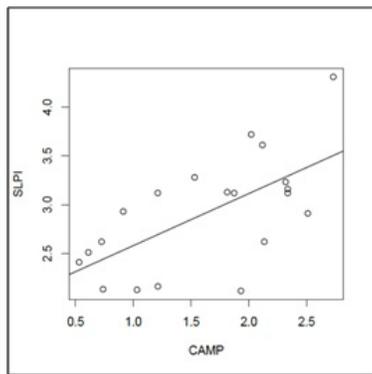


Fig. 5. The correlation between SLPI levels and CAMP levels in the studied group of patients.

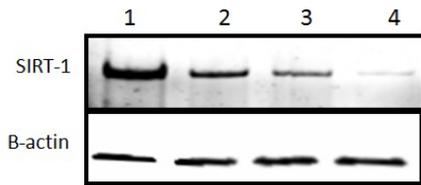


Fig. 6. The scanning densitometry results of SIRT-1 versus  $\beta$ -actin protein levels in colonic tissues of severe (1), moderate (2), mild (3) IBD and control tissue subjects (4).

Multivariate regression analysis was performed using the studied parameters to predict those with IBD among the 2 groups. Results revealed that age, BMI, IL-17 and CAMP can significantly predict the presence of IBD (Table 9).

Table 9.

Multivariate regression analysis

Variable	Estimate	Std. Error	z- value	p-value
Intercept	11.45687	7.42840	1.542	0.1230
IL 17	58.70451	27.03552	2.171	0.0299
Age	- 0.07364	0.07473	- 0.985	0.3244
BMI	- 0.63515	0.30921	- 2.054	0.0400
CAMP	46.56846	17.5345	1.345	0.0189

Discussion

In our research, we studied a group of 20 patients, together with 20 subjects served as controls; 2 patients in the study group were diagnosed as CD, while 18 were diagnosed as UC. Analysis of the age and sex of the study group showed that the mean age of patients was 33.8 years, with a significant male predominance, which is similar to a finding reported in another study done by Brahmania and Bernstein.<sup>(19)</sup> Although our results may agree with the known IBD peak in the fourth decade of life and do not agree with the known female predominance, it is important to stress that our patients do not represent a random sample of the general population of IBD patients and that they were included in our study according to specific inclusion criteria.

The diagnosis of IBD in our study was based on standard clinical and endoscopic findings. All patients complained of diarrhea, abdominal cramps, easy fatigability; 60% of them experienced more than 6 motions per day, 40% showed blood in stool, 80% experienced joint pains and 87.5% of them complained of pain in 2 or more big joints. The mean CRP level was 51.9 mg/L. The mean hemoglobin level was 10.95 g/dl.

Isene et al. concluded that the cumulative prevalence of first extraintestinal manifestation (mainly arthritis) was 16.9%.<sup>(20)</sup> Dignasset et al. stated that the main symptom of UC is visible blood in the stools, which is reported in more than 95% of active disease. Rectal urgency, tenesmus and occasionally severe constipation represent the classical complaints of rectal involvement, while chronic diarrhea with nocturnal defecation and crampy abdominal pain are typical of left-sided or extensive UC.<sup>(21)</sup>

Lennard-Jones and Shivananda concluded that patients affected by Crohn's colitis often present a UC-like clinical phenotype. Blood or mucopurulent exudates with the stool can be seen in up to 40% to 50% of patients with Crohn's colitis, but less frequently than in UC subjects.<sup>(22)</sup> Other studies, by Sands and Van Asschee, also suggested that chronic diarrhea is the most common presenting symptom in classical ileo-colonic CD, followed by abdominal pain and weight loss.<sup>(23,24)</sup>

Consistent with the above clinical features, a nested, case-controlled study by Melmedet et al. showed that UC patients with either non-bloody diarrhea or weight loss as onset symptoms have an increased likelihood of subsequent change in diagnosis to CD, compared to those with none of these risk factors, and might thus warrant further diagnostic work-up.<sup>(25)</sup> This matches Ricaneket et al., in which more than 50% of the patients presented with symptoms of abdominal pain, diarrhea, and blood in stool, mainly in those diagnosed as UC; the median CRP level was 26 mg/L in those diagnosed as CD and 24 mg/L in those diagnosed as UC.<sup>(26)</sup>

In our study, colonoscopic examination showed erythema and erosions in the rectum in 90% of cases and erythema and erosions up to the splenic flexure in 70% of cases, loss of the fine vascular pattern in 90% of the cases, pseudopolyps in 50%, and ulcers in 75%; one case showed serpiginous ulcers with cobblestone appearance in the left colon and another one showed small discrete aphthous ulcers with normal adjacent mucosa in the left colon. The histological examination of

colonic biopsies taken from the patients in our study showed crypt abscesses in 90% of the cases, crypt branching and shortening in 15% of the cases, basal lymphoid aggregates in 90% of the cases, and focal inflammation and granuloma formation in 10% of the cases. Geboes and De Hertogh stated that one of the main histopathologic differences between CD and UC is the microscopic extension of inflammation. In UC, it is localized to the mucosa and submucosa, whereas CD is characterized by a transmural inflammation. As a result, in CD, deep fissuring ulcers may penetrate through the muscle layer, resulting in abscesses or fistulas between involved segments or adjacent organs.<sup>(27)</sup>

Cathelicidins are a family of peptides with established antibacterial, antiviral and antifungal effects.<sup>(28)</sup> Cathelicidin plays an important role in the pathophysiology of human disease such as Kostmann's syndrome (a severe congenital neutropenia with chronic gingivitis due to the lack of cathelicidin in immature neutrophils).<sup>(29)</sup> M. Imura et al. showed that colon epithelial cathelicidin expression is critical for barrier function, bacterial adhesion and surface epithelial cell damage in vivo.<sup>(30)</sup>

In accordance with previous reports, we found that the CAMP mRNA expression was found to be markedly elevated in patients in the study group compared to controls ( $P=0.00$ ). CAMP expression was significantly higher in patients with severe disease. The same results were found when we studied the association with joint pain; significantly higher expression of CAMP was found in those who experienced pain in 2 or more big joints ( $P<0.001$ ). This increase in CAMP levels may be due to the inflammatory nature and the breakdown of the colon epithelial barrier in IBD. AMPs, like cathelicidins, are located at epithelial surfaces.

Schauberet et al. found that cathelicidin expression was significantly increased in inflamed colon mucosa from UC patients compared to non-inflamed mucosa in the controls.<sup>(31)</sup>

During colitis, a delicate equilibrium is established between proteases and antiprotease.<sup>(32)</sup> In inflammatory states, proteases injure tissues while protease inhibitors stabilize tissue damage and facilitate healing. Elafin and SLPI are protease inhibitors that modulate inflammation via its anti-protease activity.<sup>(33)</sup> In UC patients, elafin and SLPI levels are high and may tend to act as a self-protective mechanism against colitis.<sup>(34)</sup> The systemic concentration of these antiproteases in the serum is comparably low.<sup>(35)</sup> The main proteolytic activity for elafin and SLPI is directed against human neutrophil elastase, HNE, thus, antagonizing excessive elastase release in the inflamed tissue.

Furthermore, both molecules are capable of antagonizing various other proteases; Elafin inhibits porcine pancreas elastase and proteinase-3, whereas SLPI is also a potent inhibitor of trypsin, chymotrypsin, tryptase, chymase and cathepsin G.<sup>(36-38)</sup>

In the colon, SLPI and elafin have been found in the normal epithelium,<sup>(39,40)</sup> and elafin has been shown to be increased in UC.<sup>(41)</sup> In addition to their function as antiproteases, they are effective antimicrobial peptides, being active against Gram-positive and Gram-negative bacteria, fungi, and viruses.<sup>(42,43)</sup>

In our study, the elafin and SLPI mRNA expression were markedly higher in the study group patients than in

the controls ( $P<0.001$ ). There was an association between the severity of IBD and both elafin and SLPI levels, where both elafin and SLPI mRNA expression were much higher in patients with severe disease ( $P<0.001$  for all cases). It was also shown that both elafin and SLPI mRNA expression were much higher in patients who experienced pain in 2 or more big joints ( $P<0.001$ ).

In our study, there was a positive correlation between elafin and SLPI ( $r=0.8325$ ,  $P<0.001$ ). CAMP expression levels were significantly correlated with SLPI levels ( $r=0.6373$ ,  $P<0.001$ ). A positive correlation was reported as well as between CAMP levels and elafin levels ( $r=0.6842$ ,  $P<0.001$ ).

M. Schmid et al. reported that elafin and SLPI correlated with the proinflammatory cytokine IL-8, which has been demonstrated to be a good marker for histological inflammation. They found also that elafin predominated in the epithelium, whereas SLPI was preferentially found in inflammatory cells.<sup>(44)</sup>

Another cell type that recently caught interest in conjunction with IBD is the Th17 cell. This T cell subset produces IL-17A, commonly called IL-17, and IL-17F, which exist as homo and heterodimers and share 60% amino acid sequence identity. Depending on cell type, IL-17 can therefore trigger expression of many proinflammatory cytokines, including TNF- $\alpha$ , IL-1, IL-6, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and chemokines.<sup>(45)</sup>

In our study, the Th17-associated gene expression was found to be higher in the patient group than in the controls ( $P<0.001$ ). IL-17 expression levels were slightly higher in patients with severe disease but this difference was not statistically significant.

In accordance with our study, Jiang reported that Th17 cells and Th17-related cytokines (IL-17, IL-21 and IL-22) were significantly increased in the intestinal mucosa of active IBD patients and may play an important role in disease activity and mucosal damage.<sup>(46)</sup>

There were no statistically significant differences in IL-17 expression level between those who had pain in 2 or more big joints and those who experienced pain in only one joint ( $P=0.55$ ).

SIRT-1, a NAD<sup>+</sup> dependent histone deacetylase, is involved in many pathophysiological processes, such as anti-inflammation, metabolism modulation of cell growth and anti-carcinogenesis.<sup>(47)</sup> It regulates inflammation by modulating a variety of pro-inflammatory mediators.<sup>(48)</sup>

A significant increase in TNF- $\alpha$ , IL-6, IL-1 $\beta$ , IFN- $\gamma$  and IL-17 with suppression in TIMP-3 and SIRT-1 mRNA level was observed during the Dextran Sodium Sulfate (DSS) exposure phase to induce IBD in animals, which reverts to normal towards the remission phase. Treatment with resveratrol, a SIRT-1 activator, significantly elevated SIRT-1 and TIMP-3, suppressed TNF- $\alpha$  converting enzyme (TACE) mRNA expression, and was associated with amelioration of disease.<sup>(49)</sup>

In our study, the levels of SIRT-1 mRNA expression were found to be higher in the patient group compared to controls ( $P<0.001$ ). SIRT-1 levels were slightly higher in patients with severe disease, but this difference was not statistically significant ( $P=0.52$ ). Additionally, there was a significant

positive correlation between IL-17 levels and SIRT-1 levels in patients ( $r=0.7822$ ,  $P<0.001$ ). This coincided with a recent study, which reported that Sirt1, when adjusting the pattern of cellular metabolism to nutrient availability, can regulate many metabolic functions including DNA repair, genome stability, inflammatory response, apoptosis, cell cycle, and mitochondrial functions.<sup>(18)</sup> However, our study disagreed with another study that strongly emphasized the involvement of TACE in colon inflammation and suggested that inhibition of TACE directly or indirectly via SIRT-1 activation ameliorates colitis.<sup>(49)</sup>

Multivariate regression analysis was performed using the studied parameters to predict those with IBD among the studied groups, and our results revealed that age, BMI, IL17 and CAMP can significantly predict the presence of IBD. This agrees with Lukás, who reported that the strongest predictors of disease course in CD and UC are the age at diagnosis, disease location and smoking habit.<sup>(50)</sup>

**In conclusion**, we can apply the biochemical markers IL-17, CAMP, Elafin, SLPI and SIRT-1 for the early diagnosis and treatment intervention of IBD. Our study may reveal a novel pathogenic mechanism linking the NAD-dependent deacetylase Sirt1 and its modulation of IL-17 in IBD.

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## A Complex, Multidisciplinary Approach to Prevention of Gastro-Duodenal Bleeding in Therapeutic Patients of a General Hospital

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### Abstract

**The aim** of this study was to make the prevention of erosive-ulcerous gastroduodenal bleeding (EU-GDB) more efficient in therapeutic patients admitted to a general hospital by using a multidisciplinary approach that includes a diagnostic algorithm, treatment protocol, and individual methods of EU-GDB prevention.

**Materials and Methods:** The study included 114 patients of the therapeutic, pulmonary, and cardiology departments of the Voronezh City Emergency Care Hospital. The patients had been admitted due to destabilization of their underlying conditions and displayed signs of acute gastroduodenal erosions and ulcers during their stay in the hospital. All the patients were randomly divided into two equal groups: the main group and the comparison group. A multidisciplinary approach was applied to patients of the main group (n=58; mean age, 62.64±14.37); it included early pre-clinical diagnosis of erosive-ulcerous gastroduodenal impairments (EU-GDI) by fibrogastroduodenoscopy on the second or third day after their admission to the hospital, which helped to reveal in a timely manner uncomplicated EU-GDI and directly start local treatment as a part of complex therapy. An algorithm of the procedure provides for participation of a surgeon. Powder-like biologically active granular sorbents of the new generation (ASEPTISORB-A, ASEPTISORB-D, or ASEPTISORB-DT) were applied to the revealed acute erosions and gastroduodenal ulcers during the FGDS procedure to prevent hemorrhagic complications. After manifestation of the first signs of EU-GDB: during the curative endoscopy, the use of the developed minimally invasive method of endoscopic hemostasis, which provided combined application of a local haemostatic preparation Gelplastan and ASEPTISORB-D to the defect area. In the comparison group (n=56), the traditional technique of surgical consultations “on demand” was used. FGDS was performed when first symptoms appeared. Common methods of endoscopic hemostasis without local treatment of EU-GDI and application of granulated sorbents were used in this group.

**Conclusion:** The developed program helps to prevent hemorrhagic complications, exclude emergency operations, and reduce mortality rate by 3 times. (*International Journal of Biomedicine*. 2017;7(3):204-207.)

**Key Words:** erosive-ulcerous damages • gastroduodenal bleedings • granular sorbents • endoscopic hemostasis

### Introduction

The problem of gastroduodenal bleeding (GDB) in the context of therapeutic pathology and the cardiovascular system disorders is still one of the most acute problems in clinical medicine.<sup>(1-3)</sup> Timely diagnosis of symptomatic

gastroduodenal erosions and ulcers is a challenge that still does not have a solution because 30%-90% of patients have an asymptomatic course of these conditions, 46%-58% patients do not display a typical clinical performance, and in 25%-42% of patients, symptoms of the underlying condition prevail. In most cases, acute erosions and ulcers are diagnosed only in cases of bleeding or autopsy.<sup>(1,3)</sup>

Causes of the consistently high level of mortality in therapeutic patients with GDB are reported to be, first, untimely diagnosis of gastroduodenal erosions and ulcers that result in massive bleeding; second, high operational risk in patients

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with decompensation of an underlying chronic condition in the context of anemia; third, a lack of unified and successive tactics of medical care.<sup>(1,3)</sup> Thus, prevention and treatment of erosive-ulcerous GDB (EU-GDB) in patients with therapeutic pathologies are acute problems in applied medicine.

**The aim** of this study was to make the prevention of EU-GDB more efficient in therapeutic patients admitted to a general hospital by using a multidisciplinary approach that includes a diagnostic algorithm, treatment protocol, and individual methods of EU-GDB prevention.

## Materials and Methods

The study included 114 patients of the therapeutic, pulmonary, and cardiology departments of the Voronezh City Emergency Care Hospital. The patients had been admitted due to destabilization of their underlying conditions and displayed signs of acute gastroduodenal erosions and ulcers during their stay in the hospital. The investigation was approved by local ethics committee, and written informed consent was obtained from all participants.

Reasons for hospitalization were hypertension and diabetes mellitus (27.2%); the active stage of COPD, bronchitis and pneumonia (14.9%); progressive angina pectoris (10.5%); and ischemic heart disease and heart rhythm disorders (34.2%).

Depending on the character of gastroduodenal erosive-ulcerous impairments, all patients were divided into several groups. In the first group were patients, 49(43%), with isolated or multiple erosions in the stomach and/or duodenum; in the second group were patients, 47(41.2%), with acute gastric and/or duodenal ulcers. (Unlike chronic gastroduodenal ulcers, acute gastric and/or duodenal ulcers were located on all walls of the organ.); and in the third group were patients, 18(15.8%), with ulcerous gastric and/or duodenal disease.

Erosive and ulcerous defects in therapeutic patients either showed no signs of bleeding or were the causes of hemorrhages. Among 49 patients with acute gastric and/or duodenal erosions, 18(15.8%) displayed signs of erosive bleeding. Among 47 patients with gastroduodenal ulcers, 24(20.1%) were found to have signs of acute GDB. Bleeding stigmata of peptic ulcers were classified according to the Forrest (F) classification.<sup>(4)</sup>

All the patients were randomly divided into two equal groups: the main group and the comparison group. Patients of the main and comparison groups were comparable in their age, gender, clinical performance, accompanying conditions, localization and size of erosive-ulcerous defects, and duration of observation. Similar pharmacological therapy conforming to the current standards for care of patients with therapeutic pathologies was used in both groups. Patients of both groups received antiulcerous therapy: proton pump inhibitors (omeprazole), antacids, anti-helicobacter therapy (on indications).

A multidisciplinary approach was applied to patients of the main group (n=58; mean age, 62.64±14.37); it included early pre-clinical diagnosis of erosive-ulcerous gastroduodenal impairments (EU-GDI) by fibrogastroduodenoscopy (FGDS) on the second or third day after their admission to the hospital,

which helped to reveal in a timely manner uncomplicated EU-GDI and directly start local treatment as a part of complex therapy. An algorithm of the procedure provides for participation of a surgeon. Powder-like biologically active granular sorbents of the new generation (ASEPTISORB-A, ASEPTISORB-D, or ASEPTISORB-DT) were applied to the revealed acute erosions and gastroduodenal ulcers during the FGDS procedure to prevent hemorrhagic complications. The mechanism of action of these sorbents is based on a potent draining effect, which results in complete cleaning from the wound of the wound contents: clots of blood, fragments of tissues, microorganisms, and the decay products of these contents. Next, regeneration and epithelialization processes are activated. ASEPTISORB-A contains an anesthetic agent, anilocaine, that removes the pain syndrome. ASEPTISORB-D contains an antiseptic agent, dioxydin, that prevents development of a purulent process. ASEPTISORB-DT contains dioxydin and a protein-degrading enzyme, collagenase, that lyses necrotic tissues.

Patients of the main group with acute erosions underwent local treatment by endoscopic insufflation of ASEPTISORB-A; patients with gastroduodenal ulcers less than 1.0cm in diameter were given a local treatment by pneumatic insufflation of a granular sorbent ASEPTISORB-D on the defect area. Patients with ulcerous defects 1.0 cm and more were given combined insufflations of two sorbents: first, 0.2 g of ASEPTISORB-DT was applied on the bottom of the ulcer using an insufflator; then, 0.4g of ASEPTISORB-D was applied on the whole surface of the ulcer. Second-stage treatment of the ulcerous process (after cleansing the ulcerous bottom of necrotic tissues) was done using insufflations of ASEPTISORB-D only. Local treatment of erosive-ulcerous defects was performed with a 4-5 day interval.

The plans of action were altered after manifestation of the first signs of EU-GDB: during the curative endoscopy, the use of the developed minimally invasive method of endoscopic hemostasis, which provided combined application of a local haemostatic preparation Gelplastan and ASEPTISORB-D to the defect area. A surgeon participating in this procedure helped to choose the further tactics of treatment according to the treatment protocol of patients with GDB.

The comparison group consisted of 56 patients (mean age, 62.38±14.47). In this group, the traditional technique of surgical consultations “on demand” was used. FGDS was performed when first symptoms appeared. Common methods of endoscopic hemostasis without local treatment of EU-GDI and application of granulated sorbents were used in this group.

The statistical analysis was performed using the statistical software STATISTICA 6.0. A probability value of  $P<0.05$  was considered statistically significant.

## Results and Discussion

The results obtained demonstrated that clinical and endoscopic remission in patients of the main group came earlier than in patients of the comparison group. A timely diagnostic FGDS performed as a part of multidisciplinary approach gave an opportunity to reveal acute EU-GDI without signs of bleeding (F-III) early in 41 out of 58(70.7%) patients

of the main group. To these patients, we applied individual methods of local treatment to prevent GDB with biologically active granulated sorbents according to the technique we developed. It should be noted that granulated sorbents swell directly after insufflations, forming an elastic, hardly detached hydrogel covering on the surface of the erosive-ulcerous defect, protecting it from aggressive gastric and duodenal content. This layer of hydrogel covered a defect as a sort of a curative dressing, under which the reparation process proceeded significantly more rapidly. Endoscopic investigations showed that after local treatment with granulated sorbents, all patients of the main group manifested rapid, high quality healing of erosive-ulcerous defects. Furthermore, special attention was paid to two important moments relating to preventive local treatment by granulated sorbents: first, gastric pain syndrome disappeared in all patients on the first day of treatment, and, second, no one patient of the main group displayed signs of hemorrhagic complications.

Another important peculiarity of the timely FGDS, as a part of multidisciplinary approach, appeared to be detection of acute gastroduodenal erosions and ulcers complicated by bleeding in 17(29.3%) patients. As the study demonstrated, 12(70.6%) of these 17 patients were diagnosed with bleeding in the initial stage (F-IIC), 4(23.5%) patients had bleeding with unstable hemostasis (F-IIA – F-IIB), and only 1(5.9%) patient was reported to have active bleeding (F-IA – F-IB), which demanded endoscopic arrest of the hemorrhage. All these patients of the main group were given the minimally invasive method of local hemostasis as a part of the multidisciplinary approach to prevent recurrence of bleeding; this method provided combined application of powder-like Gelplastan and ASEPTISORB-D on the defect area during the procedure of curative endoscopy. As observations demonstrated, the applied pharmaceutical complex turned into a blood-painted massive layer of hydrogel directly after pneumo-insufflation and protected a thrombosed vessel, clot or hematin from lysis, preventing repeated hemorrhages in the complex treatment with general hemostatic and antiulcerous therapy. Such a complex program of treatment resulted in a lack of hemorrhage recurrence and emergency operations in patients of the main group. Hospital length of stay (LOS) in the main group was  $8.86 \pm 1.69$  days. One (1.7%) patient of the main group, who was admitted to the department of general therapy with acute severe cardiovascular and respiratory failure and atrial fibrillation after acute myocardial infarction and an acute cerebrovascular event, died. Uncontrolled intake of warfarin resulted in GDB in this patient. GDB was arrested by the developed endoscopic technique, but the patient died in 3 hours 15 minutes after admission due to underlying cardiovascular and respiratory failure on the background of anemia.

In the patients of the comparison group, pain syndrome related to EU-GDI persisted until they were discharged from the hospital on the eighth to tenth day. During endoscopic investigation, only 32.1% patients of the comparison group displayed erosive-ulcerous gastroduodenal defects without signs of F-III bleeding. Acute gastroduodenal erosions and ulcers complicated by hemorrhages were diagnosed in the rest (67.9%) of the patients in the comparison group. Endoscopic

findings of GDB were classified as follows: F-IIC in 22 (39.3%) patients, F-IIA and F-IIB in 14(25%) patients, and F-IA in 2(3.6%) patients. Hemorrhage recurrences in patients of the comparison group were registered in 6(10.7%) patients, 2 of them were operated on during the peak of the bleeding rate. In the comparison group, 3(5.4%) patients died. The hospital LOS in the comparison group was  $12.47 \pm 3.44$  days vs.  $8.86 \pm 1.69$  days in the main group ( $P < 0.05$ ). Comparative efficiency of EU-GDB prevention in therapeutic patients of a general hospital is presented in Table 1.

**Table 1.**

**Efficiency of EU-GDB prevention in patients of the main and comparison groups**

Variable	Main group (n=58)	Comparison group (n=56)	P
Detection of acute erosions and ulcers in endoscopic investigation (day)	2nd - 3rd	6th -8th	–
Number of patients with acute erosions and ulcers without signs of bleeding, (n/%)	41/70.7%	18/32.1%	<0.05
Number of patients with bleeding in the initial stage, (n/%)	12/20.7%	22/39.3%	<0.05
Number of patients with unstable hemostasis, (n/%)	4/6.9%	14/25%	<0.05
Number of patients with active bleeding, (n/%)	1/1.7%	2/3.6%	–
Recurrence of bleeding, (n/%)	–	6/10.7%	–
Surgery, (n/%)	–	2/3.6%	–
Mortality rate, (n/%)	1/1.7%	3/5.4%	–
LOS, (days)	8.86	12.47	<0.01

Thus, timely endoscopic examination of the stomach and duodenum in therapeutic patients helped to increase the detection of acute EU-GDI by 2.2 times before the occurrence of hemorrhagic complications. Early local preventive treatment of EU-GDI according to the technique we designed gave an opportunity to prevent the development of decompensated conditions of the underlying therapeutic pathology and reduce the hospital LOS by 1.4 times. In a meta-analysis, endoscopic management was a more effective treatment than pharmacological managements or placebo for lowering the rebleeding and mortality rates in patients with GDB.<sup>(5)</sup> Early endoscopic management has been reported to decrease the length of hospitalization, in comparison with delayed endoscopy, in high- and low-risk groups.<sup>(6,7)</sup>

## Conclusion

Thus, the complex curative program we developed to prevent GDB in therapeutic inpatients is based on a

multidisciplinary approach: an improved algorithm of examination, timely diagnosis of symptomatic EU-GDI, and pharmacological therapy with early local preventive treatment of acute erosions and gastroduodenal ulcers by granulated sorbents. This program helps to prevent hemorrhagic complications, exclude emergency operations, and reduce mortality rate by 3 times.

## Competing interests

The authors declare that they have no competing interests.

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# Risk Prediction of Early and Late Acquired Glomerular and Tubular Dysfunctions in Patients with Disorders of Carbohydrate Metabolism

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## Abstract

**The purpose** of this study was to create a new system for predicting the risk of glomerular and tubular dysfunctions (GTD) in patients with disorders of carbohydrate metabolism (DCM) based on standard parameters and new molecular markers for the development of kidney damage in patients with impaired glucose tolerance (IGT) and T2DM patients with diabetic nephropathy (DN).

**Material and Methods:** The study included 69 patients: 16 patients with IGT (Group 1), 28 T2DM patients with microalbuminuria (Group 2), and 25 T2DM patients with macroalbuminuria (Group 3), according to the inclusion/exclusion criteria in the research. All patients were stratified by the MDRD. The control group (Group 4) included 11 healthy individuals. The duration of DN was 10.5 years. At the stage of data collection and screening, the standard methods of identification of IGT, DM and DN were applied. Additional methods are included quantitative analysis of the level of  $\alpha$ -GST and  $\pi$ -GST, MMP-9 in urine by ELISA.

**Result:** Based on the data of standard methods of identification of early and late acquired GTD in patients with DCM and new methods for assessment of metabolic profile of urine, we propose new prognostic markers of renal glomerular and tubular lesions in patients with IGT and T2DM. (**International Journal of Biomedicine. 2017;7(3):208-212.**)

**Key Words:** diabetic nephropathy • glomerular dysfunctions • tubular dysfunctions • molecular markers • impaired glucose tolerance

## Abbreviations

**CKD**, chronic kidney disease; **DCM**, disorders of carbohydrate metabolism; **DN**, diabetic nephropathy; **eGFR**, estimated glomerular filtration rate; **FG**, fasting glucose; **GTD**, glomerular and tubular dysfunctions; **GST**, glutathione S-transferase; **IGT**, impaired glucose tolerance; **MAU**, microalbuminuria; **MacAU**, macroalbuminuria; **MMP-9**, matrix metalloproteinase-9; **OGTT**, oral glucose tolerance test; **T2DM**, type 2 diabetes mellitus.

## Introduction

About of 20% of middle-aged people and 35% of the population of older persons is characterized by varying degrees of IGT and symptoms of insulin resistance.<sup>(1)</sup> In

accordance with the current evaluation of the International Diabetes Federation, there will be 642 million patients with diabetes mellitus by 2040.<sup>(2)</sup>

Epidemiological studies have considered MAU as risk factor for atherosclerosis, coronary artery disease, and other vascular disorders in patients with T2DM and IGT.<sup>(3-5)</sup>

In Russia, the prevalence of DN in T2DM is an average of 8%; that is 5 times lower than world values. However, active screening of T2DM patients reveals that the true prevalence

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of DN exceeds registered cases in various regions of Russia by 2-8 times.<sup>(6)</sup> The epidemiology of DN in T2DM has not been investigated sufficiently since it is extremely difficult to determine precisely the time when DN begins. The risk of MAU in prediabetic subjects is high, and probably prediabetic subjects are at higher risk of DN progression.<sup>(7)</sup>

Experimental and clinical trials performed between 1998 and 2014 showed that hyperglycemia, hyperlipidemia, a high level of creatinine in the blood, glomerular hyperfiltration, proteinuria, arterial hypertension, and anemia play an important role in the DN development in T2DM patients.<sup>(8-15)</sup>

The molecular pathogenesis of GTD in patients with DCM cannot be described only on the basis of standard clinical research methods. Modern methods and technologies of molecular analysis make it possible to discover new prognostic markers and to investigate pathways of formation of renal damage at various levels of functioning in patients with DCM.

The purpose of this study was to create a new system for predicting the risk of GTD in patients with DCM based on standard parameters and new molecular markers for the development of kidney damage in patients with IGT and T2DM patients with DN.

## Material and Methods

The study protocol was reviewed and approved by the Ethics Committee of Rostov-on-Don State Medical University and Dagestan State Medical University. All participants provided the written informed consent. The study was prospective cohort comparative with parallel design and included 69 patients: 16 patients with IGT (Group 1), 28 T2DM patients with MAU (Group 2), and 25 T2DM patients with MacAU (Group 3), according to the inclusion/exclusion criteria in the research. All patients were stratified by the MDRD. The control group (Group 4) included 11 healthy individuals. Patients corresponded to the criteria for the classification of DN and CKD.<sup>(6,16,17)</sup> The T2DM risks were evaluated with a special assessment designed by Tuomilehto (Finnish Type 2 Diabetes Risk Assessment Form). The duration of DN was 10.5 years. The duration of therapy (hypoglycemic drugs: glibenclamide, metformin, pioglitazone, insulin glargine; antihypertensive drugs: amlodipine, valsartan; hypolipidemic drug: fenofibrate; and antiplatelet drug: acetylsalicylic acid) was 9.2 years.

At the stage of data collection and screening, the standard methods of identification of IGT, DM and DN were applied: the assessment of medical history; physical examination; ambulatory blood pressure monitoring; ultrasonography of kidney (Doppler spectrum of the intrarenal arteries in conjunction with evaluation of the renal cortical echogenicity); complete blood count and urinalysis; blood glucose; OGTT; lipide profile; the serum levels of ALT, AST, pancreatic amylase, total bilirubin, creatinine, potassium, sodium, urea, and C-peptide; MAU and ketone bodies in urine; the estimation of T2DM compensation by HbA1c levels; the assessment of kidney function by eGFR according to the CKD-EPI formula.<sup>(18)</sup> Additional methods are included

quantitative analysis of the level of  $\alpha$ -GST and  $\pi$ -GST, MMP-9 in urine by ELISA. The following diagnostic medical equipment were used: SonoAce R3 (Samsung Medison, South Korea), Olympus AU640 (Olympus Corporation, Japan), Immulite 1000 Immunoassay System (Siemens, USA) "Unimed" (Russia), Randox Laboratories Ltd. (UK), Luminox MAGPIX (USA), standard reagent kits ALPCO Alpha GST ELISA (UK), Biotrak Cell Proliferation ELISA System (UK).

Statistical analysis was performed using Statsoft Statistica V 12.0. Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean $\pm$ SEM for continuous variables. Comparisons between four groups were performed with the one-way ANOVA with Tukey's post-hoc test. Group comparisons with respect to categorical variables are performed using chi-square tests with Yates correction or, alternatively, Fisher's exact test when expected cell counts were less than 5. A probability value of  $P < 0.05$  was considered statistically significant.

## Results

We have verified the next groups of patients with IGT and T2DM: a group of patients with Stage 1 of DN (pre-nephropathy stage with normoalbuminuria,  $n=16$ ) and a group of patients with Stages 2 and 3 of DN (MAU and MacAU,  $n=53$ ). Clinical and anamnestic characteristics of patients and healthy individuals are presented in Table 1.

Parameters of complete blood count and urinalysis were in the range of reference values in patients with IGT. Ultrasonographic signs of DN were detected in all patients with T2DM and DN (high resistive indices were obtained in the region of the arcuate or the interlobar arteries in patients with elevated serum creatinine levels).

There were unidirectional dynamics of mean values of parameters of blood metabolic profile in all groups of patients compared to the control group: FG (Group 1:  $6.17 \pm 0.1$  mmol/l; Group 2:  $8.1 \pm 0.4$  mmol/l; Group 3:  $8.69 \pm 0.4$  mmol/l; and Group 4:  $5.26 \pm 0.2$  mmol/l;  $F=14.6019$ ,  $P=0.0000$ ;  $P_{2,4}=0.0001$ ,  $P_{3,4}=0.0000$ ), OGTT (Group 1:  $6.9 \pm 0.2$  mmol/l; Group 2:  $8.3 \pm 0.3$  mmol/l; Group 3:  $10.0 \pm 0.7$  mmol/l; and Group 4:  $5.8 \pm 0.2$  mmol/l;  $F=11.5656$ ,  $P=0.0000$ ;  $P_{2,4}=0.0120$ ,  $P_{3,4}=0.0000$ ), and HbA1c (Group 1:  $6.91 \pm 0.2\%$ ; Group 2:  $6.97 \pm 0.3\%$ ; Group 3:  $7.68 \pm 0.3\%$ ; and Group 4:  $5.29 \pm 0.1\%$ ;  $F=8.3345$ ,  $P=0.0001$ ;  $P_{1,4}=0.0130$ ,  $P_{2,4}=0.0034$ ,  $P_{3,4}=0.0000$ ).

A significant increase in the average values of the C-peptide level in the blood was recorded in patients with IGT compared with values of this parameter in healthy individuals (Group 1:  $926 \pm 47.8$  pmol/l; Group 4:  $743.4 \pm 45.4$  pmol/l;  $P_{1,4}=0.0138$ ). Significantly increased blood levels of total cholesterol (Group 1:  $6.4 \pm 0.3$  mmol/l; Group 2:  $5.9 \pm 0.2$  mmol/l; Group 3:  $5.9 \pm 0.2$  mmol/l; and Group 4:  $5.2 \pm 0.2$  mmol/l;  $F=2.9643$ ,  $P=0.0373$ ;  $P_{1,4}=0.0197$ ) and LDL-C (Group 1:  $3.45 \pm 0.1$  mmol/l; Group 4:  $2.76 \pm 0.1$  mmol/l;  $P_{1,4}=0.0000$ ) were found in the group of patients with IGT compared to the control group. A statistically significant increased atherogenicity index was identified in groups of patients with IGT and T2DM+MacAU compared to the control group (Group 1:  $4.4 \pm 0.1$ ; Group 3:  $4.1 \pm 0.1$ ; Group 4:  $3.67 \pm 0.2$ ;  $P_{1,4}=0.0015$ ,  $P_{3,4}=0.0389$ ).

Table 1.

## Clinical and anamnestic characteristics of patients with DCM and healthy individuals

Parameter	Group 1 (n=16)	Group 2 (n=28)	Group 3 (n=25)	Group 4 (n=11)	Statistics
Sex (male/female)	5/11	3/25	9/16	3/8	
Age, years	51.1±1.9	54.0±1.9	55.8±2.0	46.2±2.8	$F=2.9173, P=0.0395; P_{1-2}=0.7645, P_{1-3}=0.4159, P_{1-4}=0.5551, P_{2-3}=0.9011, P_{2-4}=0.1053, P_{3-4}=0.0327$
BMI, kg/m <sup>2</sup>	30.6±0.8	33.4±1.1	31.7±1.4	26.6±1.3	$F=3.9390, P=0.0114; P_{1-2}=0.3961, P_{1-3}=0.9294, P_{1-4}=0.2785, P_{2-3}=0.6951, P_{2-4}=0.0062, P_{3-4}=0.0691$
The degree of obesity					
Underweight (25.0-29.9 kg/m <sup>2</sup> )	5	5	8	9	$\chi^2=14.609, P=0.00218$
Grade 1 (30.0-34.9 kg/m <sup>2</sup> )	8	13	10	2	$\chi^2=3.247, P=0.35508$
Grade 2 (35.0-39.9 kg/m <sup>2</sup> )	3	7	5	-	Yates' $\chi^2=1.935, P=0.58600$
Grade 3 (>40.0 kg/m <sup>2</sup> )	-	3	2	-	Yates' $\chi^2=0.667, P=0.88094$
Duration of IGT, months	5.3±1.3	-	-	-	
Duration of T2DM, months	-	-	20.2±3.8	-	
Duration of arterial hypertension, months	9.6±0.8	17.3±3.0	25.3±4.7	-	$F=4.0217, P=0.0225; P_{1-2}=0.3447, P_{1-3}=0.0180, P_{2-3}=0.2277$

Table 2.

## Parameters of renal function in patients with DCM and healthy individuals

Parameter	Group 1 (n=16)	Group 2 (n=28)	Group 3 (n=25)	Group 4 (n=11)	Statistics
Blood laboratory tests					
Urea, mmol/l	5.0±0.3	5.7±0.2	5.4±0.3	4.3±0.4	$F=3.5125, P=0.0192; P_{1-2}=0.3042, P_{1-3}=0.7610, P_{1-4}=0.5018, P_{2-3}=0.8276, P_{2-4}=0.0147, P_{3-4}=0.0885$
Creatinine, μmol/l	73.7±3.6	76.0±2.5	80.1±3.1	70±1.8	$F=1.6351, P=0.1883; P_{1-2}=0.9485, P_{1-3}=0.4575, P_{1-4}=0.8979, P_{2-3}=0.6909, P_{2-4}=0.6009, P_{3-4}=0.1757$
Potassium, mmol/l	4.5±0.03	4.2±0.03	4.5±0.06	4.1±0.03	$F=17.9198, P=0.0000; P_{1-2}=0.0001, P_{1-3}=NaN, P_{1-4}=0.0000, P_{2-3}=0.0000, P_{2-4}=0.5162, P_{3-4}=0.0000$
Sodium, mmol/l	147.5±0.8	150.1±0.9	139.4±0.6	147.1±0.3	$F=41.0321, P=0.0000; P_{1-2}=0.1079, P_{1-3}=0.0000, P_{1-4}=0.9921, P_{2-3}=0.0000, P_{2-4}=0.0994, P_{3-4}=0.0000$
eGFR, ml/min/1.73 m <sup>2</sup>	81.7±3.5	79.3±2.4	81.3±3.1	90.2±3.2	$F=1.7084, P=0.1724; P_{1-2}=0.9434, P_{1-3}=0.9998, P_{1-4}=0.3913, P_{2-3}=0.9510, P_{2-4}=0.1213, P_{3-4}=0.2812$
Urine laboratory tests					
Urine specific gravity, g/l	1018.8±1.7	1021.±1.2	1022.9±1.3	1017.8±1.9	$F=2.1719, P=0.0982; P_{1-2}=0.7009, P_{1-3}=0.2063, P_{1-4}=0.9791, P_{2-3}=0.7116, P_{2-4}=0.5111, P_{3-4}=0.1397$
Albumin, mg/24h	-	7.1±1.5	155.9±24.6	-	$P=0.0000$
α-GST, ng/ml	56.1±2.1	79.8±1.3	85.5±1.5	34.5±1.4	$F=166.3471, P=0.0000; P_{1-2}=0.0000, P_{1-3}=0.0000, P_{1-4}=0.0000, P_{2-3}=0.0252, P_{2-4}=0.0000, P_{3-4}=0.0000$
π-GST, ng/ml	2.0±0.03	1.4±0.03	1.4±0.04	2.4±0.1	$F=98.1620, P=0.0000; P_{1-2}=0.0000, P_{1-3}=0.0000, P_{1-4}=0.0000, P_{2-3}=NaN, P_{2-4}=0.0000, P_{3-4}=0.0000$
MMP-9, ng/ml	317.8±16.5	663.0±10.5	679.6±13.6	154.4±6.4	$F=319.0550, P=0.0000; P_{1-2}=0.0000, P_{1-3}=0.0000, P_{1-4}=0.0000, P_{2-3}=0.7364, P_{2-4}=0.0322, P_{3-4}=0.0446$

In patients with T2DM+MAU, we found a significantly increased blood level of ALT (Group 2:  $27.6 \pm 1.8$  U/l; Group 4:  $21.13 \pm 1.9$  U/l;  $P_{2,4} = 0.0000$ ). The blood level of pancreatic amylase (Group 3:  $72.9 \pm 2.3$  U/l; Group 4:  $39.3 \pm 1.8$  U/l;  $P_{3,4} = 0.0000$ ) was significantly increased in patients with T2DM+MacAU compared to the healthy individuals. The parameters of renal function in patients with DCM are presented in Table 2.

Regression analysis demonstrated the lack of influence of clinical and demographic predictors on the actual number of outcomes with the appearance of GTD determined by the combination of new molecular diagnostic parameters of urine. Therefore, the risk of GTD in patients with DCM can be assessed using new diagnostic markers, which is demonstrated by correlation analysis between interrelated metabolic parameters of blood and urine. In patients with DCM and healthy controls, correlation analysis between the level of eGFR,  $\alpha$ -GST,  $\pi$ -GST and MMP-9 in urine, on the one hand, and blood metabolic profile, parameters of kidney function, on the other hand, has revealed key laboratory tests of blood and urine predicting the occurrence and progression of GTD in IGT and T2DM with MAU and MacAU. The following correlations were found: eGFR with urea concentration in blood (Group 3:  $r = -0.59$ ,  $P < 0.001$ ), blood creatinine (Group 1:  $r = -0.93$ ,  $P < 0.001$ , Group 2:  $r = -0.68$ ,  $P < 0.001$ ) and albuminuria (Group 3:  $r = 0.48$ ,  $P < 0.05$ ); a concentration of  $\alpha$ -GST in urine with blood level of total cholesterol (Group 2:  $r = -0.39$ ;  $P < 0.01$ ), blood sodium (Group 3:  $r = -0.54$ ;  $P < 0.001$ ), blood urea (Group 2:  $r = 0.44$ ,  $P < 0.01$ ) and MMP-9 in urine (Group 2:  $r = 0.54$ ,  $P < 0.001$ ); a concentration of  $\pi$ -GST in urine with urea and creatinine in the blood (Group 3:  $r = 0.39$ ,  $P < 0.05$  and  $r = 0.42$ ,  $P < 0.05$ , respectively).

## Discussion

Determining the level of albumin in urine in patients with DCM is proven and necessary for early detection and evaluation of the progression of DN.<sup>(19)</sup> However, the detection of early changes in glomerular and tubular kidney functions in DCM patients requires the identification and introduction of new specific and sensitive diagnostic markers to the clinical practice. All these changes correlate with high expression of urine proteins, reflecting the progression of epithelial–mesenchymal transition (EMT) and changes in the extracellular matrix (ECM) of the kidneys in DCM patients.

A large number of typical morphological changes is the result of EMT: thickening of basal membranes and the glomerular mesangial matrix, and the expansion of tubulointerstitial space due to an increase in ECM. All EMT events are regulated by a variety of intracellular pathways.

The regularities of conjugate dynamics of hormonal and metabolic profiles of blood and urine have demonstrated changes in the functioning of the hepatorenal axis in patients with DCM.

Based on obtained data about the increase of the blood C-peptide level, it is necessary to note its probable protective effect in the course of development of functional and structural disorders in the renal tissue in DCM. The combined decrease in glomerular hyperfiltration and albuminuria in patients with IGT requires further studies, and additional data are required

on the modulating effects of the different ranges of C-peptide concentrations for the formation of early and late GTD in patients with DCM.<sup>(20)</sup>

The revealed dyslipidemia in patients with IGT and T2DM with the presence of signs of GTD indicated their great prognostic value in combination with other indicators of kidney function. Dyslipidemia can lead to a decrease in the release of NO synthase via the phosphatidylinositol-3-kinase pathway in the endothelium of glomerular vessels, which contributes to the impairment of the filtration function of kidneys and a reduction in eGFR.<sup>(21)</sup>

We found an increase in the concentration of MMP9 in the urine in DCM patients, the most pronounced with T2DM and MAU/MacUA. An increase in the concentration of urine MMP9 in patients with IGT, even in the absence of MAU, indicates an excessive accumulation of it in ECM, which subsequently leads to the development of DN. MMP9 is a participant in the process of remodeling in the ECM in DN.

With a greater degree of probability, the concentration of GSTs in urine may be an early predictor of acquired tubular dysfunctions in patients with DCM. GSTs are primarily the cytosolic enzymes, which are detected in high concentrations in renal tubules.  $\alpha$ -GST is primarily expressed in proximal tubules, whereas  $\pi$ -GST is expressed in the distal tubules and collecting tubules.<sup>(22)</sup> Due to the damage to tubules, the concentration of GSTs increases in urine, which has been demonstrated in our study.<sup>(23)</sup> The increase in the level of GSTs in the urine indicates the functioning of a compensatory mechanism in the response to pronounced oxidative stress in the lesion of renal tissue in DCM.<sup>(24)</sup>

Based on the data of standard methods of identification of early and late acquired GTD in patients with DCM and new methods for assessment of metabolic profile of urine, we propose new prognostic markers of renal glomerular and tubular lesions in patients with IGT and T2DM. The diagnostic markers of acquired GTD in patients with DCM interact with each other and with other molecules participating in universal pathways, which are the main causes for EMT formation and changes in ECM: Smad, p38 MAPK, TLRs, Wnt, mTOR, Notch, small GTPase and Hedgehog, and PI3K/AKT signaling pathways.

## Conclusion

We identified potential new biomarkers allowing us to predict the risk of acquired GTD in patients with DCM (IGT, T2DM). The results of the study are essential for practical implementation of non-invasive diagnostic tests for early detection of nephropathy in DCM. The study made it possible to clarify the molecular mechanisms in the development of acquired GTD in patients with IGT and T2DM. The study of signaling pathways and molecules expressing, secreting and forming ECM may be a basis for the prophylaxis of acquired GTD in patients with DCM.

## Competing interests

The authors declare that they have no competing interests.

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# Diagnostic Markers of Primary Infertility in Women of Reproductive Age with Hypothalamic Dysfunction in the Pubertal Period

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## Abstract

**The aim** of the study was to assess fertility in women of reproductive age with hypothalamic dysfunction (HD) in the pubertal period and to determine the diagnostic significance of pro-inflammatory (TNF- $\alpha$  and IL-1 $\beta$ ), anti-inflammatory cytokine (IL-10) and NF-kB activity in the diagnosis of primary infertility in these women.

**Materials and Methods:** Fertility was assessed in 86 women of reproductive age with HD in the pubertal period. A comparative characteristic of fertile women (Group 1, n=46) and primary infertility women (Group 2, n=21) with HD in the pubertal period was performed. FPG and FPI were determined after 8 to 12 hours of fasting. Serum IRI concentrations were measured using an ELISA kit. The levels of TNF- $\alpha$ , IL-1 $\beta$  and IL-10 were determined in the venous blood serum after a 12-hour fasting, as well as in uterine aspirate (UA) on the 21<sup>st</sup> day of the menstrual cycle using ELISA kits. The activity of NF-kB was determined in UA on the 21<sup>st</sup> day of the menstrual cycle using an enzyme immunoassay kit.

**Results:** BMI in Group 1 was significantly lower than in Group 2: 22.63 $\pm$ 2.68 kg/m<sup>2</sup> versus 27.05 $\pm$ 4.03 kg/m<sup>2</sup> ( $P=0.000$ ). WC in women of Group 1 was 66.11 $\pm$ 5.66 cm versus 78.52 $\pm$ 10.54 cm in Group 2 ( $P=0.000$ ); WC >80 cm was found in 2(4.4%) and 14(66.7%) women, respectively ( $P=0.000$ ). The average levels of FPG and FPI were significantly higher in Group 2. Serum levels of TNF- $\alpha$  and IL-1 $\beta$  in Group 2 were significantly higher than in Group 1. The serum level of anti-inflammatory cytokine IL-10 was significantly lower in Group 2; accordingly, the TNF- $\alpha$ /IL-10 ratio in Group 2 was 1.8 times higher than in Group 1. The IL-1 $\beta$  level in UA ( $P=0.000$ ) and the TNF- $\alpha$ /IL-10 ratio ( $P=0.02$ ) were significantly higher in women of Group 2 than Group 1, which indicated the pronounced inflammatory effects of TNF- $\alpha$  in the endometrium. In women of Group 2, the NF-kB level in UA was 1.4 times higher than in Group 1 ( $P=0.000$ ).

**Conclusion:** Every fourth woman of reproductive age with HD in the puberty period has primary infertility. The results obtained indicate the activation of the Th-1 immune response with the formation of the inflammatory reactions at the systemic level and in the endometrium. Diagnostically significant markers of primary infertility are the serum TNF- $\alpha$  level and the UA levels of IL-1 $\beta$  and NF-kB. (*International Journal of Biomedicine*. 2017;7(3):213-217.)

**Key Words:** hypothalamic dysfunction • primary infertility • endometrium • cytokines

## Abbreviations

**BMI**, body mass index; **FPG**, fasting plasma glucose; **FPI**, fasting plasma insulin; **HD**, hypothalamic dysfunction; **IRI**, immunoreactive insulin; **IR**, insulin resistance; **UA**, uterine aspirate (an aspirate from the uterine cavity); **WC**, waist circumference.

## Introduction

In recent years, more attention has been paid to the development of reproductive health and the reproductive potential of adolescent girls, as future mothers. It has been

noted that in the Russian Federation in the 16-17 age group, the prevalence of endocrine pathology is 5 times higher than in the whole population.<sup>(1)</sup> According to the data of a number of researchers, the prevalence of HD in the pubertal period among girls is from 7.1% to 25%.<sup>(2,3)</sup> On the background of HD, obesity has been diagnosed in 61.1% of adolescent girls.<sup>(4)</sup> Analysis of prospective studies shows that HD in the pubertal period leads to excess body weight in the reproductive age<sup>(5,6)</sup> and increases the frequency of reproductive disorders by 2

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times.<sup>(3,7,8)</sup> According to the data of a number of researchers, a slight inflammatory process is formed on the background of obesity.<sup>(7,9-11)</sup> Pro-inflammatory cytokines secreted by fat tissue affect the state of the endometrium and ovulation.<sup>(11-13)</sup> In this regard, studying a number of proinflammatory and anti-inflammatory cytokines at the systemic and local levels in women of reproductive age with HD in the pubertal period is the most promising way of determining the diagnostic markers of primary infertility.

The aim of the study was to assess fertility in women of reproductive age with HD in the pubertal period and to determine the diagnostic significance of pro-inflammatory (TNF- $\alpha$  and IL-1 $\beta$ ), anti-inflammatory cytokine (IL-10) and NF-kB activity in the diagnosis of primary infertility in these women.

A prospective study was conducted between 2000 and 2013. The mean follow-up was 4.7 $\pm$ 1.7 years. Of the 170 potential participants in the pubertal period, 86 were enrolled in the study at the reproductive age, and 84 were excluded from the study.

The study protocol was reviewed and approved by the Ethics Committee of Amur State Medical Academy. All participants provided the written informed consent. Inclusion criteria were age over 18 years, HD (ICD-10-CM E23.3) manifested by the neuroendocrine (excessive body weight or obesity) and neurotrophic (pink or white striae) disorders according to electroencephalography (EEG) in the pubertal period. Exclusion criteria were tubal infertility (ICD-10-CM N 97.1), previous pelvic inflammatory diseases in history (ICD-10-CM N70 - N75), women of reproductive age who do not plan pregnancy.

In order to identify diagnostic markers of primary infertility in women of reproductive age, we performed a comparative characteristic of fertile women (Group 1, n=46) and primary infertility women (Group 2, n=21) with HD in the pubertal period. In the reproductive age, fertility categories were assessed in accordance with the standardized WHO protocol No.88093.

BMI is calculated using Quetelet's formula (kg/m<sup>2</sup>). The nature of the distribution of adipose tissue was determined by WC. WC >0.80 cm showed an abdominal type of obesity according to IDF. FPG and FPI were determined after 8 to 12 hours of fasting. Serum IRI concentrations were measured using an ELISA kit (Monobind Inc., USA). To determine IR, Caro index was used (FPG(mmol/L)/IRI( $\mu$ IU/ml)); Caro index <0.33 indicates IR.

Blood samples (5 ml) for serological tests were obtained from the ulnar vein on an empty stomach at 8:00 a.m. Studies were carried out in paired sera. Samples of the sera were stored at -20°C. The levels of TNF- $\alpha$ , IL-1 $\beta$  and IL-10 were determined in the venous blood serum after a 12-hour fasting, as well as in UA on the 21st day of the menstrual cycle using ELISA kits ("Interleukin 1-ELISA-BEST", "alpha"-TNF-IFA-BEST (ZAO Vector-Best, Novosibirsk), Interleukin-10 (BenderMed Systems, USA)) according to the manufacturer's recommendations. The activity of NF-kB was determined in UA on the 21st day of the menstrual cycle using an enzyme immunoassay kit (Cayman Chemical Co., USA) according to the manufacturer's recommendations.

Statistical analysis was performed using StatSoft Statistica v6.0. The mean (M) and standard deviation (SD) were calculated. Differences of continuous variables departing from the normal distribution, even after transformation, were tested by the Mann-Whitney *U*-test. Categorical variables were analyzed using the Chi-square test with the Yates' correction. Pearson's Correlation Coefficient (*r*) was used to determine the strength of the relationship between the two continuous variables. A probability value of *P*<0.05 was considered statistically significant.

## Results and Discussion

Fertility was assessed in 86 women of reproductive age with HD in the pubertal period: 46(53.5%) women were found to be fertile, 21(24.4%) women were with primary infertility and 14(16.3%) with secondary infertility; 5(5.8%) women were with unknown fertility and a male factor of infertility. According to the goal of the study, a comparative analysis was performed between the fertile women (Group 1, n=46) and women with primary infertility (Group 2, n=21).

The average age of women in the study groups did not differ significantly: 21.91 $\pm$ 1.11 years and 21.85 $\pm$ 0.97 years. BMI in Group 1 was significantly lower than in Group 2: 22.63 $\pm$ 2.68 kg/m<sup>2</sup> versus 27.05 $\pm$ 4.03 kg/m<sup>2</sup> (*P*=0.000). WC in women of Group 1 was 66.11 $\pm$ 5.66 cm versus 78.52 $\pm$ 10.54 cm in Group 2 (*P*=0.000); WC >80 cm was found in 2(4.4%) and 14(66.7%) women, respectively (*P*=0.000).

The average age at menarche for women in Group 1 and Group 2 was 11.67 $\pm$ 0.76 years and 11.52 $\pm$ 0.92 years, respectively, and did not differ significantly. Gynecological history in women of Groups 1 and 2 was complicated by oligomenorrhea (N91.3) in 2(4.35%) and 9(42.9%), respectively (*P*=0.000), and by excessive and frequent menstruation (N92.0) in 2(4.35%) and 8(38.1%), respectively (*P*=0.001). Secondary amenorrhea (N91.1) in the reproductive age was diagnosed only in 1(2.2%) woman of Group 1. Abortion in the reproductive age with complications was found in 1(2.2%) woman of Group 1. There were no spontaneous abortions in women of both groups. Two (4.3%) women of Group 1 had a delivery in their gynecologic history, and 11(23.9%) women of reproductive age in Group 1 used combined oral contraceptives to regulate the menstrual cycle and contraception.

The levels of FPG and FPI were determined in all women of Groups 1 and 2 (Table 1).

Analysis of carbohydrate metabolism showed that the average level of FPG was significantly higher in Group 2. The FPI level in women of Group 1 was 2.2 times lower than in Group 2. A Caro index <0.33 was found in 15(71.4%) women of Group 2 and in 6(13.3%) women of Group 1 (*P*=0.000).

To identify systemic immune disorders, serum levels of pro-inflammatory cytokines were determined. Serum levels of TNF- $\alpha$  and IL-1 $\beta$  in Group 2 were significantly higher than in Group 1 (Table 2). The serum level of anti-inflammatory cytokine IL-10 was significantly lower in Group 2; accordingly, the TNF- $\alpha$ /IL-10 ratio in Group 2 was 1.8 times higher than in Group 1.

**Table 1.****Parameters of carbohydrate metabolism in women with HD in the pubertal period**

Variable	Group 1 (n=46)	Group 2 (n=21)	P
FPG, mmol/l (>6.1 mmol/l)	4.27±0.51	4.55±0.52	0.03
FPI, $\mu$ IU/ml (>20.0 $\mu$ IU/ml)	9.62±1.99	20.97±10.75	0.000
Caro index (<0.33)	0.46±0.14	0.28±0.16	0.000

**Table 2.****Serum cytokine levels in women with HD in the pubertal period**

Variable	Group 1 (n=34)	Group 2 (n=16)	P
TNF- $\alpha$ , pg/ml	24.09±2.11	28.63±3.85	0.000
IL-1 $\beta$ , pg/ml	39.77±3.27	43.08±3.39	0.001
IL-10, pg/ml	13.56±1.05	10.06±2.74	0.000
TNF- $\alpha$ /IL-10	1.78±0.22	3.17±1.35	0.000

We determined in UA the local immune disorders, the levels of the main Th-1 and Th-2 cytokines involved in the regulation of the inflammatory process and NF- $\kappa$ B, as one of the regulators controlling the cascade of reactions associated with the cytokine activations. Thus, the serum level of TNF- $\alpha$  did not differ between the two groups, but the IL-1 $\beta$  level in UA was significantly higher in women of Group 2 than Group 1 (P=0.000) (Table 3).

**Table 3.****UA cytokine levels in women with HD in the pubertal period**

Variable	Group 1 (n=34)	Group 2 (n=16)	P
TNF- $\alpha$ , pg/ml	18.95±1.05	19.73±3.91	0.28
IL-1 $\beta$ , pg/ml	28.24±1.41	30.59±2.79	0.000
IL-10, pg/ml	5.87±1.88	4.88±1.87	0.08
TNF- $\alpha$ /IL-10	3.57±1.18	4.59±1.88	0.02

The IL-10 levels in UA did not differ significantly between Groups 1 and 2, but the TNF- $\alpha$ /IL-10 ratio was 1.3 times higher in Group 2 than in Group 1 (3.57±1.18 and 4.59±1.88, respectively, P=0.02), which indicated the pronounced inflammatory effects of TNF- $\alpha$  in the endometrium. Imbalance in the production of pro-inflammatory and anti-inflammatory factors indicated the activation of the Th-1 immune response with the formation of the inflammatory reactions at the systemic level and in the endometrium, which is a factor in reducing its implantation ability. In women of Group 2, the NF- $\kappa$ B level in UA was 1.4 times higher than in Group 1: 8.76±1.74 pg/ml versus 6.33±1.0 pg/ml (P=0.000).

In Group 2, we found significant positive correlations

between BMI and serum TNF- $\alpha$  level ( $r=-0.63$ ), BMI and IL-1 $\beta$  level in UA ( $r=-0.60$ ), BMI and NF- $\kappa$ B level in UA ( $r=-0.81$ ), as well as negative correlation between BMI and IL-10 level in UA ( $r=-0.62$ ) (Table 4).

**Table 4.****Correlations between BMI and cytokine levels in the blood serum and UA**

Variable	Group 1 (n=34)		Group 2 (n=16)	
	r	P	r	P
TNF- $\alpha$ (serum)	-0.1	0.549	0.63	0.009
IL-1 $\beta$ (serum)	0.18	0.273	0.40	0.115
IL-10 (serum)	0.06	0.737	-0.40	0.121
TNF- $\alpha$ (UA)	-0.14	0.393	0.18	0.494
IL-1 $\beta$ (UA)	0.19	0.259	0.60	0.013
IL-10 (UA)	0.14	0.393	-0.62	0.009
NF- $\kappa$ B	0.23	0.149	0.81	0.0001

Thus, according to our data, an increase in BMI (1.2 times) and WC >80 cm (66.7%) against a background of an increase in PFF and insulin (2.2 times) indicated the formation of abdominal obesity in 66.7% of women and insulin resistance in 71.4% of women with primary infertility in the reproductive age with HD in the pubertal period.

As is well known, insulin stimulates the production of gonadotropic hormones in the hypothalamus, disrupting the circadian rhythm, which affects the functioning of the hypothalamo-pituitary-ovarian axis.<sup>(3,12,14)</sup> The association between obesity and insulin resistance is largely due to changes in the function of adipose tissue. According to many authors, obesity plays a significant role in reproductive disorders, leading to fertility decline.<sup>(11,14)</sup> Obesity may impair reproductive functions by affecting both the ovaries and endometrium.<sup>(15)</sup> In several studies, it is found that the risk of infertility is threefold higher in obese women than in non-obese women<sup>(16)</sup> and their fertility seems to be impaired in both natural and assisted conception cycles.<sup>(17,18)</sup> It has been shown that the probability of pregnancy is reduced by 5% per unit of BMI exceeding 29 kg/m<sup>2</sup>.<sup>(19)</sup> It has been unequivocally proven that fat is metabolically active; as a result of lipolysis, the release and production of a number of proinflammatory cytokines occur,<sup>(5,9)</sup> which is also confirmed by our research. In primarily infertile women of reproductive age, such immune disorders as increasing the proinflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  and reducing the anti-inflammatory cytokine IL-10 with an increase in serum TNF- $\alpha$ /IL-10 ratio reflect the predominant Th1-type inflammatory response with the formation of systemic inflammatory reactions. Correlation analysis showed a direct relationship between BMI and serum TNF- $\alpha$  level. TNF $\alpha$  was the first cytokine to be implicated in the pathogenesis of obesity and insulin resistance.<sup>(20)</sup> Adipose tissue expression of TNF $\alpha$  is positively correlated with adiposity and insulin resistance.<sup>(20,22)</sup> Chronic exposure to TNF $\alpha$  induces insulin resistance both in vitro and in vivo.<sup>(22,23)</sup>

Many reports have shown that TNF- $\alpha$  may have an important role in the IR pathogenesis by multiple mechanisms, such as downregulation of genes that are required for normal insulin action, direct effects on insulin signaling, induction of elevated free fatty acids via stimulation of lipolysis, and negative regulation of peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ), an important insulin-sensitizing nuclear receptor.<sup>(24-27)</sup> In addition, TNF- $\alpha$  functions as a modulator of gonadotropin release in the hypothalamus<sup>(28)</sup> and an activator of intravascular coagulation.<sup>(10)</sup> TNF- $\alpha$  is a pro-angiogenic factor and contributes to impaired vascularization of the endometrium.<sup>(28)</sup>

Investigation of the level of pro-inflammatory cytokines in UA showed the diagnostic significance of an increase in IL-1 $\beta$  level and the TNF- $\alpha$ /IL-10 ratio in women with primary infertility. We found a direct correlation between BMI and IL-1 $\beta$  level and an inverse correlation between BMI and IL-10. Based on the results of other authors, IL-1 $\beta$  increases the production of prostaglandins, causing uterine contractions, which may be important in miscarriages.<sup>(13)</sup> IL-10, which is involved in the process of hemopoiesis and angiogenesis, has a powerful anti-inflammatory and immunomodulatory effect and plays a major role in suppressing the excessive production of pro-inflammatory mediators.<sup>(29)</sup> The decrease in IL-10 level and the predominance of pro-inflammatory cytokines over anti-inflammatory cytokines can alter the direction of the mother's immune response with the formation of the inflammatory reactions in the endometrium, which is a factor in reducing its implantation ability.

The correlation analysis also showed a direct, strong correlation between BMI and NF- $\kappa$ B in UA. NF- $\kappa$ B as a transcription factor is involved in the control of a large number of normal cellular and organismal processes, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis.<sup>(30)</sup> These capacities of NF- $\kappa$ B can be used in the diagnosis of the implant ability of the endometrium.

**In conclusion**, every fourth woman of reproductive age with HD in the puberty period has primary infertility. The results obtained indicate the activation of the Th-1 immune response with the formation of the inflammatory reactions at the systemic level and in the endometrium. Diagnostically significant markers of primary infertility are the serum TNF- $\alpha$  level and the UA levels of IL-1 $\beta$  and NF- $\kappa$ B.

## Competing interests

The authors declare that they have no competing interests.

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## Monitoring Extremely Preterm Birth in the Republic of Sakha (Yakutia)

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### Abstract

The monitoring of outcomes of very early premature births in the Republic of Sakha (Yakutia) (RS(Y)) for 2014-2016 is presented in this article. (**International Journal of Biomedicine. 2017;7(3):218-220.**)

**Key Words:** extremely preterm birth • premature amniorrhexis • extremely low birth weight • routing of pregnant women

### Abbreviations

**PB**, preterm birth; **EPB**, extremely preterm birth; **ELBW**, extremely low birth weight; **TOPFA**, termination of pregnancy for a fetal anomaly; **PCNCM**, Perinatal Center of the National Center of Medicine; **VPB**, very preterm birth.

### Introduction

A large proportion of perinatal losses are due to the outcomes of preterm labor. According to WHO, Every year, an estimated 15 million babies are born preterm (before 37 completed weeks of gestation), and this number is rising.<sup>(1)</sup> Prematurity is the single most important cause of death in the first month of life and is a factor in over 75% of pediatric deaths in the neonatal period. As the second leading cause of death in children under five years old, prematurity remains a global health problem.<sup>(2,3)</sup> As practice has shown, the only effective way to reduce perinatal losses and improve the outcomes of premature births is to route patients with preterm labor to large perinatal centers for observation, as those centers have sufficient equipment for nursing for deeply premature infants.

In 2012, Russia adopted the WHO criteria for a live birth. According to the Order of the Ministry of Health and Social Development of Russia (No. 1687n, December 27, 2011) “On the medical criteria of birth, form of birth certification and the procedure for its issuance”,<sup>(4)</sup> for the first time in the history

of domestic medicine, the following criteria for liveborn babies were adopted: gestational age of 22 weeks and more; the body weight of the newborn of 500 g or more (or less than 500 g for multiple births) or, if the child’s weight at birth is unknown, the body length of the newborn of 25 cm or more, if the newborn shows signs of live birth (breathing, heartbeat, umbilical cord pulsation, voluntary muscle movements). According to the Order of the Ministry of Health of RF (№15-4-10/2-9480, December 17, 2013),<sup>(5)</sup> the clinical protocol for the management of preterm birth has been introduced into the clinical practice in all medical institutions since 2013.

Later, on September 21, 2015, the clinical protocol “Organization of medical evacuation in case of premature birth”<sup>(6)</sup> was developed and recommended. In each subject of Russia, the performance of these clinical protocols is monitored monthly.

RS(Y) (Fig. 1) is the largest subject of Russia, occupying about 3.1 million square kilometers, and more than 40% of its territory lies above the Arctic Circle. On 01/01/16, the population of Rs(Y) numbered 959,600 people, with a population density of 0.3 inhabitants per 1 km<sup>2</sup>. On 01/01/16, the female population numbered 493,987, and 242,282 women were of fertile age. Annually, the birth rate exceeds the death rate, which provides a positive coefficient of natural growth rate, which at the beginning of 2016 was 7.66‰.

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**Fig. 1.** Geographical location of the RS (Y) on world map.

A particular feature of the region is the difficult climatic conditions. More than half of the territory is in the remote areas with only seasonal transport service; about 76% of 34 districts do not have reliable transport links with the center of the Republic and surrounding regions. The organization of coordinated work to provide obstetric and gynecological care in the region requires great attention and continuity of various services.

The obstetric and gynecological service of RS(Y) is represented by a three-level structure that includes 37 maternity hospitals. Only PCNCM belongs to the third-level group; the second-level group is represented by 6 City Maternity Departments in multi-profile hospitals; the first-level group is represented by 30 Maternity Departments in the Central District Hospitals. In total, there are 379 beds for pregnant and parturient women, 408 beds for pregnancy pathology and 503 gynecological beds.

The aim of this study was to investigate all cases of EPB at all levels of the obstetric and gynecological service, identify routing errors, and analyze the outcomes.

## Materials and Methods

The study was designed as a population-based descriptive study, based on the results of a longitudinal analysis of national and regional reports of the Yakut healthcare services and an analysis of medical records describing all case histories of childbirth and newborns.

## Results and Discussion

The number of childbirths in the RS(Y) in the 5-year period between 2011 and 2016 did not change significantly (Table 1), but in 2016, there were 15,429 births, thus, the birth rate decreased by 0.8%. In 2011, before the adoption of the new live birth criteria, the share of preterm delivery in the total delivery structure was 5.4%; this figure increased to 6.9% in 2012. This increase of 1.5% was due to EPB previously registered in the structure of late miscarriages in the period up to 28 weeks. In the following years, this parameter has been at the previous level: 2012 – 0.6%, 2013 – 0.5%, 2014 – 0.4%, 2015 – 0.5%, 2016 – 0.5%) (Tab. 1).

**Table 1.**

**The number of births in RS (Y) in 2011-2016**

Variable	2011	2012	2013	2014	2015	2016
Term birth	15309 (94.6%)	15650 (92.4%)	15407 (92.9%)	15705 (92.6%)	15134 (92.4%)	14266 (92.4%)
PB	884 (5.4%)	1160 (6.9%)	1078 (6.5%)	1172 (6.9%)	1159 (7.1%)	1075 (6.9%)
EPB	-	112 (0.6%)	93 (0.5%)	71 (0.4%)	86 (0.5%)	84 (0.5%)
Total	16193	16922	16578	16948	16379	15425

Preterm infants with ELBW caused a sharp increase in the perinatal mortality rate from 8.4% in 2011 to 13.0% in 2012. This was due to an increase in the rate of early neonatal mortality (3.0% in 2011 and 4.6% in 2012), indicating that patients with threatening PB were not routed to third-level hospitals and that resuscitation departments for the treatment of such patients were not available. In the following years, there was a decrease in the rate of early neonatal mortality, which reduced perinatal mortality (Table 2).

**Table 2.**

**Structure of perinatal mortality in the RS (Y) in 2011-2016**

Variable	2011	2012	2013	2014	2015	2016	
Stillbirth	Born dead, ‰	5.4	8.4	5.22	6.4	6.5	6.4
	TOPFA, ‰	-	-	1.08	0.4	0.7	0.7
Early neonatal mortality, ‰	3.0	4.6	4.5	3.2	3.4	2.5	
Total Perinatal mortality, ‰	8.4	13.0	10.8	10.0	10.6	9.6	

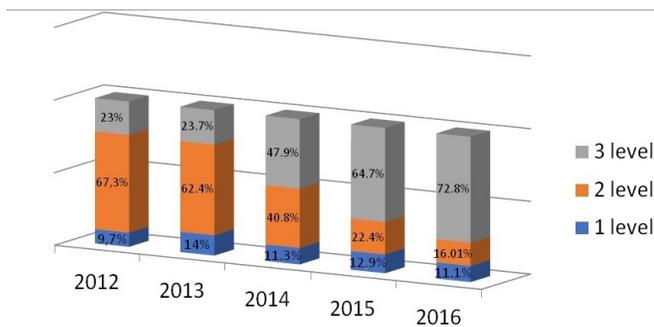
Unfortunately, this is impossible to say about stillbirth, which has no tendency to decline (5.4‰ in 2011, 8.4‰ in 2012, 6.3‰ in 2013, 6.8‰ in 2014, 7.2‰ in 2015, and 7.1‰ in 2016). In its structure, termination of pregnancy for a fetal anomaly (TOPFA) after 22 weeks of gestation comprises a considerable part (1.08‰ in 2013, 0.4‰ in 2014, 0.7‰ in 2015, and 0.7‰ in 2016) (Table 2). This is partly due to untimely prenatal diagnosis.

In 2016, after introduction of the clinical protocol on the routing of pregnant women with threatening premature births, 117 pregnant women with threatening premature births were transported to PCNCM (58/49.57% women from medical organizations of the second level and 59/50.42% women from medical organizations of the first level). Due to timely routing, the proportion of PB in PCNCM is increasing every year (Table 3). In the structure of EPB, the share of PCNCM had also increased significantly by 2016. The increase in this indicator was due to the timely evacuation of pregnant women from the second-level obstetric hospitals. At the same time, these indices practically did not decrease in the first-level obstetric hospitals (Fig. 2).

**Table 3.**

**The frequency of preterm delivery in maternity hospitals of RS(Y) in 2011-2016**

Variable	2012	2013	2014	2015	2016
The 3rd –level group	331 (28.5%)	411 (38.1%)	487 (41.5%)	545 (47%)	530 (49.3%)
The 2nd –level group	616 (53.1%)	514 (47.7%)	528 (45.1%)	467 (40.3%)	451 (41.9%)
The 1st –level group	213 (18.4%)	153 (14.2%)	157 (13.4%)	147 (12.7%)	94 (8.7%)
Total of PB	1160	1078	1172	1159	1075



**Fig. 2.** Structure of EPB according to the level of obstetric hospitals in RS(Y) in 2011-2016.

On average, 17% of babies were born through the birth canal, and 71.4% by cesarean section. Tokolisis, taking into account contraindications, was carried out in 44% of cases by nifedipine and ginipral, and in 8% of cases with atosiban; 45% of patients received full prevention of respiratory distress syndrome.

The total number of EPB cases in PCNMC was 60. A total of 69 children were born, taking into account multiple pregnancy (7 sets of twins and 1 of triplets) and 6 babies as a result of IVF (in vitro fertilization). Live births occurred in 44 cases: 48 liveborn infants, including 5 sets of twins, in one case from twins with one stillborn. In the case of live births, an emergency Cesarean section was performed in 36 mothers, while 8 mothers gave birth through natural birth canals. Liveborn babies born at more than 26 weeks of gestation accounted for 34, and at less than 26 weeks of gestation, 14 babies. The total number of perinatal deaths was 25, of which 21 were stillbirths and 4 were early neonatal death. Survival analysis showed that 12 children died in the first month of their life: 4 children born at 22–26 weeks of gestation and 8 children at more than 26 weeks of gestation. Eight of the 12 children died between the 2nd and 4th weeks of life, 3 of them at 22-25 weeks of gestation, and 5 at more than 26 weeks of gestation. A total of 19 children died in the first year of their life.

The percentage of EPB cases in the second-level obstetric hospitals was 16.01% in 2016, 22.4% in 2015, 40.8% in 2014, 62.4% in 2013, and 67.3% in 2012. Alertness and timely evacuation provided a decrease in the EPB level

at the second level of obstetric service. On average, 69.2% of mothers gave birth through natural birth canal. Cesarean section was performed in 30.8% of cases. The causes of EPB were congenital malformations of fetus and antenatal fetal death in 61.5% of cases, fetal growth retardation in 7%, threatening condition of the fetus in 14% of cases, and spontaneous vaginal delivery in 23% of cases. The total number of EPB cases was 13. The number of perinatal deaths was 10, including 9 stillborns and 2 cases of early neonatal death. Only one child was discharged home.

The total number of EPB cases in the first-level obstetric hospitals was 9, including twin delivery. On average, 77.8% births were spontaneous, complicated with infection and premature amniorrhexis, and occurred through the natural birth canal. Cesarean section was performed in 22.2% of cases due to premature abruption of a normally located placenta. Among 10 newborns, 5 were stillborn and 5 babies died in the early neonatal period.

## Conclusion

Thus, our analysis shows that the timely carrying out of such activities as a full survey of a pregnant woman and clear observance of the terms for combined first trimester screening and prenatal ultrasound diagnosis (FMF certificates) reduce the rate of PB and stillbirth due to timely detection and termination of pregnancy with severe congenital malformation of the fetus. Also, the doctor's vigilance and strict adherence to clinical recommendations (at preterm delivery) and routing of pregnant women with threatening premature birth will allow avoidance of PB at the first level, which is the main task facing the obstetric and gynecological service of RS(Y) in 2017.

## Competing interests

The authors declare that they have no competing interests.

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## Impact of Acute Deltamethrin Poisoning on Rat Adrenal Glands: Biochemical and Pathomorphological Study

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### Abstract

**Background:** Deltamethrin is known all over the world as an effective preparation for the control of insects. In connection with this, its role as a chemical stressor increases. The aim of the study was to determine the features of the functioning and structure of adrenal glands (AG) after a single administration of synthetic pyrethroid deltamethrin in experimental animals at a dose of 17.4 mg/kg (1/5 LD<sub>50</sub>).

**Material and Methods:** For the experiment, 88 male Wistar rats with a body weight of 240±10 g were divided into 8 groups of 10–12 animals each. Groups 1, 3, 5, and 7 were control groups, which were administered physiological solution intragastrically. The animals in Groups 2, 4, 6, and 8 received a single dose (17.4 mg/kg) of deltamethrin, which corresponds to 1/5 LD<sub>50</sub>. In the serum of rats, the content of ACTH, progesterone, DHEAS, corticosterone and aldosterone was determined by EIA. Histological preparations of adrenal glands were stained with H&E, picrofuxin according to Van Gieson, and with Bismarck brown according to Shubich. On frozen sections, lipids were detected by Sudan Black B.

**Results:** One day after intoxication, we identified an increase in adrenal mass, edema of the parenchyma and blood capillary overflow, and a large number of lipids in corticocytes. In the blood serum, the concentration of ACTH and corticosteroids increased, but their level decreased in the adrenal cortex. After 3 days, the concentration of corticosterone in the blood serum of the experimental animals remained above the control value, but the content of other hormones decreased. At the border of the cortex and the medulla of the adrenal glands, there were mast cells in a state of degranulation; the amount of lipids decreased with time. In the subsequent terms of the study, a decrease in the weight of AG with a decrease in the concentration of hormones in the blood serum and adrenal tissue was detected.

**Conclusion:** The intoxication of rats with deltamethrin causes morphofunctional changes in AG that characterize the development of the stress response. The hormonal background is not restored within a month, which indicates the possibility of developing post-toxic complications. (*International Journal of Biomedicine*. 2017;7(3):221-225.)

**Key Words:** pesticides • pyrethroids • deltamethrin • adrenal glands • steroid hormones

### Abbreviations

AG, adrenal glands; ACTH, adrenocorticotrophic hormone; BW, body weight; DHEAS, dehydroepiandrosterone sulfate; EIA, enzyme immunoassay; H&E, hematoxylin and eosin.

### Introduction

Synthetic pyrethroids are drugs of neurotropic action. They disrupt the functioning of the sodium, chloride and calcium

channels of neurons.<sup>(1)</sup> These effects can be accompanied by the development of other undesirable effects that involve the reproductive,<sup>(2,3)</sup> immune,<sup>(4,5)</sup> cardiovascular,<sup>(6,7)</sup> respiratory<sup>(8,9)</sup> and other systems, and also contribute to a high risk of cancer development.<sup>(10)</sup> People contact pyrethroids when they are used in industrial activities and households, as well as from consumption of vegetables, fruits and water contaminated with their residues.<sup>(11)</sup>

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The world market of pyrethroids was estimated at more than 2.5 billion dollars in 2016 (according to Statistics MRC), and by 2023 will exceed 4 billion dollars.<sup>(12)</sup> Among pyrethroids, deltamethrin is one of the most popular and widely used insecticides in the world.<sup>(13)</sup> It is used in agriculture and veterinary medicine for the destruction of pests of plants and ectoparasites of animals. In this connection, there is an increasing interest in studying its toxicity for animals and humans.<sup>(14-17)</sup> Adrenal glands (AG) play an important role in maintaining homeostasis during the influence of chemical stressors. They participate in the implementation of adaptive mechanisms in the hypothalamic-pituitary-adrenal axis,<sup>(18-20)</sup> coordinating the work of all organs and systems in a stressful situation.

The aim of the study was to determine the features of the functioning and structure of AG after a single administration of synthetic pyrethroid deltamethrin in experimental animals at a dose of 17.4 mg/kg (1/5 LD50).

## Material and Methods

For the experiment, 88 male Wistar rats with a body weight of  $240 \pm 10$  g were divided into 8 groups of 10–12 animals each. Groups 1, 3, 5, and 7 were control groups, which were administered physiological solution intragastrically. The animals in Groups 2, 4, 6, and 8 received a single dose (17.4 mg/kg) of deltamethrin, which corresponds to 1/5 LD50. To determine the dynamics of the content of adrenal hormones in the blood and glandular tissue, rats were withdrawn from the experiment step by step: rats in Groups 1 and 2 - one day after the experiment, in Groups 3 and 4 - after 3 days, in Groups 5 and 6 - after seven days, and in Groups 7 and 8 - after 30 days of the deltamethrin administration. In the course of the experiment, the preparative form of deltamethrin was used under the trade name “Butox 50” (Intervet, Netherlands). The study protocol was reviewed and approved by the Ethics Committee of the Omsk State Medical University. All stages of the experiment were carried out in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

In the serum of rats, the content of ACTH, progesterone, DHEAS, corticosterone and aldosterone was determined. AG of animals were removed and weighed. The right adrenals were homogenized at  $0-2^{\circ}\text{C}$  and the content of steroid hormones was determined in the resulting homogenate. The content of ACTH, progesterone, corticosterone and aldosterone was determined by direct competitive ELISA with the antibodies immobilized on the solid phase. The amount of DHEAS was determined by a “sandwich” solid-phase enzyme immunoassay. For the EIA, the kits of Cusabio Biotech Co. Ltd (China) and DRG International Inc (USA) were used.

The left AG were fixed in a 4% neutral solution of formaldehyde. Histological preparations were stained with H&E, picrofuxin according to Van Gieson, and with Bismarck brown according to Shubich. On frozen sections, lipids were detected by Sudan Black B. The study of histological preparations was carried out using the Altami BIO 1 microscope (Altami, Russia).

Statistical analysis was performed using the statistical software «Statistica» (v6.0, StatSoft, USA). The results are presented as *Me* (median), *Q1* (lower quartile), and *Q3* (upper quartile). The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. A probability value of  $P < 0.05$  was considered statistically significant.

## Results

We recorded the change in body weight (BW) of rats as a result of exposure to a toxic dose of deltamethrin. A day after the beginning of the experiment, rats of the Groups 1 and 2 had the same BW. However, on the third day there was a decrease in BW by 3.7% in animals subjected to intoxication, compared to the corresponding control group (Fig. 1). In subsequent observation periods, the BW of rats subjected to deltamethrin was lower than in control animals. In this case, a change in the weight of AG was noted. Thus, on the third day of the observation, the weight of the AG increased, but on the 30th day it decreased compared to the corresponding control group (Fig. 2). Accordingly, the mass index of AG showed a statistically significant increase on the third day of observation, but had decreased by the 30th day (Fig. 3).

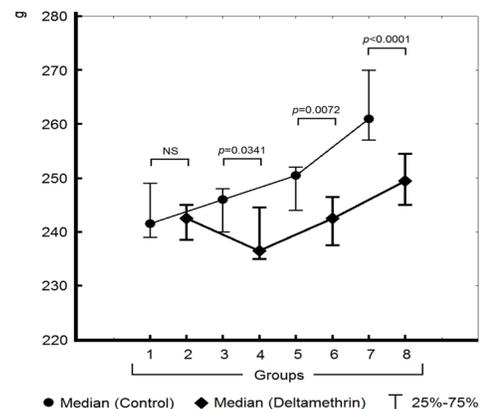


Fig. 1. Changes in BW of rats after single administration of deltamethrin.

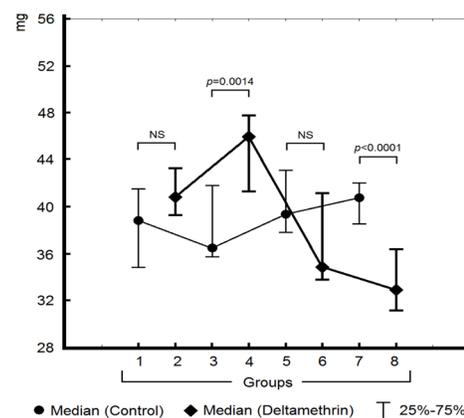
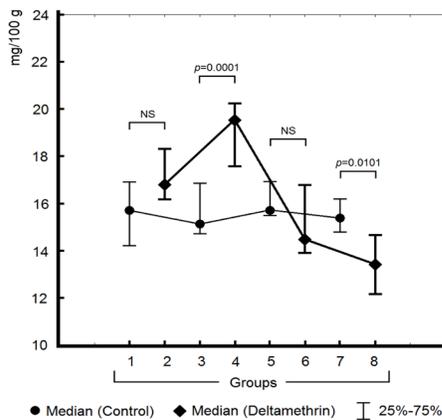
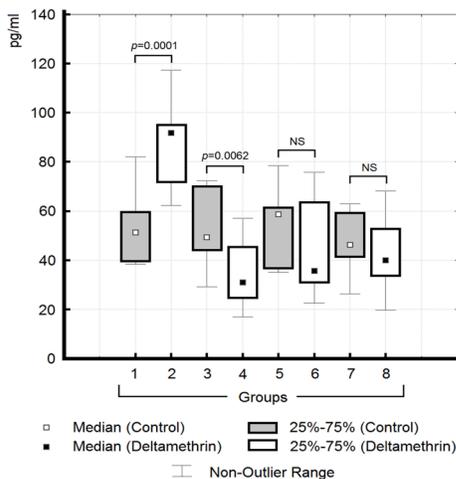


Fig. 2. Changes in adrenal mass in rats subjected to deltamethrin intoxication.



**Fig. 3.** Mass index of adrenal glands in rats subjected to deltamethrin intoxication.

A single deltamethrin administration at a dose of 1/5 LD50 led to an increase in serum ACTH level after the first day of the experiment. Thus, the content of this hormone in the blood of rats in Group 2 was 78.9% higher than in the first control group (Fig. 4). However, on the third day of the experiment, we observed a decrease in the ACTH level of rats subjected to deltamethrin intoxication. In later periods of the experiment, there were no statistically significant differences in the content of ACTH in the animals of the experimental and control groups.



**Fig. 4.** ACTH level in the blood serum of rats subjected to deltamethrin intoxication.

Intoxication with deltamethrin was accompanied by disruptions in the synthesis of steroid hormones. A decrease in the level of progesterone in the serum was observed on Days 7 and 30 of the experiment (Table 1). In the body of males, this hormone is a precursor in the synthesis of corticosteroids and androgens,<sup>(21)</sup> so reducing its concentration in adrenal tissue in males is the most dangerous. The DHEAS level in the blood serum of rats characterizes the androgen-synthesizing function of AG. One day after the experiment, there was an increase in the level of this hormone in the blood. The DHEAS level in the serum of the rats of Group 2 was 46.2% higher than in Group 1 (Table 1). Simultaneously, a decrease in the content of this

hormone in the adrenal tissue was found. So, in Group 2, on the first day after deltamethrin introduction, this indicator was 26.2% below the control level. At other times of observation, there were no statistically significant differences in the DHEAS content in adrenal tissue and in the blood of experimental and control animals.

Despite the restoration of the androgen-synthesizing function of AG after a single deltamethrin administration, the synthesis of gluco- and mineralocorticoids was disrupted. The first day of the experiment showed an increase in the level of corticosterone and aldosterone in the blood of rats subjected to deltamethrin administration. The serum level of corticosterone and aldosterone in the rats in Group 2 was higher by 30.5% and 61.2%, respectively, than the control values (Table 1). On the third day, the level of corticosterone remained elevated, and the aldosterone content returned to normal value. Seven days after deltamethrin intoxication, the serum level of corticosterone normalized, but the aldosterone level decreased. However, at the final stage of the experiment, on the 30th day, we registered a deficiency of both hormones in the blood of experimental animals. The serum level of corticosterone and aldosterone in the rats of Group 8 was lower by 36.7% and 43.8%, respectively, in comparison with the corresponding parameters in the corresponding control group (Table 1).

Along with a change in the level of corticosteroids in the blood of rats subjected to acute intoxication with deltamethrin, we found a change in the content of these hormones in adrenal tissue. One day after the experiment, the corticosterone level was significantly reduced, on the third day it returned to normal value, followed by the development of a persistent deficit on the seventh day, and did not reach the control level until the end of the experiment (Table 1). The concentration of aldosterone in the adrenal tissue on the first day after deltamethrin administration was close to the normal level; on the third day it decreased sharply and after 7 days increased again, but remained below the control level. Thirty days later, aldosterone deficiency was pronounced in adrenal tissue.

The change in the concentration of hormones in the blood serum and adrenal tissue was accompanied by an increase in the mass of AG in the first 3 days of the experiment, followed by its decrease. At this period, during the histological examination, we found that edema of the organ parenchyma and the blood capillaries were overfilled. The glomerular and fascicular zones were enlarged. Cells of the glomerular zone were a rounded shape; the contours of the cells were distinctly expressed. The cells of the fascicular zone were polygonal, large, with large rounded nuclei. Corticocytes of the reticular zone also contained the large rounded nuclei in which chromatin accumulations were distinctly expressed. A histochemical study revealed a large number of lipid droplets in the cytoplasm of the cells of the fascicular and glomerular zones. At the border of the cortex and the medulla of AG, mast cells were recorded, some of them in the degranulation stage. In subsequent periods of the experiment, the borders of the glomerular, and especially fascicular zones, narrowed, the size of the corticocytes decreased, and the lysed and pycnotic nuclei were found. The lipid content in the cells was reduced. The weight of AG was already sharply decreased 7 days after intoxication and had not reached the initial values at the end of the experiment.

Table 1.

The dynamics of the concentration of hormones in the blood serum and adrenal tissue in rats after a single administration of deltamethrin at a dose of 17.4 mg/kg (1/5 LD50), Me ( $Q_1-Q_3$ )

Group	Progesterone		DHEAS		Corticosterone		Aldosterone	
	Serum	AG	Serum	AG	Serum	AG	Serum	AG
	ng/ml	ng/mg tissue	ng/ml	ng/mg tissue	nmol/l	ng/mg tissue	pg/ml	ng/mg tissue
Day 1								
Group 1 n=10	9.40 (7.20–10.7)	1.77 (1.44–2.14)	130 (95.8–154)	7.57 (6.32–8.82)	417 (378–500)	17.9 (13.0–19.3)	98.0 (70.1–106)	1.22 (0.907–1.33)
Group 2 n=12	10.0 (6.55–11.8) NS	1.05 (0.687–1.24) $P=0.0031$	190 (132–198) $P=0.0373$	5.59 (4.63–6.17) $P=0.0112$	544 (487–662) $P=0.0043$	10.0 (6.92–10.7) $P<0.0001$	158 (128–169) $P<0.0001$	1.05 (0.657–1.19) NS
Day 3								
Group 3 n=10	6.22 (3.94–11.1)	1.71 (1.27–1.86)	141 (107–150)	8.42 (6.64–9.50)	395 (366–470)	16.4 (13.6–17.8)	77.7 (73.1–95.1)	1.26 (1.03–1.53)
Group 4 n=12	3.17 (2.15–7.26) NS	0.741 (0.384–0.806) $P<0.0001$	116 (101–149) NS	7.17 (6.09–8.01) NS	524 (414–600) $P=0.0271$	19.3 (14.8–20.8) NS	99.0 (76.1–118) NS	0.400 (0.274–0.575) $P<0.0001$
Day 7								
Group 5 n=10	8.55 (6.37–10.7)	1.53 (1.20–2.22)	101 (80.4–139)	7.23 (5.90–8.90)	428 (368–502)	17.5 (11.5–19.3)	85.7 (76.8–105)	1.05 (0.916–1.36)
Group 6 n=12	3.71 (1.92–4.67) $P<0.0001$	0.633 (0.431–1.45) $P=0.0079$	96.0 (84.2–118) NS	7.81 (5.25–7.91) NS	425 (338–471) NS	7.35 (6.41–14.1) $P=0.0092$	62.7 (48.7–81.6) $P=0.0077$	0.877 (0.766–1.29) NS
Day 30								
Group 7 n=10	7.67 (5.21–11.8)	1.63 (1.21–1.82)	135 (101–144)	6.77 (5.58–8.49)	466 (304–496)	15.3 (11.1–21.1)	88.0 (78.5–125)	1.16 (1.12–1.38)
Group 8 n=12	3.21 (2.14–6.16) $P=0.0128$	0.809 (0.719–1.48) $P=0.0157$	123 (96.0–137) NS	6.19 (4.68–6.45) NS	295 (221–348) $P=0.0152$	10.9 (5.41–13.1) $P=0.0227$	49.5 (40.3–86.6) $P=0.0157$	0.814 (0.513–0.876) $P<0.0001$

## Discussion

Our study allowed us to evaluate the morphological changes and functional activity of AG after a single administration of deltamethrin in a toxic dose. During the period of observation, we found a decrease in BW of animals after intoxication, and an increase in adrenal mass during the initial period of the experiment, followed by a decrease. With this regularity, not only the absolute but also the relative mass of the adrenal glands changed, as evidenced by their mass indices.

During the first day after deltamethrin administration, an active secretion of DHEAS, corticosterone and aldosterone into the blood was found, which was accompanied by a decrease in the content of these hormones in adrenal tissue. This indicates the development of the stage of anxiety under stress, as a result of which the internal reserves of the organism are mobilized. This confirms the increase in the mass of AG and its functional zones responsible for the synthesis of steroid hormones. Reducing the amount of lipids in corticocytes 3 days after intoxication is associated with activation of the process of corticosteroid formation after exposure to deltamethrin. Depletion of lipid stocks in the fascicular zone of the adrenal glands characterizes the state of stress in animals<sup>(22)</sup> and is observed at increased secretion of ACTH.<sup>(23)</sup>

With the passage of time, the biosynthesis of DHEAS was recovered against the backdrop of the development of a deficit of corticosterone and aldosterone. A low level of progesterone in rats with acute intoxication with deltamethrin was observed throughout the study period. This could be one of the reasons for lowering the synthesis and secretion of corticosterone and aldosterone, since progesterone is an intermediate metabolite in the biosynthesis of these hormones. In addition, a deficit of corticosteroids may be due to other causes. First, in conditions of acute poisoning with deltamethrin due to liver damage, the synthesis rate and the transport of cholesterol, which is the substrate for the synthesis of all steroid hormones, can be disrupted. The hepatotoxic effect of synthetic pyrethroids is presented in the studies of a number of authors.<sup>(14,15)</sup> Secondly, the deltamethrin intoxication contributes to the enhancement of free radical processes against the background of a decrease in the reserves of the antioxidant system,<sup>(16,17,24)</sup> which is an inhibitory factor for steroidogenesis enzymes.<sup>(25)</sup> Thirdly, there may be absent the stimulus from the pituitary and hypothalamus. The level of ACTH in the long-term follow-up was close to the control values, although a deficiency of corticosterone should cause an increase in ACTH secretion by the feedback principle.

Thus, our results of the study allow us to conclude that a

single administration of deltamethrin to rats at a dose of 17.4 mg/kg (1/5 LD50) causes morphofunctional changes in AG, characterizing the development of the stress response. One day after intoxication, a large amount of ACTH, corticosterone and other adrenal hormones are released into the blood, and the absolute and relative mass of AG increases. Over time, the index of adrenal weight decreases in animals, which is accompanied by a decrease in the level of corticosteroids. The death of animals during the experiment was not observed. However, the studied indicators did not recover to the level of control values within a month, which indicates the possibility of developing post-toxic complications.

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## Competing interests

The authors declare that they have no competing interests.

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## Associations of Polymorphic Variants of the Biotransformation Genes with the Components of the Glutathione System in Men with Infertility

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### Abstract

**The aim** of this research was to investigate the glutathione system components and their association with polymorphisms GST genes in men with infertility.

**Materials and Methods:** One hundred and sixty Russian men of reproductive age (Caucasians) who came to the public health institution Republican Perinatal Center in Ulan-Ude with an infertility problem of one year and more after marriage were included in the main group. The control group included 104 men with proven fertility. DNA samples were genotyped for polymorphisms in *GSTP1*, *GSTT1* and *GSTM1* genes and activity of glutathione system enzymes was determined.

**Results:** The most informative genetic and metabolic indicators in Caucasian males with infertility were combinations of the null genotypes *GSTT1*(\*0/\*0)+*GSTM1*(\*0/\*0) associated with a decrease of GST activity in blood and ejaculate and an increase of GSH and GPO in the blood. Another combination is *GSTP1*(Ile105Val)+*GSTP1*(Ala114Val), which is associated with suppression of the blood and ejaculate GPO activity and a decrease in blood concentration of GSH. (**International Journal of Biomedicine. 2017;7(3):226-230.**)

**Key words:** male infertility • *GSTP1* • *GSTM1* • *GSTT1* • glutathione

### Abbreviations

**AFO**, active forms of oxygen; **DNA**, deoxyribonucleic acid; **GST**, glutathione S-transferase; **GPO**, glutathione peroxidase; **GR**, glutathione reductase; **OS**, oxidative stress.

### Introduction

Genetic factors cause 30%–50% of cases of male infertility in different forms.<sup>(1-3)</sup> The development of molecular biology, biotechnology and gene engineering evoked significant progress in the research mechanisms to control gene expression, which are involved in physiological and pathological processes. Studies aimed at a search for

associations between peculiarities of gene polymorphisms and different forms of reproductive function disorder are becoming more relevant.<sup>(4-6)</sup> Much attention is given to study of the polymorphic variants of the “susceptibility” genes, which, in contrast to mutations, are not evident in the phenotype, but they are not always neutral and often lead to an appearance of metabolic products with modified physical and chemical features and parameters of functional activity.<sup>(7)</sup> Genetic polymorphisms of biotransformation enzymes determine the intensity of the accumulation of genotoxic metabolites in the cells, participating in DNA damage and control of the enzymes, which detoxify free radicals and enable homeostasis in cells and tissues. Susceptibility of the organism to adverse

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effects of the environment is highly dependent on the activity of the xenobiotic detoxification enzymes.<sup>(8,9)</sup> Depending on genetically determined peculiarities of xenobiotic biotransformation, its interaction with receptors and enzyme systems, detoxification enzymes define the body's reaction to various toxic substances and pharmaceuticals.<sup>(10-14)</sup>

The glutathione S-transferase (GST) genes (*GSTM1*, *GSTT1*, and *GSTP1*) are involved in the detoxification of a broad range of toxic substances. Polymorphisms in GST genes can affect the expression levels of the GST enzymes. Since GST enzymes play a vital role in cellular defense against environmentally toxic compounds, polymorphisms of GST genes can increase susceptibility to diseases caused by such xenobiotics. *GSTM1* and *GSTT1* genes show deletion polymorphism.<sup>(15)</sup> These homozygous gene deletions, called null genotypes, are denoted as *GSTM1*\*0/\*0 and *GSTT1*\*0/\*0. The percentage of individuals who do not express the *GSTM1* enzyme due to a homozygous gene deletion is higher in Caucasians and Asians than in Africans.<sup>(16-18)</sup> About 60% of Asians, 40% of Africans and 20% of Caucasians do not express the *GSTT1* enzyme.<sup>(19)</sup> The *GSTP1* gene has polymorphism loci within its coding region: *GSTP1 Ile105Val* polymorphism in exon 5 and *GSTP1 Ala114Val* polymorphism in exon 6.<sup>(20,21)</sup> Polymorphisms within *GSTP1* are also associated with alterations in enzyme activity.

The aim of this research was to investigate the glutathione system components and their association with polymorphisms GST genes in men with infertility.

## Materials and Methods

One hundred and sixty Russian men of reproductive age (Caucasians) who came to the public health institution Republican Perinatal Center in Ulan-Ude with an infertility problem of one year and more after marriage were included in the main group. The control group included 104 men with proven fertility. All men had a laboratory and clinical examination by an andrologist, including an ultrasonic scan of scrotum and prostate. Macroscopic and microscopic examination of ejaculate was performed in accordance with with the WHO recommendations (2010). The study was conducted in accordance with ethical principles of the Declaration of Helsinki (2000) and approved by the Republican Perinatal Center (Ulan-Ude) Ethics Committee. Written informed consent was obtained from all participants.

Patients with the genetic causes of infertility were excluded from the research (AZF-deletions, CFTR-mutations, mutational changes of the number of CAG repeats, controlled by androgen receptors).

Concentration of the reduced (GSH) and oxidized (GSSG) glutathione was determined by the method of P. Hissin.<sup>(22)</sup> The activity of GST, GPO and GR was determined using Randox reagents.<sup>(23-25)</sup> The concentration of conjugates during the reaction was registered spectrophotometrically with a wavelength of 340 nm using a Shimadzu RF-1501 spectrofluorophotometer.

DNA samples were genotyped for polymorphisms in *GSTP1*, *GSTT1* and *GSTM1* genes. DNA was isolated from

venous blood samples using the sorbent method with the certified reagent kit DNA-Sorb-B (Central Research Institute of Epidemiology, Moscow, Russia). Genetic polymorphism of insertion/deletion (I/D) in the *GSTT1* and *GSTM1* genes was determined by PCR in the automatic thermocycler Tercyc using the reagent kit of Central Research Institute of Epidemiology (Moscow, Russia).

Deletion status of *GSTM1* and *GSTT1* was simultaneously determined by a multiplex polymerase chain reaction method.<sup>(26)</sup> To determine the genotypes at codon 105 and 114, respectively, the exon 5 and exon 6 of the *GSTP1* gene were amplified using the relevant primers. Amplification products were detected in 3% agarose gel; the electrophoresis results were registered and documented with the help of the system of computer gel documentation GelDoc. *GSTM1*+, *GSTT1*+ (wild type) and *GSTM1*\*0 and *GSTT1*\*0 homozygotes (null genotype) were analyzed.

The statistical analysis was performed using the statistical software STATISTICA 6.1 (StatSoft Inc., USA). Intergenic interaction of the polymorphic variants of the examined genes was estimated with the help of the bioinformatic method of multifactor modelling of genomic interactions – multifactor dimensionality reduction (MDR) in the open access program MDR 3.0.2. The MDR-method allows assessment simultaneously affecting the disease interaction of all examined alleles of the gene polymorphic variant, and decreases the dimension of the number of calculated parameters on the basis of creation of new variables, with assessment of how a combination of genotypes impacts the risk of developing disease.

## Results and Discussion

We conducted a comprehensive search using the MDR-method with assessment of all possible combinations of the DNA markers and defined crucial models of intergenic interactions (polymorphic locus combination) for men with infertility defined (Fig. 1).



**Fig. 1.** Dendrogram of intergenic interactions of polymorphic locus combination in men with infertility.

As we can see from the dendrogram and Table 1, the individual marker of development of reproductive disorders for Russian men is carriage of the combination of the polymorphisms *GSTT1*(\*0/\*0)+*GSTM1*(\*0/\*0) and *GSTP1*(*Ile105Val*)+*GSTP1*(*Ala114Val*).

Established synergic interactions of the polymorphic variants of xenobiotic detoxification genes lead to complete absence of the relevant protein or to appearance of enzymes with modified, usually lower, levels of activity. Taking

into account these data, we performed an analysis of the functionality of the glutathione system in men with infertility.

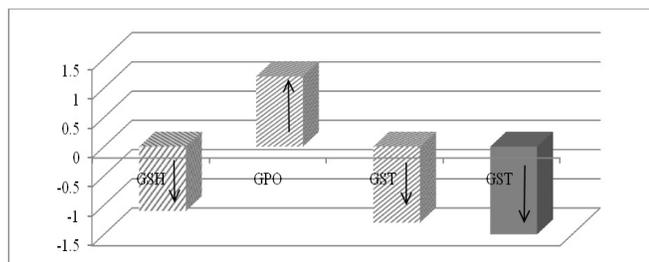
**Table 1.**

**Entropy value of polymorphic variants of xenobiotics detoxification system genes in various combinations in men with infertility**

Polymorphic variants of genes	Caucasians (n=164)
GSTT1(*0/*0)+GSTM1(*0/*0)	0.70 %
GSTP1(Ala114Val)+GSTM1(*0/*0)	0.69%
GSTP1(Ile105Val)+GSTP1(Ala114Val)	1.06%
GSTP1(Ile105Val)+GSTT1(*0/*0)	0.22%

Activities of the antioxidant enzymes are balanced and closely connected to each other.<sup>(19)</sup> Disproportion in the enzyme components of the antioxidant defense may lead to additional generation of AFO and be one of the causes of OS.<sup>(20)</sup> Not only do GST enzymes catalyze glutathione accession to the electrophilic center of various chemical compounds, making them less toxic, but also have some peroxidase activity, which plays an important role in intracellular fixation and transportation of a large amount of both endogenous and exogenous compounds.

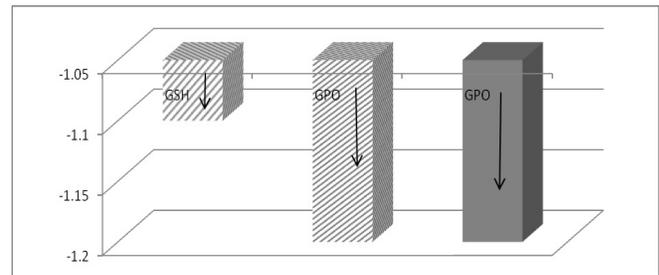
According to our results, in the main group, those who carried a combination of the null genotypes *GSTT1(\*0/\*0)+GSTM1(\*0/\*0)* demonstrated a statistically significant decrease in serum levels of GSH ( $P=0.003$ ) and GST ( $P=0.004$ ), an increase in serum level of GPO ( $P=0.04$ ), and a decrease in GST level ( $P=0.01$ ) in the ejaculate (Fig. 2).



**Fig. 2.** Changes in levels of GST enzymes in the main group, those who carried a combination of the null genotypes *GSTT1(\*0/\*0)+GSTM1(\*0/\*0)* ( $P<0.05$ ). serum ejaculate

Reduction in hydroperoxides content by GPO and GST activity prevents the progression of peroxidation and the appearance of its secondary metabolites. GSTs are an abundant family of dimeric proteins, which have the capacity to conjugate glutathione (GSH) with a variety of electrophilic compounds, primarily produced from exogenous xenobiotics by biotransformation but which can also arise from endogenous substances.

In the main group, those who carried a combination of polymorphisms *GSTP1(Ile105Val)+GSTP1(Ala114Val)* demonstrated a statistically significant decrease in serum levels of GSH and GPO ( $P=0.04$ ) and a decrease in GPO level ( $P=0.02$ ) in the ejaculate (Fig. 3).



**Fig. 3.** Changes in levels of GST enzymes in the main group, those who carried a combination of *GSTP1(Ile105Val)+GSTP1(Ala114Val)* ( $P<0.05$ ). serum ejaculate

The discovered changes of the components of the glutathione system in males with infertility characterize the functional load augmentation in antioxidant systems. GSTs cannot neutralize the toxic impact of various hydrophobic and electrophilic compounds because of their broad substrate specificity and involvement in the metabolism of many endogenous and exogenous electrophilic compounds by conjugation with glutathione.

Two main blood enzymes of the glutathione system associated both with carriage of the combination of the null genotypes *GSTT1(\*0/\*0)+GSTM1(\*0/\*0)* and *GSTP1(Ile105Val)+GSTP1(Ala114Val)* polymorphisms were reduced, but in the ejaculate only one enzyme encoding the mentioned polymorphisms was reduced, which can be considered as a compensatory ejaculate reaction to the impact of damaging factors.

## Conclusion

In human testis, reactive oxygen species are involved in the pathogenesis of male reproductive processes by inducing OS that can damage male germ cellular lipids, protein, and DNA.<sup>(8,10,11,27,28)</sup> Antioxidants, such as *GSTM1* and *GSTT1*, can reduce the toxic effects of OS on male germ lines, suggesting that these two antioxidants genes may play a protective role against OS in spermatogenesis.<sup>(12)</sup> Thus, the deletion polymorphisms of *GSTM1* and *GSTT1* are considered as candidates for genetic susceptibility factors for male infertility. The results showed that the null genotype of *GSTM1* is associated with male infertility, especially in Caucasians and Chinese, indicating that the null genotype of *GSTM1* could increase the risk of male infertility.<sup>(11,12)</sup>

The interaction between genetic factors (some gene mutations) and the environmental factors (xenobiotics) may play a role in the impaired spermatogenesis and affect the male reproductive function.<sup>(29,30)</sup> The intake of xenobiotics is different due to diet and pollution conditions, which may result in the different effect of the null genotype of *GSTT1* on male infertility among populations.<sup>(12)</sup>

Genetically determined imbalance in the system of the glutathione-dependent antioxidant defense determines lipid peroxidation activation and facilitates a significant weakening of metabolic and detoxifying functions of the

organism. As a result, the susceptibility of cells to operations with xenobiotics' harmful influence is significantly increasing, adversely affecting spermatogenesis.

In our study, the most informative genetic and metabolic indicators in Caucasian males with infertility were combinations of the null genotypes *GSTT1*(\*0/\*0)+*GSTM1*(\*0/\*0) associated with a decrease of GST activity in blood and ejaculate and an increase of GSH and GPO in the blood. Another combination is *GSTP1*(Ile105Val)+*GSTP1*(Ala114Val), which is associated with suppression of the blood and ejaculate GPO activity and a decrease in blood concentration of GSH.

Genetically determined peculiarities in how the xenobiotic biotransformation system functions make each individual unique with regard to their adaptive capacity - resistance or sensitivity to the damaging exo- and endogenous factors. Identification of carriage of the polymorphic variants of the *GSTT1* and *GSTM1*, as well as determination of the enzymes of the thiol-disulfide system, can be recommended for additional estimation of the risk of developing a disorder of the reproductive functions in males.

## Competing interests

The authors declare that they have no competing interests.

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## Anthropometric Characteristics of Young Women in Yakutia, Depending on the Type of Sexual Dimorphism and Ethnos

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### Abstract

The aim of this study was to assess the anthropometric data of girls and young women in Yakutia, depending on the type of sexual dimorphism and ethnos. The study included 2,081 girls and young women aged from 16 to 20 years. All those examined were born and permanently resided in the territory of the Republic of Sakha (Yakutia); among them there were 1284 Yakuts and 797 Russians. Anthropometric measurements were carried out according to the method of V.V. Bunak (1941), adopted in the Institute of Anthropology of MSU. Absolute values of the main components of the body (the total amount of fat, muscle tissue, bone tissue) were calculated by the formula Matiegka (1921). The body type was determined in accordance with the Tanner index. Statistical analysis was performed using statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL) The data obtained, depending on the type of sexual dimorphism, indicate the different maturation rates of the morphofunctional structures in Yakut and Russian women in the extreme natural climatic conditions of Yakutia. (**International Journal of Biomedicine. 2017;7(3):231-235.**)

**Key Words:** somatotype • human physique • Tanner index • Yakutia

### Introduction

The history of classification and analysis of the human physique can be traced back to ancient times. Hippocrates, a great Greek philosopher and physician of the fifth century BC, described two different types of bodies. The first he called *habitus phthisicus*, which was long and thin, and dominated by a vertical dimension; the second he called *habitus apoplecticus*, whose main physical characteristic was a short, thick body that was strong in the horizontal dimension. While the former were assumed to have a greater susceptibility to tuberculosis, the latter were very much prone to the diseases of the cardiovascular system. In the 1940s, the famous psychologist William Sheldon proposed the word “somatotype” to describe a body-type using his method of classifying the human physique.<sup>(1)</sup> He claimed that there are 3 such somatotypes: endomorphy, mesomorphy, and ectomorphy. Most people are unique combinations of these three body types. Sheldon stands out as the pivotal

researcher in this field even though his method is not widely used. In biomedical practice, there are many methods of somatotyping, among which the method of J.Tanner is most suitable for determining the type of physique of women.<sup>(2)</sup> Sexual dimorphism can be characterized as gynecomorphic, mesomorphic and andromorphic somatotypes.

Preserving the health of young women, as future mothers, is one of the priorities of modern medicine. According to a number of authors, the adolescent period in the ontogenetic cycle of a person is characterized by the stability of physiological parameters and the development of the reproductive system.<sup>(3,4)</sup> At the same time, there are some studies devoted to the ethno-territorial features in the formation of the morphofunctional status of human. Researchers note the different rates of physical development and reproductive system development among residents of different regions and different ethnic groups.<sup>(5,6)</sup>

Currently, a number of studies are being carried out in Yakutia aimed at studying the population from the standpoint of biomedical anthropology.<sup>(7,8)</sup> However, the morphofunctional features of girls of different ethnic groups living in the extreme climatic conditions of Yakutia have not been studied enough. That lack determined the relevance of this study.

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The aim of this study was to assess the anthropometric data of girls and young women in Yakutia, depending on the type of sexual dimorphism and ethnoses.

## Materials and Methods

The study included 2,081 girls and young women aged from 16 to 20 years. All those examined were born and permanently resided in the territory of the Republic of Sakha (Yakutia) (RS(Y)); among them there were 1284 Yakuts and 797 Russians.

In accordance with the age period of human ontogeny, the examined persons belonged to the youthful period of human development (16-20 years). The examination was not conducted during pregnancy, or in the presence of acute diseases and exacerbation of chronic diseases at the time of examination. Ethnicity of the study participants was determined based on personal data. The examined persons were full-time and part-time students of higher and secondary special educational institutions.

Anthropometric measurements were carried out according to the method of V.V. Bunak, adopted in the Institute of Anthropology of MSU.<sup>(9)</sup> Body length (BL) was measured using a Martin metal anthropometer with an accuracy of 0.1 cm. Body weight (BW) was measured without clothing using medical scales with an accuracy of 50 g. The skinfold thickness was measured using the Holtain Tanner/Whitehouse Skinfold Caliper (United Kingdom) with a contact surface area of 90 mm<sup>2</sup> and a pressure of 10 g/mm<sup>2</sup> with an accuracy of 1 mm. In addition, we measured the skin fat folds of the shoulder (the front and back surfaces), forearm, back, chest, abdomen, hip and lower leg. The circumference dimensions of shoulder, forearm, wrist, hip, lower leg, thorax, buttocks and girth over the ankles were determined using centimetric tape. The diameters of the shoulders and the pelvis were measured with a large caliber compass; the distal diameters of the shoulder, wrist, hip, and ankle with a sliding compass. The accuracy of the instruments used was verified after every 100 measurements, using a special calibration block.

The body surface area was calculated by Isaacson's method.<sup>(10)</sup> Absolute values of the main components of the body (the total amount of fat, muscle tissue, bone tissue) were calculated by the formula Matiegka (1921).<sup>(11)</sup>

The total amount of fat was determined by the formula:

$$D = d \times S \times k, \text{ where}$$

D - Total amount of fat (kg)

d - Average skin and subcutaneous adipose layer thickness (mm), S - body surface (cm<sup>2</sup>),

k - Constant equal to 0.13

The average skin and subcutaneous adipose layer thickness was calculated according to the formula:

$$d = \frac{d_1 + d_2 + d_3 + d_4 + d_5 + d_6 + d_7 + d_8}{16}, \text{ where}$$

d1- d8 - thickness of the skin fat folds of the shoulder (the front and back surfaces) forearm, back, abdomen, hip, lower leg and chest (mm).

The amount of muscle tissue was determined by the formula:

$$M = L \times r^2 \times k, \text{ where}$$

M - Absolute mass of muscle tissue (kg)

L- Body length (cm)

r- Average value of the circumference dimensions of the shoulder, forearm, hip, lower leg without skin and subcutaneous adipose layer thickness (cm);

$$r = \frac{\sum \text{girths (shoulder, forearm, hip, lower leg)} / 25.12 - \sum \text{skin and subcutaneous adipose layer thickness (shoulder, forearm, hip, lower leg)} / 100}{k}$$

k- Constant equal to 0.5

The absolute mass of bone tissue was calculated by the formula:

$$Q = L \times O^2 \times k, \text{ where}$$

Q - Absolute mass of the skin tissue (kg)

L - Body length (cm), O<sup>2</sup> - square of the average value of the distal diameters of the shoulder, forearm, hip, and lower leg

k - Constant equal to 1.2

To compare the relative values of the body components, the values of the components as a percentage of the body weight were determined.

The body type was determined in accordance with the Tanner index (sexual dimorphism index, ISD),<sup>(12)</sup> calculated by the formula:

$$ISD = 3 \times BAD - IPD, \text{ where}$$

BAD - bisacromial diameter (shoulder width), cm;

IPD - Intercrestal pelvic diameter, cm.

The values of ISD < 73.1 characterized the gynomorphic body type; ISD 73.1-82.1 the mesomorphic body type; and ISD > 82.1 the andromorphic body type.

The study protocol was reviewed and approved by the Ethics Committee of Yakut Research Centre for Complex Medical Problems of the Siberian Branch of the RAS. Written informed consent was obtained from each patient

Statistical analysis was performed using statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL). Representativeness of the sample size was determined by the formula:<sup>(13)</sup>

$$N = z^2 \times p(1-p) / e^2, \text{ where}$$

z - a 95% confidence Level (as a z-score)

p - percentage Value (as a decimal)

e - margin of error (as a decimal) ±5%

N - population size

Variables were presented as median and interquartile ranges (IQR). The Kruskal Wallis test was used to compare medians among comparison groups. Categorical variables were analyzed using the Chi-square test with the Yates' correction. A probability value of  $P < 0.05$  was considered statistically significant.

## Results and Discussion

BL and body weight BW of the examined girls and young women of the Yakut and Russian nationality were

Yakut, 159.5 cm [156.2;163.5] and 52.0 kg [48.0;57.0]; and Russian, 163.6 cm [157.0;166.8] and 56.0 kg [50.0;60.0].

Among the Yakut women, we found the gynomorphic type in 19.3% of cases, the mesomorphic type in 72.7% of cases, and the andromorphic type in 8.0% of cases; among Russian women: 16.0%, 70.5%, and 3.5%, respectively (Tables 1 and 2).

In this period of human age, according to a number of scientists, there are no longer any age-related body transformations.<sup>(3,4)</sup> At the same time, there are scientific data about the possible immaturity of morphological structures and the unfinished processes of constitution formation in adolescent girls.<sup>(14)</sup> Studies of anthropometric indices and rates of development of secondary sexual characteristics of Yakut girls testify to later terms of sexual and physical development in comparison with the average indicators of other regions of Russia.<sup>(6,15)</sup>

Thus, BL and BW of Yakut women were significantly ( $P<0.001$ ) less than those of Russian women in Yakutia, which is consistent with the conclusions of other researchers on the smaller dimensions of representatives of Mongoloid ethnic groups in comparison to Caucasians.<sup>(6,17)</sup>

An analysis of the distribution of body types according to the Tanner index revealed that mesomorphic body type dominates (72.7% in Yakuts and 70.5% in Russians) in both ethnic groups. The proportion of mesomorphic and gynomorphic types, depending on the ethnos, did not differ significantly. The extreme variant of sexual dimorphism in the form of andromorphy was smaller in both ethnic groups, but it was more often found among Russian women than among Yakut women ( $\chi^2=13.313$ ,  $P<0.01$ ).

A similar distribution of body types was observed among young women in other regions of Russia: Caucasian women of the Baikal region (Irkutsk)<sup>(18)</sup> and women of Khakassia.<sup>(5)</sup> At the same time, among students of the Voronezh State Medical Academy named after N.N. Burdenko, the gynomorphic and mesomorphic types were determined in most cases with the same frequency.<sup>(14)</sup>

Our study revealed that the parameters of overall dimensions, shoulder and pelvis diameters, and absolute and relative parameters of the body components of Yakut women of different body types did not differ significantly. The obtained data testify to later physical and sexual development among Mongoloid women.<sup>(5,8)</sup>

**Table 1.**

**Anthropometric data of the Yakut girls and young women by Tanner index**

Variable	Gynomorphic body type Me [LQ; UQ] (n=248)	Mesomorphic body type Me [LQ; UQ] (n=933)	Andromorphic body type Me [LQ; UQ] (n=103)
	1	2	3
Body length, cm	160.0 [155.4; 164.0]	159.4 [156.4; 163.2]	158.0 [155.2; 164.6]
	$H=0.434$ ; $P=0.805$		
Body weight, kg	52.0 [48.0; 56.5]	52.0 [48.0; 57.0]	50.0 [48.0; 56.0]
	$H=1.154$ ; $P=0.562$		
Shoulder diameter, cm	34.0 [33.2; 34.5]	34.8 [33.5; 35.5]	35.0 [33.5; 36.0]
	$H=1.240$ ; $P=0.538$		
Intercristal pelvic diameter, cm	27.0 [25.4; 28.0]	27.0 [26.0; 28.0]	27.0 [26.0; 28.0]
	$H=1.895$ ; $P=0.388$		
Body components			
Absolute value of fat mass, kg	14.46 [11.85; 16.70]	14.37 [11.84; 16.89]	14.60 [11.63; 18.40]
	$H=0.472$ ; $P=0.790$		
Body fat percentage, %	27.49 [24.13; 30.46]	27.43 [24.00; 30.82]	28.86 [24.80; 32.04]
	$H=3.199$ ; $P=0.202$		
Absolute mass of muscle tissue, kg	20.44 [18.08; 22.47]	20.46 [18.69; 23.20]	20.90 [19.06; 23.35]
	$H=2.955$ ; $P=0.228$		
Muscle tissue percentage, %	38.72 [35.11; 42.15]	39.51 [36.99; 42.20]	41.43 [38.16; 42.70]
	$H=5.194$ ; $P=0.074$		
Absolute value of bone mass, kg	7.83 [7.09; 8.83]	7.95 [7.23; 8.76]	8.04 [7.43; 8.76]
	$H=2.019$ ; $P=0.364$		
Bone mass percentage, %	15.00 [13.62; 16.60]	15.50 [14.00; 16.62]	15.80 [14.50; 16.70]
	$H=5.273$ ; $P=0.072$		

Table 2.

*Anthropometric data of the Russian girls and young women by Tanner index*

Variable	Gynomorphic body type Me [LQ; UQ] (n=127)	Mesomorphic body type Me [LQ; UQ] (n=562)	Andromorphic body type Me [LQ; UQ] (n=108)
	1	2	3
Body length, cm	163.0 [155.5; 164.8]	163.2 [157.0; 166.8]	164.8 [163.1; 170.6]
	$H=27.134; P<0.001$		
Body weight, kg	50.0 [49.0; 62.0]	55.4 [51.0; 59.0]	58.0 [55.0; 61.0]
	$H=28.205; P<0.001$		
Shoulder diameter, cm	32.4 [32.0; 33.0]	35.0 [34.0; 35.0]	37.0 [36.2; 37.5]
	$H=324.206; P<0.001$		
Intercristal pelvic diameter, cm	26.0 [25.5; 27.8]	27.0 [26.0; 27.2]	27.0 [25.2; 29.2]
	$H=5.764; P=0.056$		
Body components			
Absolute value of fat mass, kg	14.36 [9.34; 17.06]	14.10 [11.28; 17.70]	16.21 [12.83; 17.71]
	$H=19.634; P<0.001$		
Body fat percentage, %	25.53 [18.67; 29.08]	25.78 [21.60; 30.74]	27.88 [24.59; 29.48]
	$H=6.873; P=0.032$		
Absolute mass of muscle tissue, kg	21.63 [19.83; 24.02]	23.32 [20.86; 25.11]	24.35 [21.08; 26.10]
	$H=10.700; P=0.010$		
Muscle tissue percentage, %	42.60 [40.14; 46.26]	41.27 [38.64; 42.83]	44.10 [42.83; 47.50]
	$H=19.739; P<0.001$		
Absolute value of bone mass, kg	7.88 [6.71; 9.21]	8.38 [7.45; 9.21]	9.06 [8.57; 9.55]
	$H=39.593; P<0.001$		
Bone mass percentage, %	15.00 [12.34; 17.00]	15.60 [13.10; 17.00]	15.75 [14.80; 16.48]
	$H=7.411; P=0.025$		

The anthropometric indices of Russian women, in contrast to those of Yakut women of the same age group, differed depending on the type of sexual dimorphism, indicating that they had reached the definitive dimensions of the anthropometric parameters of the body. Our data showed that BL and BW were significantly greater in Russian women with the andromorphic type of physique compared to women with the gynomorphic and mesomorphic body types ( $P<0.001$ ). The shoulder diameter in the subjects with andromorphic type was greater than in women with other types of physique.

The presence of features of anthropometric indices that depend on the type of sexual dimorphism has been demonstrated in the works of other authors.<sup>(5,14)</sup> There were statistically significant differences in BW in women with an extreme degree of inversion in the form of andromorphy in the absence of significant differences in BL, compared to women with gynomorphic and mesomorphic body types.

Women with a gynomorphic body type had smaller BW compared to those of other types of sexual dimorphism on the background of statistically insignificant differences in BL. Indicators of the body composition in women with gynomorphy were also significantly smaller.

## Conclusion

The anthropometric study revealed that the overall body sizes and indices of the body composition of Yakut girls and young women of different body types according to ISD did not differ significantly, but in Russians, these indices had significant differences according to the body types. The data obtained, depending on the type of sexual dimorphism, indicate the different maturation rates of the morphofunctional structures in Yakut and Russian women in the extreme natural climatic conditions of Yakutia.

## Competing interests

The authors declare that they have no competing interests.

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## Prevalence of Viral Hepatitis in Pregnant Women in the Republic of Sakha (Yakutia)

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### Abstract

The prevalence of viral hepatitis in pregnant women in the Republic of Sakha (Yakutia) (RS(Y)) for 2007-2016 is presented in this article. (**International Journal of Biomedicine. 2017;7(3):236-239.**)

**Key words:** chronic hepatitis B • chronic hepatitis C • mother-to-child transmission • vaccination

### Abbreviations

**AHB**, acute hepatitis B; **CVH**, chronic viral hepatitis; **CHB**, chronic hepatitis B; **CHC**, chronic hepatitis C; **CHD**, chronic hepatitis D; **HCV**, hepatitis C virus; **HBV**, hepatitis B virus; **MTCT**, mother-to-child transmission

### Introduction

The Republic of Sakha (Yakutia) is a disadvantaged region with a high incidence of viral hepatitis.<sup>(1,2)</sup> In 2016, according to the register of chronic viral hepatitis (CVH), 14,391 cases of CVH, excluding virus carriers of hepatitis B (570 people), were registered in the Republic of Sakha (Yakutia) [RS(Y)]: CHB – 6404, CHC – 6224, CHD – 889, chronic hepatitis of mixed etiology – 821, and unknown etiology – 57, including 544 patients with liver cirrhosis and 59 patients with primary liver cancer. Thus in the overall structure of CVH, HBV infection was dominant, amounting to 44% of case.<sup>(2,3)</sup> HBV infection is the most common form of chronic hepatitis worldwide and a potentially preventable global health problem. The World Health Organization (WHO) estimates more than 2 billion people have been infected with HBV, 360 million people are chronically infected, and 600 000 people die annually from complications of HBV-related liver disease.<sup>(4)</sup>

In endemic regions, perinatal transmission of the virus is of great importance in the spread of HBV infection: 25% of carriers are infected in the perinatal period.<sup>(5-9)</sup> Of the many ways of transmitting infection, adverse outcomes often occur when infections develop through sexual contact and from mother to fetus, which require the development of effective measures to prevent transmission pathways, especially mother-to-child transmission (MTCT). MTCT is responsible for more than one third of chronic HBV infections worldwide. An estimated 15%–40% of persons chronically infected develop HBV-related complications, such as cirrhosis and hepatic carcinoma, and 25% die from these complications. MTCT can occur during pregnancy or during delivery.<sup>(10)</sup>

**The aim** of the study was to investigate the prevalence of viral hepatitis in pregnant women in RS(Y).

### Materials and Methods

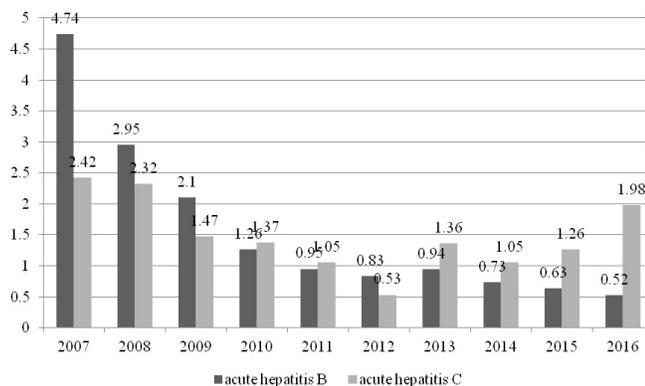
For the retrospective epidemiological analysis, we used the data of the official statistics of RS(Y) and the Center of Hygiene and Epidemiology of RS(Y). To estimate the prevalence of HBV and HCV infections in RS(Y), we used the

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data of the official register, and we analyzed the histories of pregnancy and childbirth at the National Center of Medicine (Yakutsk). A complete range of clinical, laboratory and instrumental, serological, and molecular-biological methods was evaluated.

## Results and Discussion

Our estimation of acute viral hepatitis (B and C) between 2007 and 2016 showed a significant reduction in the incidence of these forms. In 2016, the incidence rate of AHB was 0.52 per 100,000, which was 9.1 times less than in 2007. Since 2012, the incidence of AHC has increased by 1.45%, compared to 2012 (Fig. 1). The reduction in the incidence can be explained by the introduction of the compulsory vaccination against HBV and improvement of a complex of measures aimed at preventing the parenteral transmission of infection in health institutions and institutions of non-medical profile, strengthening the fight against drug abuse, increasing public awareness of how HBV and HCV infection are transmitted and how to prevent infection from these viruses.<sup>(1)</sup>

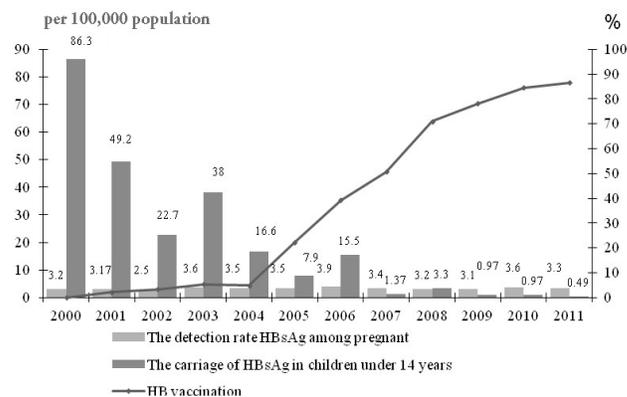


**Fig. 1.** The incidence rate of AHB and AHC in RS(Y) between 2007 and 2016 (per 100,000 population).

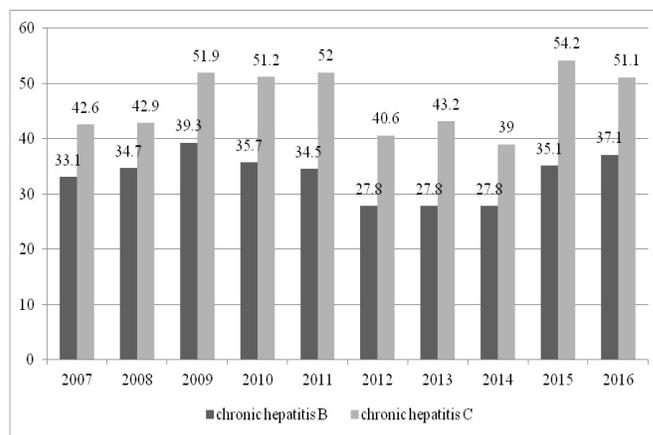
The frequency of pregnant women with HBs antigenemia in the territory of RS(Y) in 2005-2011 ranged from 3.1% to 3.9%; the total of 234,140 samples of sera were examined and HBsAg was identified in 7646(3.27%). Despite this, the absolute number of the seropositive persons with HBV infection increased by 1.9 times from 2005 to 2011 (Fig. 2).

Against the background of the specific immunization, the level of HBsAg carriage among children up to 14 years of age decreased from 86.3 per 100,000 in 2000 to 0.49 per 100,000 in 2011, which demonstrated the effectiveness of vaccination against HBV infection, as evidenced by a very high inverse correlation ( $r=-0.96$ ,  $P<0.001$ ) between the indicators of HBsAg carriage and vaccination coverage.

However, the incidence of CVHs B and C has no tendency to decrease. In the period from 2007 to 2009, the incidence of CHB increased, achieving the maximum level of 39.3 cases per 100,000 population. From 2010 to 2014, the incidence of CHB decreased to 27.8%, but since 2015, the incidence of CHB has again started increasing, up to 37.1% in 2016 (Fig. 3).



**Fig. 2.** The detection rate of HBsAg among pregnant women and the level of HBsAg carriage among children up to 14 years of age against the background of HBV vaccination in RS(Y).



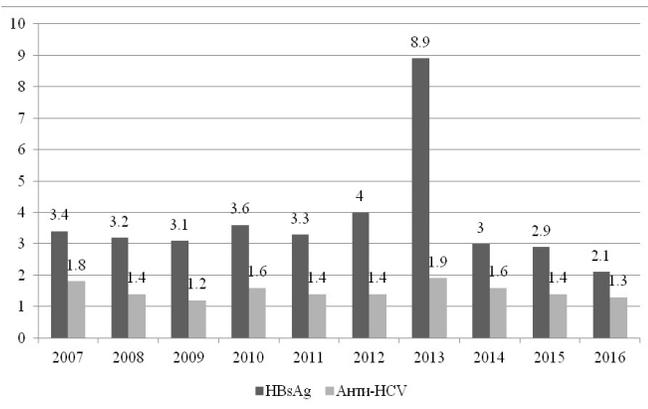
**Fig. 3.** The incidence of CVHs B and C in RS(Y) between 2007-2016 (per 100,000 population).

Thus, the detection of HBsAg among pregnant women in the period 2007 to 2016 ranged from 3.4% to 2.1% (Fig. 4). HBsAg was detected in 16,359(3.9%) out of 422,272 serum samples. The detection rate of antibodies to HCV among pregnant women during this period revealed that 1.3% of women surveyed were anti-HCV positive. Thus, HBsAg among pregnant women was 3.0 times more prevalent than antibodies to hepatitis C.

In the period between 2007 and 2017, the detection of HBsAg in newborns from women with symptomatic HBV infection and women with asymptomatic forms of HBV infection constituted 2.1% on average and fluctuated in different years from 0 to 15%. In the previous period, from 2000 to 2003, an examination of 1832 newborns at high risk for HBV infection found HBsAg in 120(6.5%) children. The decrease in this indicator demonstrates the effectiveness of specific prevention guards; the rate of anti-HCV among infants did not show a trend towards reduction.<sup>(1,11)</sup>

The results of newborn screening for HBV infection has to be compared with the dynamics of the incidence of this

infection in children during the first year of life. Therefore, in RS(Y) among groups of children up to 1 year in the period between 2003 and 2010, there were no cases of acute hepatitis, but HBsAg carriage was identified. In 2011 and 2012, 2 cases of acute hepatitis B (one for each year) were identified in children under 1 year born to mothers with active HBV infection. This fact confirms the need for comprehensive examination of children born to HBsAg-positive mothers, and for an individualized approach to immunization and subsequent longitudinal research data for children up to 1 year.



**Fig. 4.** The detection rate of HBsAg and antibodies to HCV among pregnant women in RS(Y) between 2007 and 2016 (%).

In RS(Y), pregnant women in antenatal clinics undergo an examination for the presence of serum markers of viral hepatitis B and C. Upon detection of HBsAg, all sera are subjected to HBV DNA by PCR, as well as HBsAg and HBeAg quantification by the chemiluminescent immunoassay method. In addition, it is recommended to perform a liver fibroelastometry.

All pregnant women with HBV infection have to be included in the national registry for CVHs, and thereafter, dynamic monitoring has to be performed, with the necessary complex of diagnostic measures and, according to the indications, antiviral therapy has to be performed.

Acute viral hepatitis in pregnant women usually manifests with more pronounced symptoms than in non-pregnant women. According to A.Rakhmanova,<sup>(5)</sup> there were no deaths from HAV infection; all deaths were due to HBV infection. In total, over the past 3 years, maternal mortality due to viral hepatitis has had a tendency to decrease from 1.79% to 0.21%.

All newborns born to women with HBsAg undergo vaccination on an individual scheme according to G. Poland et al.<sup>(12)</sup> According to Global Hepatitis Report (WHO), infants born to mothers who are positive for both HBsAg and HBeAg are at a higher risk of acquiring infection (transmission risk 70%–100% in Asia and 40% in Africa) than those born to HBsAg-positive mothers who have lost the HBeAg (5%–30% in Asia and 5% in Africa).<sup>(13)</sup> Caesarean section reduces the perinatal transmission of HBV infection from HBsAg-positive women to their infants.<sup>(14)</sup>

The basis of prevention of perinatal HBV infection is an active vaccination and passive immunization with hepatitis B immune globulin, which protect newborns from infection in 90%–95% of cases.

Vaccination is the most effective measure to reduce the global incidence of hepatitis B. In 1991, the World Health Organization (WHO) recommended that all countries introduce a policy of universal hepatitis B vaccination to prevent and control HBV infection and its long term sequelae on a global scale. Compared to other healthcare interventions, vaccination is an economically advantageous option, both in terms of cost-effectiveness and benefit-cost ratios.<sup>(15)</sup> WHO recognizes the importance of hepatocellular carcinoma and other HBV-related diseases as global public health problems and reiterates its recommendation that hepatitis B vaccines should be included in national immunization programmes.<sup>(16)</sup>

## Conclusion

Thus, because of active vaccination and passive immunization, there is a trend to reduce the incidence of HBV infection; however, the incidence rate is 2–3 times greater than national data. Thus, our study on the prevalence of parenteral viral hepatitis B and C among pregnant women and newborns in RS(Y) showed that hepatitis B prevalence is still high, indicating a high risk of MTCT of hepatitis B.

Enforced testing for HBsAg of blood donations has to be a universal and important requirement to prevent infections in clinical settings, as well as maintaining asepsis in invasive techniques and vaccination for high-risk groups. Screening pregnant women for HBV infection, providing infant postexposure prophylaxis, and maternal treatment with antiviral medications are strategies for reducing MTCT transmission rates and the global burden of new chronic HBV infections. Administration of hepatitis B immune globulin (HBIG) and hepatitis B (HepB) vaccine within 24 hours of birth, followed by completion of the vaccine series, is a leading way to prevent MTCT.<sup>(16)</sup> Attaining a better understanding of the mechanisms of MTCT and implementing existing policies on maternal screening and infant follow-up are critical for further reductions in MTCT transmission.

## Competing interests

The authors declare that they have no competing interests.

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## Ranking of Administrative Districts of Almaty City by Incidence of Hepatitis B and C

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### Abstract

The incidence of acute and chronic hepatitis B and C in the districts of Almaty city over the past 10 years is presented in this article. (**International Journal of Biomedicine. 2017;7(3):240-242.**)

**Key words:** acute hepatitis B • acute hepatitis C • chronic hepatitis B • chronic hepatitis C • vaccination

### Abbreviations

**AHB**, acute hepatitis B; **AHC**, acute hepatitis C; **CVH**, chronic viral hepatitis; **CHB**, chronic hepatitis B; **CHC**, chronic hepatitis C; **HBV**, hepatitis B virus; **HCV**, hepatitis C virus.; **MYI**, multi-year indicator.

### Introduction

Hepatitis B and C viruses are widely distributed among the population around the world. The World Health Organization (WHO) estimates more than 2 billion people have been infected with HBV, 360 million people are chronically infected, and 600 000 people die annually from complications of HBV-related liver disease.<sup>(1,2)</sup> Globally, an estimated 71 million people have chronic hepatitis C infection. HCV is approximately 10 times more infectious than HIV through percutaneous blood exposures and has been shown to survive for weeks in syringes.<sup>(3-5)</sup> A significant number of those who are chronically infected will develop cirrhosis or liver cancer. Approximately 399 000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma.<sup>(6)</sup> The number of deaths due to hepatitis C is at an all-time high in the US and exceeds those attributable to 60 other infectious diseases including HIV and tuberculosis, according to the Centers for Disease Control and Prevention.<sup>(7)</sup> We analyzed

the incidence of acute and chronic hepatitis B and C in the districts of Almaty city over the past 10 years.

### Materials and Methods

For the retrospective epidemiological analysis, we used the data of the official registration of the Department of Sanitary and Epidemiological Surveillance of Almaty for HBV and HCV in the intensive indicators (hepatitis incidence per 100,000 population) from 2003 to 2014 and the percent (%) indicators by stratifications (age and socio-occupational groups) used with dichotomous variables.

The incidence of individual nosological forms of hepatitis in their total sum was expressed in the extensive (relative) indices. The behavior of the epidemic process was assessed by the annual dynamics of the cumulative incidence of hepatitis B and C.<sup>(8-10)</sup>

### Results and Discussion

Table 1 shows the average incidence rate for AHB in Almaty's adult population at 4-year intervals for 2003-2014: the AHB incidence by city areas steadily decreased

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by 3 or more times. These data conclusively demonstrate the epidemiological effectiveness of HBV vaccination.

According to the indicators of the average multi-year incidence of AHB, the studied districts ranged in the following order (by rank): the Bostandyksky (5.4<sup>0</sup>/<sub>0000</sub>), Auezovsky (4.8<sup>0</sup>/<sub>0000</sub>), Zhetysusky (4.3<sup>0</sup>/<sub>0000</sub>), Almalinsky (4.5<sup>0</sup>/<sub>0000</sub>), Turksibsky (4.1<sup>0</sup>/<sub>0000</sub>), Medeusky (2.8<sup>0</sup>/<sub>0000</sub>), and Alatausky (2.1<sup>0</sup>/<sub>0000</sub>) districts.

**Table 1.**

**The average incidence rate for AHB by the districts of Almaty for 2003-2014**

Districts	MYI at 4-year intervals ( <sup>0</sup> / <sub>0000</sub> )			Rate of decrease	MYI ( <sup>0</sup> / <sub>0000</sub> )
	2003-2006	2007-2010	2011-2014		
Almalinsky	9.3	3.1	1.1	8.4	4.5
Turksibsky	6.6	3.6	2.2	3.0	4.1
Zhetysusky	8.9	2.9	4.15	7.7	4.3
Bostandyksky	9.3	3.5	3.6	2.6	5.4
Medeusky	4.4	3.1	0.92	4.7	2.8
Auezovsky	6.2	6.5	1.9	3.2	4.8
Alatausky	-	4.5	1.6	2.8	2.1

The results of a similar analysis for AHC incidence are shown in Table 2. Thus, during 2003-2014, AHC indicators for districts ranged from 0.25<sup>0</sup>/<sub>0000</sub> to 2.7<sup>0</sup>/<sub>0000</sub>. According to the average multi-year incidence, the Auezovsky district (1.3<sup>0</sup>/<sub>0000</sub>) took the first place. During subsequent years, these indicators were equal to zero in some districts or were very low due to registration of single cases of AHC. Apparently, comparatively more pronounced indicators in 2003-2006 are associated with deficiencies of the specific qualities (sensitivity, specificity, etc.) of the first batches of diagnostic test systems for ELISA.

**Table 2.**

**The average incidence rate for AHC by the districts of Almaty for 2003-2014**

Districts	MYI at 4-year intervals ( <sup>0</sup> / <sub>0000</sub> )			Trend	MYI ( <sup>0</sup> / <sub>0000</sub> )
	2003-2006	2007-2010	2011-2014		
Almalinsky	1.8	0.0	1.3	1.38↓	1.03
Turksibsky	0.25	0.06	0.0	-	0.08
Zhetysusky	0.35	0.17	0.0	-	0.17
Bostandyksky	0.8	0.4	0.0	-	0.4
Medeusky	0.45	0.85	0.2	2.25↓	0.5
Auezovsky	2.7	0.9	0.3	9.0↓	1.3
Alatausky	-	0.5	1.6	0.3↑	0.7

However, it should be noted that unlike with AHB—in which the IgM antibody to the hepatitis B core antigen is diagnostic of acute infection and precedes the appearance of IgG—with HCV infection the IgM antibody responses are variably detected in both acute and chronic phases.<sup>(11)</sup> Anti-HCV IgM cannot therefore serve as a diagnostic marker of acute HCV infection.<sup>(12)</sup>

Thus, the assessment of dynamics of incidence of AHB and AHC showed that the incidence rate for AHB dropped sharply, and isolated cases of AHC were in fact cases of CHC. Based on these considerations, our studies in the regions were focused mainly on the problems of chronic hepatitis C, as the most relevant infection for public health.

The analysis of the incidence of CHB and CHC by the districts of Almaty for 2003-2014 is presented in Tables 3 and 4. During the analyzed period, in the study areas, with the exception of the Medeusky and Auezovsky districts, there was a decrease in the incidence of CHB in the range from 1.2 to 3.6 times. In the Medeusky and Auezovsky districts, we observed that the incidence rate of CHB increased by 1.2 and 1.5 times, respectively.

**Table 3.**

**The average incidence rate for CHB by the districts of Almaty for 2003-2014**

Districts	MYI at 4-year intervals ( <sup>0</sup> / <sub>0000</sub> )			Trend	MYI ( <sup>0</sup> / <sub>0000</sub> )
	2003-2006	2007-2010	2011-2014		
Almalinsky	6.4	4.2	3.9	1.6↓	4.8
Turksibsky	2.2	2.8	0.6	3.6↓	1.9
Zhetysusky	0.15	2.1	-	-	1.4
Bostandyksky	6.2	6.1	5.7	1.2↓	6.0
Medeusky	6.2	6.4	7.2	1.2↑	6.6
Auezovsky	5.8	8.7	12.5	2.0↑	9.0
Alatausky	-	3.7	1.9	1.9↓	1.8

**Table 4.**

**The average incidence rate for CHC by the districts of Almaty for 2003-2014**

Districts	Multi-year indicators at 4-year intervals ( <sup>0</sup> / <sub>0000</sub> )			Trend	MYI ( <sup>0</sup> / <sub>0000</sub> )
	2003-2006	2007-2010	2011-2014		
Almalinsky	3.9	3.1	4.1	1.05↑	3.7
Turksibsky	2.5	1.4	2.9	1.3↑	2.3
Zhetysusky	2.1	0.2	-	-	1.4
Bostandyksky	8.9	9.5	9.9	1.1↑	9.4
Medeusky	3.5	1.3	11.5	3.3↑	5.4
Auezovsky	7.9	13.1	21.3	2.6↑	11.6
Alatausky	-	1.3	1.9	1.4↑	1.05

Thus, CHC was registered in all districts of the city. Moreover, during the observed period, there was a tendency for the CHC incidence to grow from 1.1 to 3.3 times. The most noticeable growth was observed in the Medeusky (3.3 times) and Auezovsky (2.6 times) districts.

The analyses of the multi-year incidences of CHB and CHC, with rank positions for districts, are presented in Tables 5 and 6. In the ranking of districts with a decrease in the multi-year incidence of CHB and CHC (per 100 thousand population), the first three ranks were occupied by the same

districts in which the incidence rate for CHB and CHB was the highest. These results may indicate the identity of the mode of transmission for HBV and HCV in these regions.

**Table 5.**

*The 2003-2014 multi-year incidences of CHC with rank positions for districts*

Districts	MYI ( $^{\circ}/_{0000}$ )	Rank number
Auezovskiy	11.6	1
Bostandykskiy	9.4	2
Medeuskiy	5.4	3
Almalinskiy	3.7	4
Turksibskiy	2.3	5
Zhetysuskiy	1.4	6
Alatauskiy	1.05	7

**Table 6.**

*The 2003-2014 multi-year incidences of CHB with rank positions for districts*

Districts	MYI ( $^{\circ}/_{0000}$ )	Rank number
Auezovskiy	9.6	1
Medeuskiy	6.6	2
Bostandykskiy	6.0	3
Almalinskiy	4.8	4
Turksibskiy	1.9	5
Alatauskiy	1.8	6
Zhetysuskiy	1.4	7

## Conclusion

Thus, as a whole, we observed a tendency toward a gradual decrease in the incidence of CHB in the districts of Almaty city. Assessment of the hepatitis C incidence by the cumulative indices reflects the disturbing epidemiological situation for this disease that requires the effective and quality monitoring of hepatitis C.

## Competing interests

The authors declare that they have no competing interests.

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# Complex Sanitary and Hygienic Characteristics of the Quality of the Megacity Environment

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## Abstract

The complex assessment of habitat factors that affect the level of sanitary-epidemiological well-being and health status of the population of Almaty city over the past 10 years is presented in this article. (**International Journal of Biomedicine**. 2017;7(3):243-247.)

**Key Words:** air quality • atmospheric pollution • maximum permissible concentration • harmful emissions

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## Abbreviations

**API**, air pollution index; **BOD**, biochemical oxygen demand; **IAP**, index of atmospheric pollution; **MPC**, maximum permissible concentration; **MPCad**, average daily MPC; **MR**, maximum repeatability; **SI**, standard index; **SP**, suspended particulates; **TPP**, thermal power plant.

## Introduction

One of the most important scientific problems of our time, which is both theoretical, fundamental and applied, and lies at the intersection of a number of branches of science and practice, is the question of the possibility of and mechanisms for regulating the level of public health. One of these mechanisms, of course, is the improvement of the quality of the habitat. Ecological and hygienic problems associated with the intensive growth of cities and urban populations are among the significant phenomena of modern times. In large industrial cities, where production facilities of various profiles are concentrated, significant amounts of various chemical substances are simultaneously emitted into the environment, among which is a high proportion of highly toxic compounds of toxicity classes 1 and 2.<sup>(1)</sup> The rapid growth of motor vehicles in megacities causes additional environmental risks to public health.<sup>(2-5)</sup> This problem is the most difficult in a

modern metropolitan area, including territories with different levels and patterns of industrialization and varying population density, significantly differing in architectural and planning characteristics, transport load, remoteness from green areas, etc.<sup>(6)</sup> Almaty city can serve as a typical example of such a metropolis. To address the issue of prioritizing activities aimed at minimizing environmental risks in large urban agglomerations, it is necessary to identify all major sources of environmental pollution, including sources in the adjacent areas, in connection with the possibility of spatial distribution of pollution.

The aim of our study was to conduct a complex assessment of habitat factors that affect the level of sanitary-epidemiological well-being and health status of the population of the megalopolis.

## Material and Methods

In accordance with the purpose of the work, we carried out hygienic studies of the state of the habitat of Almaty in general and in the administrative districts of the city. Almaty is currently a major administrative center; its territory is divided

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into 8 districts: the Alatausky, Almalinsky, Auezovsky, Bostandyksky, Medeusky, Zhetysusky, Nauryzbaysky and Turksibsky districts.

In the course of the scientific and technical program for the study, all districts of the city were selected, with the exception of the Nauryzbaysky district, since this district was included in the city boundaries only in July 2014.

To adequately assess the air quality in Almaty in general and in the city's territorial-administrative districts, the results of instrumental measurements of the leading atmospheric pollution for the period 2007-2016 were analyzed. The sources of the study were:

- Annual report form 8
- Annual statistical collections "Environmental Protection in Almaty city for 2007-2010 and 2011-2015"
- Information bulletins on the environmental state of the Republic of Kazakhstan for 2010-2016
- Monitoring data of the Republican State Enterprise "Kazhydromet"

Analysis of the state of atmospheric air pollution included qualitative and quantitative data on emissions of harmful substances from various types of sources (stationary and mobile). We calculated the average annual, average daily and maximum single concentrations of priority pollutants at the sampling points. The level of atmospheric pollution was estimated according to complex index,  $IAP_5$ , which is based on the level of 5 atmospheric pollutants with the highest normalized values for MPC, taking into account their hazard class: sulfur dioxide ( $SO_2$ ), nitrogen dioxide ( $NO_2$ ), carbon monoxide (CO), phenol, and formaldehyde.

## Results and Discussion

In recent years, deteriorating air basin quality in Almaty city has become a serious threat to human health. The city's atmosphere is polluted by emissions from various types of economic activities, including industry and transport.

For the period 2007-2016, the number of stationary sources (industrial enterprises, individual residential sector and TPP-2), which have harmful emissions, decreased by 22.4%. Their share in the total annual emissions of pollutants is 19.8%-21.6% of all stationary and mobile sources.

Consequently, the leading source of pollution is the exhaust gases of motor vehicles, the specific level of which is up to 80% of all emissions into the city air. The situation is aggravated by the large share of old cars with a service life of more than 10 years, the technical condition of which in most cases does not meet hygienic standards. The growth in the level of air pollutants is facilitated by the use of low-quality fuels and lubricants when servicing motor vehicles. Transport pollution of the atmosphere, according to its effects on the human body, is divided into toxic, carcinogenic and irritating. Class I air pollutants include CO, oxides of nitrogen, oxides of sulfur, hydrocarbons and lead. Benzo[a]pyrene (BaP), di- and trichloromethane, benzene, acetaldehyde and formaldehyde are carcinogens. Because of emissions at the level of breathing, these substances are much more dangerous than industrial and energy toxicants, which are dispersed by high smokestacks for considerable distances. Oxides of sulfur and hydrocarbons are irritants. The degree of influence on the human body of all of the above components of the exhaust gases of road transport depends on their concentration in the atmosphere and the duration of exposure.

All of the above toxicants are present in the atmosphere of the metropolis. Thus, the main air pollutants in Almaty, which determine the greatest contribution to environmental damage and health risk to the population, are  $NO_2$ ,  $SO_2$ , CO, formaldehyde, BaP, lead and SP (dust, soot), average annual concentrations of which in the surface layer of the atmosphere exceed the MAC by 2-5 or more times, especially under unfavorable meteorological conditions. In the cold season of the year (the first quarter and the end of the fourth quarter), the level of all priority pollutants reaches its seasonal maximum. In January, the average monthly concentration of  $NO_2$  reaches 5.1 MPC, CO - 3.4 MPC, SP - 1.2 MPC, and formaldehyde - 2.9 MPC (Table 1, Fig. 1).

The dynamics of the average monthly levels of atmospheric pollution in Almaty city (the frequency of exceeding MPCad) for 2013-2015 is shown in Fig. 1. In accordance with the data in Table 1, a high concentration of formaldehyde persisted in the second quarter; only in the hottest months of summer did it decrease, by 2.2 times. A similar pattern was observed in all the years of the study period.

**Table 1.**

**The dynamics of the average monthly levels of atmospheric pollution in Almaty city (the frequency of exceeding MPCad) according to "Kazhydromet"**

Variable	2014			2015								
	October	November	December	January	February	March	April	May	June	July	August	September
	Quarter IV			Quarter IV			Quarter II			Quarter III		
SP	0.9	0.8	0.3	1.1	1.2	1.0	0.9	0.9	0.8	1.2	1.3	1.3
$NO_2$	1.4	1.4	2.6	3.1	3.3	3.5	2.7	2.5	2.7	2.4	2.4	2.2
$SO_2$	1.1	0.3	0.4	0.3	0.3	0.4	1.2	0.5	1.4	1.3	1.5	1.0
Formaldehyde	4.2	3.3	4.6	4.6	4.3	4.6	5.2	5.3	5.4	5.7	1.4	1.5
SI (in respect to $NO_2$ )	7.9	5.6	11.8	8.7	9.3	8.7	4.9	4.6	4.8	4.6	4.0	2.9
MR. %	92.3	93.3	98.6	100.0	98.6	100.0	97.4	89.9	97.4	93.6	47.8	22.7

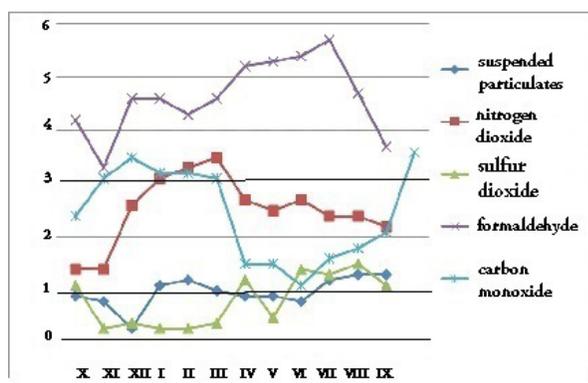


Fig. 1. The dynamics of the average monthly levels of atmospheric pollution in Almaty city (the frequency of exceeding MPC<sub>ad</sub>) for 2013-2015.

Only for 2015, according to the data of the RSE “Kazgidromet” the number of cases of the MPC excess was 5,897 for CO<sub>2</sub>, 2,970 for SO<sub>2</sub>, 589 for NO<sub>2</sub>, and 96 for SPs.

The highest rates were recorded for NO<sub>2</sub>, belonging to the UN Class 2.3 substances—toxic gases. This gas exceeded the maximum one-time MPC by 11.76 times; 30,330 cases of excessive MPC were recorded. In 673 cases, it was more than 5 MPC and in 105 cases more than 10 MPC. According to the automatic observation post in Almaty, only in 2015 were there 105 cases of high air pollution. Indicators of air pollution were as follows: SI - 11.8, MR - 88.8%, which corresponds to very high degree of atmospheric air pollution – the fourth degree (SI>10, MR>50). The average annual IAP<sub>5</sub> in 2014 was 10.0.

The overall assessment of air pollution in 2015 showed a positive trend. In 2015, the atmospheric pollution indicators decreased compared to 2014: IAP<sub>5</sub> - 7.6 vs. 11.8, SI - 8.7 vs. 11.8, and MR - 47.8% vs. 88.8%. Despite this, the indicators characterizing the level of atmospheric pollution in the city are within the limits of a high level of contamination (IAP<sub>5</sub> within 7-13, SI>10, MR within 20%-49%). The air of the city remains the most polluted by nitrogen dioxide. In general, the average concentrations by pollutants were as follows: NO<sub>2</sub> - 2.6 MPC<sub>ad</sub>, formaldehyde - 1.5 MPC<sub>ad</sub>, and SO<sub>2</sub> - 1.2 MPC<sub>ad</sub>. The content of heavy metals and other pollutants did not exceed MPC.

IAP<sub>5</sub> in the metropolitan area for 2002-2015 was characterized by wavy dynamics (Fig. 2). After a rise in the level of atmospheric pollution in 2006-2008, the indicator fell down in 2011, then the growth was again recorded at 1.3 times: from 9.23 to 11.8.

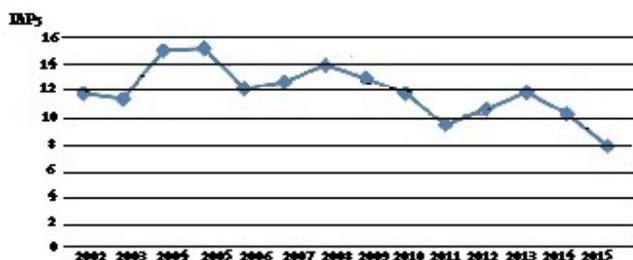


Fig. 2. The dynamics of atmospheric pollution in Almaty city according to IAP<sub>5</sub> for 2002-2015.

In the last three years of the study period, the indicator fell to 7.6 in 2015. On average, for the study period, the level of IAP<sub>5</sub> was 11.52±1.39, which corresponds to the third degree of atmospheric pollution. Although, the IAP<sub>5</sub> level exceeding 14 is considered dangerous for human life, in conditions of weak natural ventilation inherent to Almaty, these indicators reflect a direct negative impact on the health of the population and require urgent solutions.

A significant reduction in air pollution could be achieved by updating the vehicle fleet and more strict exhaust emission control. To solve the problems of optimizing traffic in the city, an important role is played by the construction of transport interchanges. Monitoring of air quality along the motorways after traffic interchanges were commissioned showed a decrease by more than 11% in the number of indicators of harmful substances exceeding MPC. This fact illustrates especially graphically the concentration of nitrogen dioxide in the ambient air in the area of traffic congestion (the intersection of Furmanov and Satpayev streets) and the area of the interchange (Furmanov and Al-Farabi streets) located two blocks to the south. The concentration of nitrogen dioxide in the first case was 8.94 MPC, which is almost 23 times higher than in the interchange area (0.39 MPC).

The territory of a large industrial city, such as Almaty, is not homogeneous in terms of its sanitary-hygienic situation. In this regard, the population living in various regions of the city is experiencing a different intensity of the impact of a variety of negative factors. The reasons for this lie in the varying degrees of the development of industrial production in the regions, the presence of thermal power plants and other sources of pollution, geographical location and the “rose of wind.”

Data on the distribution of the amount of air pollutants from stationary sources in the districts of Almaty for the period 2007-2016 are presented in Table 2. Thus, the highest level of atmospheric pollution, on average, for 2007-2015 was observed in the Zhetysusky district (6516.14±1303.54 tons/year), which amounted to more than half (52.13%) of the average city level.

Table 2.

Rank distribution of Almaty districts by the amount of emissions from stationary sources for the period 2007-2015

Districts	Emission (tons/year)		Share of total urban emissions (%)	
	average level for the period	Rank number	average level for the period	Rank number
Alatausky	227.00±74.44	7	1.81±0.63	7
Almalinsky	683.29±110.23	6	5.46±1.32	6
Auezovsky	1694.71±735.52	3	13.55±6.15	3
Bostandyksky	713.29±104.00	5	5.70±0.68	5
Zhetysusky	6516.14±1303.54	1	52.13±7.11	1
Medeusky	770.43±138.11	4	6.16±1.15	4
Turksibsky	1913.28 ±339.48	2	15.30±1.44	2
Almaty city	12500.14±1104.21	-	100.00	-

According to the level of air toxins emitted from stationary sources, the studied districts ranged in the following order: the Zhetyysusky ( $52.13 \pm 7.11\%$ ), Turksibsky ( $15.30 \pm 1.44\%$ ), Auezovsky ( $13.55 \pm 6.15\%$ ), Medeusky ( $6.16 \pm 1.15\%$ ), Bostandyksky ( $5.70 \pm 0.68\%$ ), Almalinsky ( $5.46 \pm 1.32\%$ ), and Alatausky ( $1.81 \pm 0.63\%$ ) districts. The lowest average indicator was recorded in the Alatausky district with a level of emissions of  $227.0 \pm 74.44$  tons/year (1.81%), which can be explained by the absence of large industrial enterprises in the area.

The main impact on the general air pollution of the Alatausky district, as well as the city as a whole, is caused by the emissions of the thermal power complex (TPP-2) located near the western border of the district. The main fuel used by TPP-2 is Ekibastuz coal with an ash content of 34.4%. With total emissions of 39.5 thousand tons per year, up to 15,642 tons of harmful substances are carried to the city's territory with winds from the north, west and northwest, which exceeds the volume of emissions from all stationary sources of the thermal power and industry enterprises located in the city territory. In addition, a significant part of the private sector that occupies a significant part of the territory of the Alatausky district is not gasified and is a source of solid fuel combustion products, which is confirmed by its exceeding by 1.2-1.6 times the MPC level of CO and by 11.2 times the MPC level of the dust in the air of the district in the cold season. With incomplete combustion and thermal decomposition of fuel hydrocarbons, suspended substances (soot) are formed, which are particles of solid carbon, on the surface of which BaP can be adsorbed. In this case, the deposition of soot particles on airway and alveolar surfaces has an even more negative effect on the body, stimulating the development of malignant neoplasms.

The leading causes of atmospheric air pollution from stationary sources are obsolete technologies of many industries, insufficient quantity and low efficiency of existing dust and gas cleaning installations, disturbances in the technological mode of operation, and the use of low-quality coals in power engineering. The rapid growth of the car fleet in megacities has led to an increase of more than 50% in the amount of harmful impurities in the air and an increase in the noise levels on urban highways by 5-10 dB. One of the negative characteristics of traffic noise is its spread over vast areas and almost constant impact throughout the day. Strong and prolonged noise has a harmful effect on the human body as a whole, causing irritation, deteriorating well-being and speeding up the fatigue growth process. The share of transport in the noise impact on the population of the Auezovsky and Medeusky districts, where large transport routes are located, reaches 80% and is 50-55 dB in the daytime and 40-45 dB at night.

The average values of gamma-ray background of the surface layer of the atmosphere along the metropolitan areas were in the range between 0.10 and 0.31  $\mu\text{Sv/h}$ . On average, the gamma-ray background was 0.16  $\mu\text{Sv/h}$  and was within acceptable limits.

In the territory of Almaty, there are 22 rivers and 4 riverbeds of artificial origin. The total length of riverbeds is

225.8 km. The total area of the water mirror is 1,116 hectares. The largest rivers are the Ulken Almaty (the length of 29 km), the Kishi Almaty (the length of 28 km) and the Esentai River (the length of 25 km). Monitoring of water resource quality at 3 water bodies in Almaty for 2013-2015 showed that no significant changes in the state of these water bodies were recorded during the 3-year period. During the observation period, the water temperature was in the range from 5.1 to 4.2°C; the hydrogen index in all samples taken did not exceed 7.8. The concentration of oxygen dissolved in water ranged from 10.1 mg/dm<sup>3</sup> (the Kishi Almaty) to 11.1 mg/dm<sup>3</sup> (the Esentai River). BOD<sub>5</sub> was 1.5 mg/dm<sup>3</sup> and 1.4 mg/dm<sup>3</sup>, respectively. In the Ulken Almaty, similar indicators were in the range between 1.5 mg/dm<sup>3</sup> and 10.2 mg/dm<sup>3</sup>. Excesses of MPC in water of all three water bodies were recorded for substances from groups of the biogenic substances (nitrogen nitrite - from 1.51 MPC to 1.9 MPC; fluorides did not exceed 1.1 MPC) and heavy metals. The copper level was 1.27-1.50 MPC. The highest levels of copper and manganese were recorded in the Kishi Almaty - 1.77 MPC and 2.3 MPC, respectively. These indicators were lower for the Ulken Almaty (copper - 1.27 MPC, manganese - 1.58 MPC) and the Esentai River (copper - 1.5 MPC, manganese - 1.35 MPC). In Lake Ulken Almaty, the water temperature was in the range from 5.1 to 8.3 °C, the hydrogen index was 8.0; the concentration of dissolved oxygen in the water was 9.3 mg/dm<sup>3</sup> and BOD<sub>5</sub> - 0.4 mg/dm<sup>3</sup>. Substances from the group of heavy metals exceeded MPC (copper - 1.3 MPC). Pollution of water bodies in Almaty by cadmium and zinc was minimal.

Thus, **in summary**, we made following conclusions from our analyses:

- In 2007-2015, the air pollution of the city was characterized as high and very high. During the follow-up period, the mean values of SI, MR and IAP<sub>5</sub> were  $11.5 \pm 0.62$ ,  $88.8 \pm 4.2\%$  and  $11.5 \pm 0.91$ , respectively. In the dynamics of observation, there was a tendency to improve the situation in 2015: IAP<sub>5</sub> decreased by 1.55 times, SI - by 1.36 times and MR - by 1.86 times. Despite this, the studied indicators remained within a high level of contamination.

- Between 2007 and 2014, the number of stationary sources (industrial enterprises, individual residential sector and TPP-2) with harmful emissions decreased by 22.4%. Their share in the total annual volume of emissions of pollutants was 19.8%-21.6% of all stationary and mobile sources. Consequently, the leading sources of pollution are the exhaust gases of motor vehicles, which constitute about 80% of all air pollution.

- The main pollutants of Almaty's air, which determine the largest contribution to environmental damage and health risk to the population, are NO<sub>2</sub>, SO<sub>2</sub>, CO, formaldehyde, BaP, lead, and SP (dust, soot), whose mean annual concentrations in the surface layer of the atmosphere exceed MPC by 2-5 or more times, especially under unfavorable meteorological conditions. In the cold season of the year (the first quarter and the end of the fourth quarter), the level of all priority pollutants reaches its seasonal maximum. In January, the average monthly concentration of NO<sub>2</sub> reaches 5.1 MPC, CO - 3.4 MPC, SP - 1.2 MPC, and formaldehyde - 2.9 MPC. IAP<sub>5</sub> in

the metropolitan area was characterized by wavy dynamics for 2013-2015 with the expressed tendency to a decrease in 2015: 11.8 vs. 7.6 in 2014 with a negative rate of a gain (-) of 35.6%.

- According to the level of air toxins emitted from stationary sources, the studied districts ranged in the following order: the Zhetyysusky ( $52.13 \pm 7.11\%$ ), Turksibsky ( $15.30 \pm 1.44\%$ ), Auezovsky ( $13.55 \pm 6.15\%$ ), Medeusky ( $6.16 \pm 1.15\%$ ), Bostandyksky ( $5.70 \pm 0.68\%$ ), Almalinsky ( $5.46 \pm 1.32\%$ ), and Alatausky ( $1.81 \pm 0.63\%$ ) districts.

- We found a steady increase in emissions of pollutants in the Auezov and Alatau districts with the greatest average daily concentrations of dust,  $\text{NO}_2$ , CO, and lead. Excesses of MPC by 1.2-1.6 times for CO and 11.2 times for dust in the air of the Alatau region testifies to the predominant use of solid fuel in the heat sources (low-quality coal, wood, household waste).

- The share of transport in the noise impact on the population of the Auezovsky and Medeusky districts, where large transport routes are located, reaches 80% and is 50-55 dB in the daytime and 40-45 dB at night.

## Competing interests

The authors declare that they have no competing interests.

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## Psychosomatic Aspects of Chronic Heart Failure

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### Abstract

**The purpose** of this study was to investigate the psychosomatic correlations in CHF patients with anxiety and depressive disorders (DDs).

**Materials and Methods:** This study involved 102 patients (60 men and 42 women) with CHF functional class (FC) II-III (NYHA) in age from 41 to 65 (mean age, 56.3±2.7). 6MWT as a sub-maximal exercise test was used to determine functional capacity in patients. Patients' subjective assessment of dyspnea was conducted using the modified VAS in the form of a 10 cm long straight line. The personality characteristics were studied using the FPI. Depressive disorders were assessed by HAM-A and HAM-D. The average score of DD severity in the general group of patients was 11.36±2.24 points, which corresponds to a moderate depressive episode, according to HAM-D. Data analysis revealed that the degree of depressive disorders in the examined patients was a direct function of CHF severity. The severity of dyspnea was associated not only with the clinical-instrumental characteristics of CHF (LVEF, 6MWT), but also with the personality and the behavioral and psychological peculiarities of the patients: smoking, hypochondriac type of attitude towards the disease, sleep disorders, severity of depression and anxiety.

**Conclusion:** The obtained data demonstrate that mental disorders observed in CHF patients aggravate the severity of the patients' condition and decrease their adaptive capability, thus adversely affecting the course and outcome of the underlying disease. (*International Journal of Biomedicine. 2017;7(3):248-250.*)

**Key Words:** congestive heart failure • depression • anxiety • psychosomatic disorders

### Abbreviations

**6MWT**, the 6-minute walk test; **CHF**, chronic heart failure; **DD**, depressive disorder; **FPI**, the Freiburg Personality Inventory; **HAM-A**, the Hamilton Anxiety Rating Scale; **HAM-D**, the Hamilton Depression Rating Scale; **LVEF**, left ventricular ejection fraction; **QL**, the quality of life; **VAS**, the Visual Analogue Scale.

### Introduction

Chronic heart failure (CHF), being the final stage of most cardiovascular diseases, is a serious healthcare problem due to its high prevalence, progressive course and lower life expectancy.<sup>(1)</sup> Along with CHF, one of the most incapacitating diseases is depression. Epidemiological studies have demonstrated that the prevalence of depression

in patients visiting general practitioners varies from 5% to 57% depending on the assessment methods.<sup>(2,3)</sup> In addition, depression is hard to diagnose because its symptoms – lassitude, fatigability, apathy – are quite prevalent in the population, which impedes adequate treatment. CHF and depression have several common pathophysiological mechanisms: They are associated with activation of the sympathetic nervous system, hypercoagulation, and with enhanced production of pro-inflammatory cytokines, including interleukins (IL) 1, 3, 6, TNF- $\alpha$ .<sup>(4)</sup> The high social importance of the problem of early diagnosis and adequate treatment of psychic pathology comorbid with CHF necessitates research to improve therapeutic intervention in this patient category

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of psychosomatic correlations and types of attitudes towards the disease, as well as psychopathological manifestations and their mutual influences.<sup>(5)</sup> Thus, the urgency of the research is determined by the necessity to study psychosomatic correlations in CHF patients to increase the efficiency of preventive and curative interventions and patients' QL. The purpose of this study was to investigate the psychosomatic correlations in CHF patients with anxiety and depressive disorders.

## Materials and methods

### Patients

This study involved 102 patients (60 men and 42 women) with CHF functional class (FC) II-III (NYHA) in age from 41 to 65 (mean age,  $56.3 \pm 2.7$ ). The investigation was approved by local ethics committee, and written informed consent was obtained from all participants.

The inclusion criteria were CHF FC II-III (NYHA) and stable condition within over 1 month prior to study entry.

The exclusion criteria were acute decompensated heart failure; CHF FC IV; dysthymia, cyclothymia, bipolar disorder, schizophrenia; anti-depressant and anxiolytic drug therapy resistance (no effect after two previous therapy courses in adequate doses lasting more than one month); therapy with inhibitors of monoamine oxidase and neuroleptics of prolonged action; myocardial infarction (MI) within 3 months prior to study entry; severe cardiac arrhythmias and conduction disorders; chronic alcoholism.

CHF was diagnosed in the presence of two key criteria: 1) specific CHF symptoms (exertional dyspnea and/or dyspnea at rest, fatigability and limited physical activity, bilateral ankle edema), and 2) objective proofs that these symptoms are associated with damage to the heart rather than with any other organs (for example, lung diseases, anemia, renal failure).

Cardiovascular diseases prior to CHF development were as follows: arterial hypertension in 63(61.8%) patients, MI in anamnesis in 32(31.4%) patients, and stable angina in 48(47.1%) patients.

### Methods

6MWT as a sub-maximal exercise test was used to determine functional capacity in patients. Patients' subjective assessment of dyspnea was conducted using the modified VAS in the form of a 10 cm long straight line. The initial point of the line (0) means no dyspnea, the final point (10) means intolerable dyspnea. The patient marks a point on the line that corresponds with his or her perception of dyspnea.

The clinical-psychopathological method included a detailed analysis of general anamnestic data (social status, complaints during examination and their characteristics, occupational hazards, comorbidities, smoking status), disease course peculiarities (disease onset, duration, hospitalization frequency), and description of psychopathological symptoms and syndromes, and their dynamics, in the process of treatment. The experimental-psychological methods used questionnaires. The personality characteristics were studied using the FPI. The intensity of depressive disorders was assessed by HAM-A

and HAM-D. The statistical analysis was performed using the statistical software STATISTICA 6.0. A probability value of  $P < 0.05$  was considered statistically significant.

## Results and Discussion

The most frequent manifestations of CHF were dyspnea and palpitation on exertion, as well as fatigability. Nocturnal cough was revealed in 30% of the patients and paroxysmal nocturnal dyspnea in 24%. Analysis of FPI data showed that typical personality traits of the CHF patients were neuroticism, depressiveness, emotional lability, and decreased sociability. Most patients (54%) displayed a combination of two dominating types of attitude towards the disease. In 30% of the cases, one dominating type was successfully revealed; in 16% of patients, it was impossible to unambiguously define the prevailing types of attitudes towards the disease. Among mixed types, most frequently encountered were anxious-neurasthenic (13%), neurasthenic-depressive (10%), paranoiac-depressive (8%), and egocentric-hypochondriac (6%) types.

The clinical-psychological testing showed that all CHF patients had depressive disorders of various degrees of manifestation. The average score of DD severity in the general group of patients was  $11.36 \pm 2.24$  points, which corresponds to a moderate depressive episode, according to HAM-D. For a more detailed analysis of affective disorders in the examined cohort, we assessed the manifestation of each symptom according to HAM-D and HAM-A. The degree of anxiety manifestation was  $17.6 \pm 3.2$  points, which corresponds to a high degree. Most expressed were such symptoms as agitation, general somatic symptoms, daily fluctuations, and somatic anxiety. Less expressed were the following symptoms: retardation, gastro-intestinal somatic symptoms, suicidal intentions, depersonalization and derealization, and obsession and compulsion.

Data analysis revealed that the degree of depressive disorders in the examined patients was a direct function of CHF severity. The increase in the severity of depressive disorders with the increase of the CHF FC may be explained by more severe symptoms, in particular, severe dyspnea/suffocation that grows as the disease progresses. Under a stable condition of the circulatory system, the degree of anxiety and depressive disorders was directly proportional to the duration of the disease. The highest indices of anxiety and depression severity were obtained in patients who continued smoking even after the onset of the CHF symptoms. We established that the severity of dyspnea was associated not only with the clinical-instrumental characteristics of CHF (LVEF, 6MWT), but also with the personality and the behavioral and psychological peculiarities of the patients: smoking, hypochondriac type of attitude towards the disease, sleep disorders, severity of depression and anxiety. Psychosomatic disorders in CHF patients may aggravate their subjective perception of respiratory discomfort, which is reflected in QL and frequency of drug administration (Table 1). Thus, our study revealed certain correlations between the indices of anxiety and depressive disorders, clinical-instrumental

data, types of attitudes towards the disease, personality characteristics, and QL. The obtained data demonstrate that mental disorders observed in CHF patients aggravate the severity of the patients' condition and decrease their adaptive capability, thus adversely affecting the course and outcome of the underlying disease.

**Table 1.**

**Impact of behavioral, clinical-instrumental and psychological parameters on the severity of dyspnea in CHF patients**

Variable	Investigated factors	F- criterion	P
Dyspnea	Smoking	4.98	0.0007
	LVEF	7.21	0.0001
	6MWT	5.36	0.0002
	Total anxiety score	4.12	0.0055
	Total depression score	3.69	0.022
	Hypochondriac type of attitude towards the disease	4.23	0.009
	Insomnia	6.12	0.0001
	Gender	3.24	0.038

#### **In conclusion:**

- Personality features typical of CHF patients are neuroticism, signs of depressive reaction in emotional state and behavior, lower communication need, and severe mood swings. Prevalent attitudes of CHF patients towards the disease are anxiety and hypochondria, followed by neurasthenia and depression, and then least frequent, egocentrism and paranoia.
- The CHF patients are characterized by lower QL. The score of their mental health, vitality and social

functioning does not reach even half of the absolute norm; the indices of general health and physical functioning make less than one third of the normal values; and the levels of emotional and physical role functioning have minimal values.

- Finally, the study disclosed the basic factors associated with CHF severity: the intensity of clinical symptoms (dyspnea, rapid fatigability, decompensation and hospitalization frequency), smoking, LVEF, marital status, and psychosomatic factors (anxiety and depressive disorders).

#### **Competing interests**

The authors declare that they have no competing interests.

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## Assessment of Special Endurance of Athletes in Freestyle Wrestling at the Preparatory Stage of a Sports Cycle

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### Abstract

We evaluated the special endurance (SE) of freestyle wrestlers with the help of complex tests of special working capacity, while simultaneously monitoring the change in levels of lactate concentration and LPO in the blood at the preparatory stage of the annual sport cycle. A total of 23 athletes with high sports qualification participated in the experiment. Athletes were tested in the field by “repeated marginal load” tests, including the operational test (OT) developed by the authors. The most noticeable shifts in the studied indicators were observed after the test load of OT, which indicates an increase in the glycolytic capacity of the athlete’s body. Thus, our study shows the possibility of using our OT to analyze the anaerobic performance of elite athletes in freestyle wrestling. (**International Journal of Biomedicine. 2017;7(3):251-253.**)

**Key Words:** freestyle wrestling • special endurance • lactate • lipid peroxidation

### Abbreviations

**EI**, endurance index; **LPO**, lipid peroxidation; **LMAO**, low molecular weight antioxidants; **MDA**, malondialdehyde; **OT**, operational test; **SE**, special endurance.

### Introduction

At present, in the theory of sports training of athletes in freestyle wrestling, the growth of sports achievements is associated with an increase in the technical preparedness of athletes. The Churapcha State Institute of Physical Education and Sports (ChSIPES), actively conducts research to optimize the technical training of athletes engaged in freestyle wrestling. The trainers of ChSIPES, summarizing the long-term experience, identify the priority means of technical training of athletes and construct the programs, taking into account the specifics and traditions of the institute. Improving the technical and tactical training of athletes in freestyle wrestling requires the development of SE, given the increased intensity

of competitive activity. In accordance with the specifics of the wrestling match, the increase of SE is due to an accelerated involvement of anaerobic glycolytic processes in the energy supply of the work. It is possible to assess the level of development of the SE of athletes in freestyle wrestling by the indicator of the magnitude of the acidotic shifts in a situation as close as possible to a competitive one.<sup>(1)</sup> At the same time, the intensity of anaerobic glycolytic processes reflects the concentration of lactate in the blood. The analysis of data in the literature indicates that the most suitable conditions for physical exercise, in which the maximum realization of the anaerobic glycolytic potential occurs, is an intermittent load with a repeated short-term series of the maximum possible load lasting no more than 60 seconds for each exercise, with constant (1-3 minute or shorter) rest intervals.<sup>(2)</sup> With intensive physical load, a limiting factor, along with an increase in lactate concentration, is the accumulation of LPO products. In accordance with V. Lankin et al.,<sup>(3)</sup> moderate activation

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of LPO processes in response to the adverse factor is one of the adaptation mechanisms. However, the accumulation of products of free radical oxidation is the most important disadaptation factor; it reduces the motor abilities of the organism, its endurance and physical working capacity.

The topic of this study is relevant because coaches and athletes need a selection of simple and effective medico-biological methods for assessing the SE of athletes throughout the training process.

The purpose of our work was to assess the SE of freestyle wrestlers with the help of complex tests of special working capacity while monitoring the level of changes in lactate concentration and the LPO level in the blood at the pre-competition stage of the annual training cycle.

## Material and Methods

The study involved 23 students of ChSIPES with the sports qualifications of the candidates for Master of Sports and Master of Sports aged between 18 and 23, who are engaged in freestyle wrestling. The study protocol was reviewed and approved by the Ethics Committee of the North-Eastern Federal University. All participants provided the written informed consent.

To determine the concentration of lactate in the peripheral blood, a portable device, Accutrend Lactate (Roche Diagnostics), was used, which allows one to determine the level of lactate in a training hall for 1 minute. The blood test was performed 3 minutes after the exercise. The LPO intensity was determined according to the serum MDA level. MDA content in serum was determined by spectrophotometric method by reaction with thiobarbituric acid at  $\lambda=532$  nm. The content LMAO was determined by by ortho-phenanthroline colour method.<sup>(4)</sup> o-Phenanthroline quantitatively forms complex with  $Fe^{2+}$ , which get disrupted in the presence of chelating agents. The antioxidant interfered with the formation of ferrous-phenanthroline complex which is spectrophotometrically read at 510 nm.

Blood for examination was taken from the ulnar vein in the morning before and at the end of training sessions. To assess SE, a complex test was performed according to VF Boyko,<sup>(5)</sup> in which, in the interval mode, the athlete performs 3 specific loads of a different nature with maximum available intensity during 20 seconds with intervals of rest of 10 seconds. The EI was calculated as the ratio of the average values between data obtained during the second and third minute of work and data recorded during the first minute. The second type of test was OT developed by the ChSIPES trainers for the rapid assessment of special technical preparedness. This is a set of special exercises performed three times: the 10 throws through the back with capturing a hand ("poker"), taking into account the performance time; the 10 sit-ups on one leg, taking into account performance time;; the 10 jumps imitating the protection action against the passage to the feet ("throwing off the legs"), taking into account performance time; the 10 throws through the hip, taking into account performance time; the 10 torso lifts, lying on the back, hands behind the head ("torso lifts"), taking into account performance time. A conditional

unit (CU), the ratio of the average data of the first approach to the average data of the second and third approaches, was also calculated.

Results were statistically processed using the *software* package BIostat 3.03 and Microsoft Excel 2007. The mean (M) and standard error of the mean (SEM) were calculated. Student's paired t-test was used to compare the differences between the paired samples. A probability value of  $P<0.05$  was considered statistically significant.

## Results and Discussion

Based on the results of pedagogical testing of the level of special technical preparedness of wrestlers at the beginning and at the end of training camps, a reliable increase in the parameters of the complex test was revealed in 5 students out of 23; the remaining students did not show any significant differences in the studied parameters. There was also no significant shift in lactate concentration after the test. According to EI in the complex test, a slight increase in this value was observed. It is possible that in the performance of this test, the athletes were not motivated to perform the limit work, and the results obtained cannot show the degree to which they realized their functional capabilities.

In the OT, the students showed improved results (Table 1). According to throws through the back with capturing a hand, the gain was 15 seconds, on average, "torso lifts" - 18 seconds, "throwing off the legs" - 10 seconds. In general in the OT, the coefficient increased by 20% ( $P<0.05$ ). The average lactate value after the test also increased by 11% ( $P<0.05$ ), which may indicate an increase in the anaerobic glycolytic capacity of the bodies of athletes. An increase in the blood lactate level after the test, with improved test results, indicates a predominantly glycolytic anaerobic orientation of this test load.

**Table 1.**

### Results of tests of special working capacity

Test	At the beginning of training cycle		At the end of training cycle	
	Test Score	lactate, mmol/l	Test Score	lactate, mmol/l
Complex test, <sup>(5)</sup> EI	0.68±0.06	11.8±1.1	0.71±0.09*	12.5±1.2*
OT, CU	0.72±0.05	16.5±1.3	0.89±0.08*	18.5±2.6*

\*-  $P<0.05$  - compared with the beginning of the training cycle

From the literature, it is known that in people adapted to physical exertion, the level of LPO is lowered and the amount of antioxidants in muscles is increased. In athletes adapted to speed-power loads, an increase in the intensity of LPO is found.<sup>(6)</sup> Earlier, we found an increase in the LPO level and a decrease in the degree of antioxidant protection in the blood of athletes compared with the control group.<sup>(7)</sup> To determine the impact of LPO processes on the performance of athletes, we determined the MDA content and the total activity of LMAO

in the blood at the beginning and at the end of this stage of the training cycle. The results showed a significant increase in the blood concentration of MDA and LMAO by 10% and 6%, respectively, indicating an increase in the intensity of the “LPO-antioxidants” system, which may have a negative effect on the general and special performance of wrestlers.

## Conclusion

The results of assessing the SE of freestyle wrestlers in two tests give reason to assume that the OT mainly reflects the anaerobic performance of athletes. Improving the results according to data of this test and deeper acidotic changes in the testing of athletes indicate an increase in the glycolytic capacity and SE of athletes at this stage of the training cycle. The increased tension in the “LPO-antioxidants” system in the body of wrestlers requires antioxidant correction of LPO activation caused by intense physical exertion. It is beneficial to test the special physical working capacity of wrestlers with biochemical control of lactate concentration changes at different stages of training in order to assess the dynamics of the development of SE and to manage the training process.

## Competing interests

The authors declare that they have no competing interests.

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CASE REPORT

# Hemoperitoneum Secondary to Arterial Rupture of Subserosal Uterine Leiomyoma

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## Abstract

Uterine leiomyomas (ULs) affect up to 80% of women during their reproductive years. Though relatively benign, they can have life-threatening complications. This case report presents a 50-year-old, postmenopausal female who presented with severe abdominal pain secondary to massive hemoperitoneum. Upon exploratory laparotomy, a large, pulsing artery was seen on the uterus, consistent with a ruptured subserosal uterine fibroid. This case offers unique insight into the presentation and management of this surgical emergency. (**International Journal of Biomedicine. 2017;7(3):254-256.**)

**Key Words:** uterine fibroid • menorrhagia • hemoperitoneum • emergency

## Introduction

Uterine leiomyomas (ULs) are benign tumors of smooth muscle that affect up to 80% of women during their reproductive years. The prevalence of these tumors peaks in the perimenopausal years and declines in post-menopausal women.<sup>(1)</sup> ULs are often asymptomatic but can present with menorrhagia, pelvic pressure, heaviness, urinary urgency and frequency, or even constipation. The symptomatology depends inherently on the size and location of each tumor. Treatment for severely symptomatic patients is often with uterine artery embolization, laparoscopic myomectomy, or open myomectomy for younger patients desiring to preserve their fertility. However, total abdominal hysterectomy is regarded as completely curative. This case describes a 50-year-old woman who presented with severe abdominal pain and was discovered to have a massive hemoperitoneum secondary to a bleeding subserosal fibroid.

## Case Report

A 50-year-old G2P1011 presented to the emergency department for severe, intractable, right-sided abdominal pain and vomiting since the morning. The pain was sharp, 10/10, and radiating to her right shoulder. She denied any fever,

chest pain, diarrhea, vaginal bleeding, hematochezia, and hematemesis. Her past medical history was significant only for fibroids with no past surgical history. She had a significant family history of cancer on her paternal side.

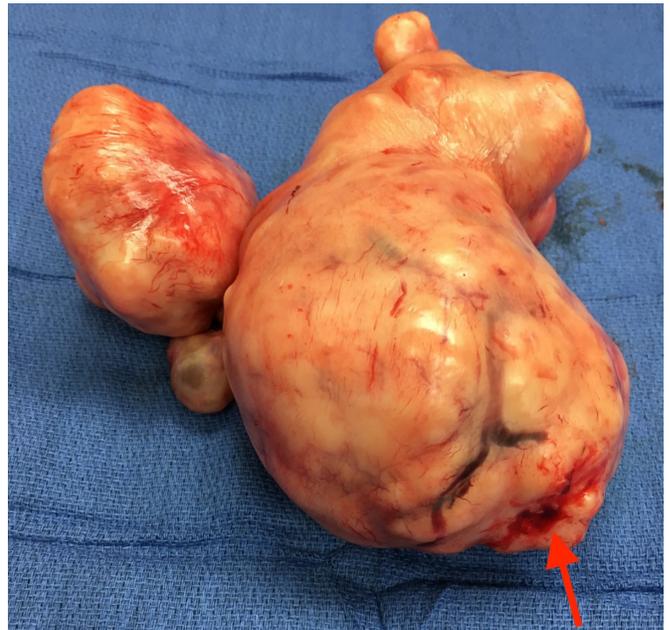
Her hemoglobin had been trending downwards, falling from 11.8 g/dL to 10.5 g/dL in 10 hours. In addition, her white blood cell count was elevated to 20.9 K/uL. All other labs were within normal limits. An abdominal CT scan revealed a large, complex, solid cyst with high attenuation—highly suggestive of ovarian malignancy. The mass was poorly delineated from the uterus, and extensive intraabdominal fluid collection was reported. The fluid was measured to be 59 Hounsfield units—consistent with intraabdominal hemorrhage. The patient was taken immediately to the OR for a diagnostic laparotomy.

Intraoperatively, three liters of blood were evacuated from the peritoneum. The uterine anatomy was grossly distorted (Figure 1) and a pulsing vessel on the uterine fundus was noted (Figure 2). A total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. The ovaries were grossly normal in size, shape, and appearance (Figure 3). The uterus measured 15×15×6 cm with the largest leiomyoma measuring 12×7 cm. Pathology of the specimen confirmed numerous subserosal leiomyomas with no abnormal pathology in the ovaries. The patient was transferred to the surgical intensive care unit for monitoring of hemodynamic stability. However, there were no acute events post-operatively. The remainder of the patient's hospital course was uneventful and the patient was safely discharged home.

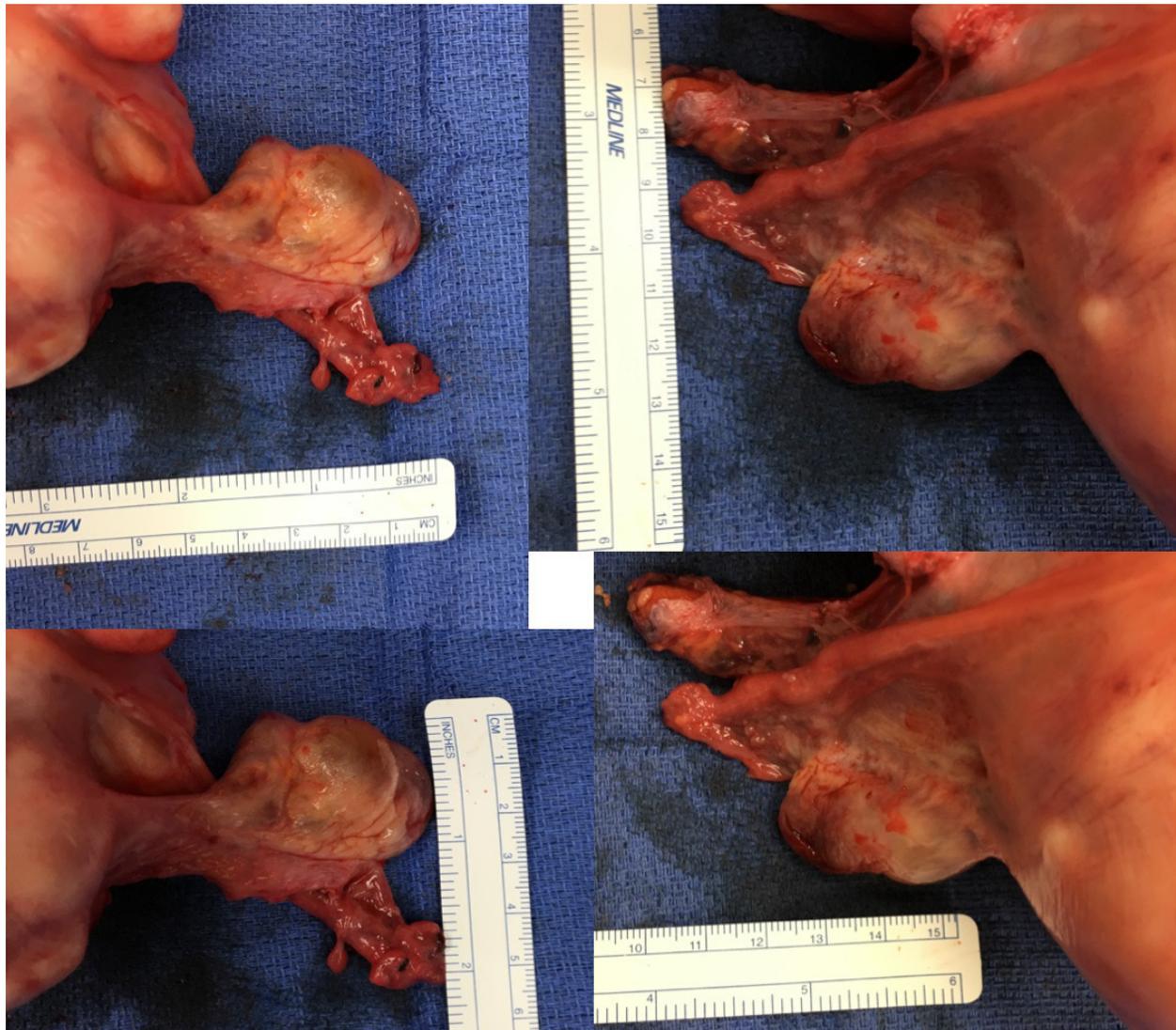
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**Fig. 1.** Surgical specimen showing gross distortion of uterine anatomy.



**Fig. 2.** Red arrow pointing to ruptured subserosal uterine fibroid with pulsing vessel.



**Fig. 3.** Grossly normal appearing ovaries with no obvious deformity.

## Discussion

This case offers unique insight into the potentially malignant presentation of a relatively benign condition. Thus far, hemoperitoneum secondary to a uterine leiomyoma has only been described anecdotally. We were only able to find 5 such previous case reports describing a rupture of subserosal fibroids with venous bleeding.<sup>(2-6)</sup> Two other case reports were found that did not specify whether the source of the bleeding was venous or arterial.<sup>(7-9)</sup> This dearth of examples demonstrates the rarity of this potentially morbid presentation.

Additionally, hemoperitoneum secondary to an arterial source is even rarer than to a venous source. To our knowledge, there have been only 2 such cases<sup>(10,11)</sup> describing arterial rupture of a uterine fibroid. These few cases offer insight into the presentation and the need for emergency intervention. Following stabilization, the patient should be taken immediately to the operating room for exploratory laparotomy to identify the source of the bleeding and to proceed with a total abdominal hysterectomy.

An important differential to be mindful of in a perimenopausal female with extensive abdominal fluid collection is ovarian malignancy. Rupture of granulosa cell tumors has been estimated to be between 10% and 35% and is associated with presenting complaints of abdominal pain, distension, and hypotension consistent with hemoperitoneum.<sup>(12)</sup> Additionally, rupture of fibrothecoma, clear cell tumors, and ovarian adenocarcinoma have been identified as causing hemoperitoneum.<sup>(13-15)</sup> Thus, malignancy is a key differential to keep in mind in a patient presenting with signs and symptoms of intraabdominal hemorrhage.

Further research could offer insight in to the presentation and the prevalence of hemoperitoneum secondary to uterine leiomyomata. Additionally, these investigations could shed light on the average age of presentation, analyze risk factors for the development of hemoperitoneum, and report on outcomes in patients with this presentation. Though malignancy is a key differential to keep in mind, it is possible that hemoperitoneum secondary to carcinoma is more likely in older, postmenopausal populations. Given this patient's CT scan and sudden presentation, intraabdominal hemorrhage secondary to carcinomatosis was the leading diagnosis. However, it is possible that the patient's age and lack of identifiable risk factors made leiomyomatosis a more relevant and thoughtful diagnosis.

The presentation of this case and the small, incidental occurrence of similar cases previously raises important questions regarding the morbidity and mortality of uterine fibroids. As a uterine leiomyoma can cause a life-threatening crisis and surgical emergency, further study into the epidemiology and postoperative outcomes is warranted.

## Competing interests

The authors declare that they have no competing interests.

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CASE REPORT

## HIV-associated Tuberculosis: Clinical Case

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### Abstract

This case report presents a 35-year-old female who presented with HIV-associated multidrug-resistant tuberculosis. Resistant tuberculosis in HIV-infected patients with immunodeficiency is extremely difficult for effective treatment. Nevertheless, our clinical case showed that the cure of tuberculosis is possible in the late stages of HIV infection with the correct choice of treatment tactics, taking into account the resistance of MBT with the subsequent use of antiretroviral therapy. The progression of HIV infection, against the background of the effective antituberculous therapy, was associated with the patient's refusal to treat the underlying disease. (**International Journal of Biomedicine. 2017;7(3):257-259.**)

**Key Words:** human immunodeficiency virus • multidrug-resistant tuberculosis • antiretroviral therapy • antituberculous therapy

### Abbreviations

**AIDS**, acquired immune deficiency syndrome; **ARVT**, antiretroviral therapy; **CHC**, chronic hepatitis C; **HIV**, human immunodeficiency virus; **MBT**, Mycobacterium tuberculosis; **MDR-TB**, multidrug-resistant tuberculosis; **RS(Y)**, the Republic of Sakha (Yakutia); **TB**, tuberculosis.

### Introduction

According to UNAIDS, there were approximately 36.7 million people worldwide living with HIV/AIDS at the end of 2015. An estimated 2.1 million individuals worldwide became newly infected with HIV in 2015.<sup>(1)</sup> Since the beginning of the epidemic, more than 70 million people have been infected with the HIV virus and about 35 million people have died of HIV.<sup>(2)</sup>

However, between 2000 and 2015, the number of new HIV infections in the world decreased by 35% and the AIDS-related death rate decreased by 28%, which saved the lives of 8 million people. On the contrary, in the Russian Federation, the number of HIV-infected people is increasing annually.<sup>(3)</sup> As of December 31, 2016, according to official data, 103,438 new HIV infections among the citizens of Russia were

reported, which is 5.3% more than in 2015. The total number of reported cases of HIV infection among citizens of Russia has reached more than 1.1 million people, of whom 243,836 (21.8%) died from various causes. According to the data of the Yakut Republican Center for AIDS Prevention and Control, 2,022 HIV-infected persons have been registered since 1996 in RS(Y), and for the 6 months of 2017, 97 new cases of HIV infection were detected.

Along with HIV infection, Russia is one of 22 countries with a high burden of TB (1990–2014). In 2014, according to WHO, the prevalence rate of TB in Russia was 109[49–192] per 100,000 population, and HIV prevalence in incident TB cases was 4.6[3.8–5.3]%. Worldwide, 9.6 million people are estimated to have fallen ill with TB in 2014. Globally, 12% of the 9.6 million new TB cases in 2014 were HIV-positive. TB now ranks alongside HIV as a leading cause of death worldwide. In 2014, TB killed 1.5 million people (1.1 million HIV-negative and 0.4 million HIV-positive).<sup>(4)</sup>

In RS(Y), 827 persons with HIV infection are currently registered, and 180 of them receive preventive therapy against

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tuberculosis. In 2015, 42 patients with HIV in combination with TB were registered.

Globally, people living with HIV are 26 times more likely to develop TB disease than those who are HIV-negative.<sup>(5)</sup> The interdependence of the two epidemic processes is multifactorial in nature, based on the disturbances in the immune response.<sup>(6)</sup> TB in HIV-infected patients usually tends to progress and disseminate.<sup>(7)</sup> TB can occur early in the course of HIV infection and throughout all stages of the disease. Although TB can be a relatively early manifestation of HIV infection, it is important to note that the risk of developing TB, and of disseminated infection, increases as the CD4 cell count decreases. Even with effective immune reconstitution with ART, the risk of TB generally remains elevated in HIV-infected patients above the background risk of the general population, even at high CD4 cell counts.<sup>(8-10)</sup>

The presentation of TB is affected by the extent of HIV-related immunosuppression. In patients with CD4 counts of  $>350$  cells/ $\mu$ L, the clinical and radiographic presentation is similar to that of patients without HIV infection. However, as immunosuppression advances, the radiographic presentation becomes less typical and extrapulmonary and disseminated disease becomes more common. However, in patients with primarily extrapulmonary involvement or disseminated disease, the CD4 cell count may be much lower.

Similarly, TB may negatively impact the natural history of HIV infection. Several studies have indicated that TB co-infection increases the risk of HIV progression and death, particularly in persons with untreated HIV disease.<sup>(11,12)</sup> The effect of TB on HIV disease progression is hypothesized to be attributable to increased immune activation<sup>(13)</sup> and increased expression of the CCR5 and CXCR4 co-receptors on CD4 cells.<sup>(14)</sup> In addition, the HIV and tuberculosis combination is related closely to resistance of MBT to anti-tuberculosis drugs. The combination of HIV infection and multidrug-resistant tuberculosis (MDR) is characterized by an extremely unfavorable course.<sup>(3,15)</sup>

In medical practice, priority is the early therapy of tuberculosis with subsequent antiretroviral therapy. WHO recommendations on the interventions needed to prevent TB in HIV-positive people and to reduce the impact of HIV among TB patients were first issued in 2004, and are collectively known as collaborative TB/HIV activities.<sup>(4)</sup>

## Case report

A 35-year-old woman with the antibodies to HIV infection detected in June 2011 was registered in the Yakutsk AIDS-Center in 2012. The infection was associated with intravenous drug use for 5 years. CHC was detected in 2012, but the antiviral treatment for CHC was not received.

In 2015, she was hospitalized in the infectious department of YaGKB with a diagnosis of active cytomegalovirus infection on the background of HIV-infection stage 4B. She had a fever of 39 °C, abdominal pain, a loose stool 1-2 times a day, and general pronounced weakness. At the examination, CD4 counts of  $>289$  cells/ $\mu$ L, RNA-HIV - 627,000 copies/ml. The patient refused ARVT.

At a repeated examination on August 24, 2016, the diagnosis was as follows: «HIV infection, stage 4B, the progression phase in the absence of ARVT; AIDS. Cytomegalovirus infection; and the Epstein-Barr infection. CHC».

Complaints: a fever of 39 °C, abdominal pain, loose stools 1-2 times a day, marked weakness, cough, and weight loss. Health deteriorated during the last month, when these symptoms appeared.

Objective data: General condition was of moderate severity. Consciousness was preserved. The position in bed was passive. Skin was clean, dry, and pale. Local cyanosis and rash were absent. Lips were covered with crusts. Sclera of the eyes was a normal color. Subcutaneous fatty tissue was poorly developed. Edemas and bedsores were not present. Submandibular, cervical and inguinal lymph nodes were enlarged to 0.5 cm, dense, and painless. Throat was slightly hyperemic, tonsils not enlarged. The frequency of respiratory movements was 18 per minute. During auscultation, wheezing was not present. Heart sounds were rhythmical, muffled. Abdomen was soft, sensitive along the intestine, peristalsis preserved. The liver edge had a soft-elastic consistency. Spleen was not enlarged. The stool was regular and liquid; color was brown without pathological impurities. Urination was free, painless. Diuresis was adequate. Neurological status: meningeal symptoms were absent.

Laboratory test data: The total blood test (08.25.16): leukocytes -  $3.36 \times 10^9/l$ , erythrocytes -  $5.5 \times 10^{12}/l$ , hemoglobin - 137 g/l, hematocrit - 39.3%, lymphocytes - 8%, monocytes - 8.3%, neutrophils - 82.5%, eosinophils - 1.2%, basophils - 0%, platelets -  $89 \times 10^9/L$ , ESR - 32 mm/h.

Biochemical blood test (08.25.16): albumin - 34.5 g/l, ALT - 10 U/L, AST - 21.9 U/L, total bilirubin - 4  $\mu$ mol/l, cholesterol - 2.01 mmol/l, creatinine - 79  $\mu$ mol/l, glucose - 4.78 mmol/l, total protein - 80.9 g/l, urea - 4 mmol/l.

Cytomegalovirus (CMV) and Epstein Barr Virus (EBV) were detected in PCR (07.21.16). In PCR (07.21.16), the number of HIV RNA copies was 2, 400, 000 copies/ml.

Immunological examination of blood (07.19.16): leukocytes -  $6.2 \times 10^9/l$ , lymphocytes 10% (19-37), CD3 - 41% (60-80), CD4 - 9% (55 cells/ml), SD8 - 30% (16-39).

EIA (08.25.2016): HbsAg - negative, anti-HBcorIgM - negative, anti-HbsAg - negative, HBeAg - negative, anti-HDV - negative, anti-HCV - positive, core (+), NS (-), anti-HCV IgM - negative.

CT chest (08.25.16): the disseminated lung process.

Ultrasound of the abdominal cavity (08.25.16): moderate lymphadenopathy, diffusive changes in the liver.

Blood for sterility and blood culture (08.26.16) was negative.

Smear from fauces for candidiasis (08.26.16): *Candida albicans*  $>1 \times 10^6/ml$ , sensitive to nystatinum.

PCR (08.26.16): CMV DNA was not detected, HSV RNA was not detected.

Sputum smear on MBT by the bactec method (08.29.16): MBTs were detected and resistant to streptomycin, isoniazid, rifampicin, kanamycin, and capreomycin. All sputum cultures were negative from September to December 2016. Sputum on

MBT by the real-time PCR method (08.26.16): MBT resistant to rifampicin.

Based on clinical and anamnestic results of the examination, the following diagnosis was constructed: "HIV infection, 4B stage, the progression phase in the absence of ARVT. AIDS. Disseminated pulmonary TB in the phase of infiltration. MBT(+). MDR-TB: streptomycin, isoniazid, rifampicin, kanamycin, capreomycin. Candidiasis of the oral mucosa. Replicative chronic viral hepatitis C."

On August 31, 2016, the patient was transferred with this diagnosis for treatment to the Department of Multiple Drug Resistance of the Yakut NPZ "Ftiziatriia", where she was treated for 75 days and received treatment according to the IV standard regime (pyrazinamide, ethambutol, amikacin, levofloxacin, cycloserine, para-aminosalicylic acid). The patient refused ARBT treatment. Due to side effects in the form of pain throughout the body, and weakness, aminosalicylic acid was replaced by prothionamide; ethambutol was canceled due to the contra-indication of the ophthalmologist. Patient was hospitalized again in March 2017 and underwent treatment according to the IV regime (levofloxacin, pyrazinamide, cycloserine, prothionamide). She received only 12 doses. On a review of the chest X-ray (13.03.2017): The dynamics were positive; we detected a compaction of foci in S6 of both lungs. At the insistence of the patient, in connection with the move to another place of residence, she was discharged on 04.12.17, while the level of CD4 decreased up to 5.2 cells, the viral load was 3,350,000 copies/ml. Patient again refused ARBT treatment.

## Conclusion

Resistant tuberculosis in HIV-infected patients with immunodeficiency is extremely difficult for effective treatment. Patients' adherence to treatment also plays a significant role in recovery. Detection and treatment of tuberculosis in HIV-infected patients at an early stage of HIV infection is most effective. Nevertheless, our clinical case showed that the cure of tuberculosis is also possible in the late stages of HIV infection with the correct choice of treatment tactics, taking into account the resistance of MBT with the subsequent use of ARVT.

Thus, patients with HIV infection require professional psychological support with training and conversation. In our clinical case, the progression of HIV infection, against the background of the effective antituberculous therapy, is associated with the patient's refusal to treat the underlying disease, which significantly worsens the prognosis.

## Competing interests

The authors declare that they have no competing interests.

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