IJBM

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ISSN 2158-0510

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International Journal of Biomedicine (ISSN 2158-0510) is published four times a year by International Medical Research and Development Corp. (IMRDC), 6308, 12 Avenue, Brooklyn, NY 11219 USA

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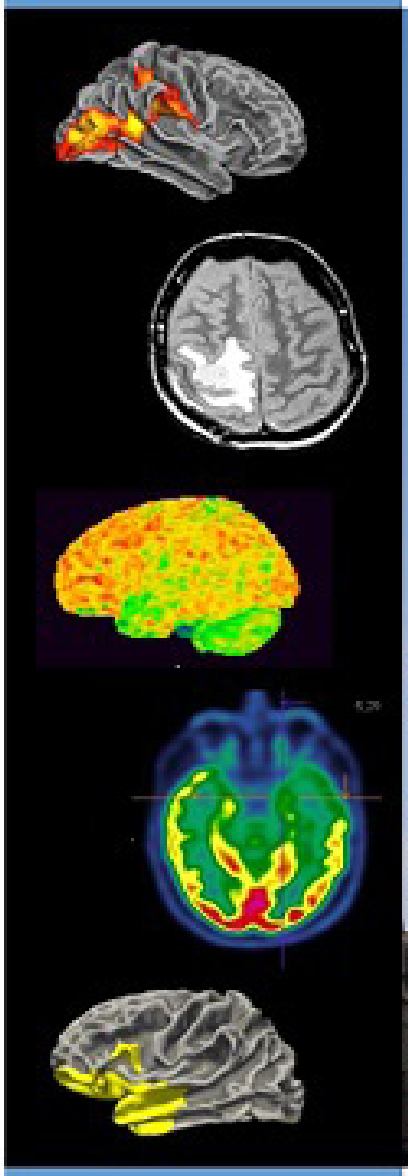

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Volume 8 Issue 3 September 2018

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ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Synchronization of Wave Flows of Arterial and Venous Blood and Phases of the Cardiac Cycle with the Structure of the Peripheral Pulse Wave in Norm: Part 2

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Abstract

Hemodynamic indices studied in practically healthy people were obtained by catheterization in various vascular areas: the chambers of the heart (ventricles, atria, coronary sinus), pulmonary trunk, aorta, inferior vena cava, superior vena cava, right hepatic vein, and sigmoid sinus. Using the mean values of the hemodynamic parameters, we constructed graphics of the "curves" of the central, arterial, and venous pressure, synchronized with each other and an ECG, and with the radial pulse wave recorded by a non-invasive method. The obtained data, which demonstrate the projection coincidences of the characteristic points of peripheral pulse wave with the key indicators of the phases of cardiac cycle, made it possible to transform the results obtained during the invasive examination into indicators of the non-invasive technique. This transformation became possible not only at the characteristic points of the deployed peripheral pulse wave, but in each anacrotic and dicrotic segment, which are understood as the projection areas of the synchronized hemodynamic and wave processes of the vascular bed. We believe it possible to catalog the forms of pulse waves, as well as their projection segments, to obtain accurate diagnostic information about the phases of cardiac cycle and organ hemodynamics in humans in norm and with pathological conditions, using a non-invasive methood based on basic information obtained by invasive methods. **(International Journal of Biomedicine. 2018;8(3):177-181.)**

Key Words: cardiac cycle • hemodynamic parameters • ECG • peripheral pulse wave

Abbreviations

Ao, aorta; **AV**, aortic valve; **CC**, cardiac cycle; **CFB,** central fibrous body; **CS**, coronary sinus; **EDP**, end-diastolic pressure; **IJV**, internal jugular vein; **IVC**, inferior vena cava; **LA**, left atrium; **LV**, left ventricle; **MV**, mitral valve; **PT,** pulmonary trunk; **PPW**, peripheral pulse wave; **PW,** pulse wave; **PV**, pulmonary valve; **RV,** right ventricle; **RA**, right atrium; **RHV,** right hepatic vein; **SS**, sigmoid sinus; **SVC**, superior vena cava; **TV**, tricuspid valve; **ZTEP**, zone of temporal equalization of pressure.

Basic Part

The aim of this study was to compare and extrapolate the results of invasive and non-invasive diagnostic techniques in humans under normal and pathological conditions.

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Methods and Discussion

The parameters of the hemodynamics of the central, arterial, and venous blood flow have been discussed in detail and repeatedly published.[1-3] Based on the mean values of hemodynamic parameters obtained by catheterization—in the heart (RV, LV, RA, LA, CS), Ao, PT, liver, kidney and SS—we constructed graphs of the sequential dynamic pressure change in the each studied point, which were synchronized with each other, ECG and with a detailed single PPW.[4] Synchronized

wave flows and PPW are shown in Table 1. Numbers and colors indicate PW (blue color) and the vessels and organs in which the catheterization was performed. In the right part (the second CC), the yellow color indicates the graphic sequence of the hemodynamic curves revealed during synchronization: ZTEPs. Separately, the list of projective coincidences of the CC phases and characteristic points of PW (10 points) is presented.

A partial discussion of the diagram of the sequences of the CC phases presented in Table 1, was given in our previous work.[5] Yellow color indicates a curve that combines the sequence of ZTEPs during CC. Recall that we understand ZTEPs (zones of intersection of hemodynamic curves) as brief (23 points of ZTEPs, marked with Roman numerals, over one CC) periods of zeroing hemodynamic gradients in the investigated zones—periods of equilibrium states of both consecutive and distributed parts of the vascular system.

We believe that ZTEPs that have a fixed sequence (indicated on the graph) form a high-speed matrix of control hemodynamic points, which ensure the sequence (phasing) and synchronism of the phase periods of CC (considering peripheral ZTEPs, possibly regulation of the vascular system as a whole). We consider it necessary to note the exact topographic coincidence of a significant part of the peak ZTEP values with all the characteristic points of the detailed peripheral pulse curve: 1-10 points (marked by vertical lines, Table 1).

The mismatch (delay) of the peak values of the arterial part of the hemodynamic curves is due to the outrunning spread of the wave impulse (PW) along the vascular bed in comparison with the speed of distribution of the arterial blood substrate after the systole of LV.

Recall that an impulse of PW reaches all metabolic zones of the body in the interval from the opening of AV to closing of AV.[6] We believe (intentionally simplifying the structure of connections of hemodynamic flows) that the centrifugal controlling informational impulse (PW), having passed the exchange zones of organs (i.e., the mosaic peripheral resistance, which depends on the activity of exchange and intensity of local blood flow), is transformed into a network of afferent information flows of feedback with the concentration in RA to prepare the next CC.

Modern methods of non-invasive examination of PW do not have a direct appeal to heart hemodynamics guided by indirect signs (including oscillations and vibrations of the chest of various genesis) of the heart functioning. We studied these methods across the whole range of available information, regardless of the method and localization of the initial information signal, and quantitatively assessed the characteristics of PW, followed by differentiation of its species, cataloging, and expert diagnostic evaluation, One of the most accurate methods is to measure the phases of CC according to a high-speed kinetocardiogram (KCG) [4] synchronous with the reference curves: ECG, phonocardiogram, and sphygmogram of the carotid artery. KCG indirectly measures the following time intervals: the duration of CC (R-R), the phase of asynchronous contraction, the phase of isometric contraction, the phase of rapid ejection, the phase of slow ejection, and the systole of the atrium, without the ability to accurately measure the inside and interphase dynamics or connections with organ hemodynamics.

Most researchers believe that the basic information about the state of health is represented by the elements of a single PW of the radial artery that has a stable character. The main informational elements are the amplitude-time parameters determined by the shape (contour) of the pulsogram (local maxima-minima inflection points). A high degree of similarity in the contour of the pulsograms of the carotid and radial arteries was established, which allowed to transfer the methods of not only contour analysis, but also of the phase analysis from the curves measured in the heart and the aortic aperture to the pulsogram of the radial artery.[7] As a mathematical apparatus, a contour pulsogram analysis was used by the methods of spline approximation and regularization. The most variable regions of PW by analyzing the statistical nature of the dynamic series of amplitude-time parameters of the characteristic points of long realizations of PW are identified. This variant of mathematical processing made it possible to use the method for contour analysis of a pulsogram (sphygmogram) as an effective analysis of biomedical information.

The method is based on detecting amplitude–time parameters in 10 informative points of PW, characterizing not only the phases of CC (9 temporal intervals corresponding to projections on time axis), but also the shape of PW. Point 10 (the end of PW) is simultaneously the starting point (Point 1) of the next wave. It was also found that the distribution of the amplitudes of the dynamic series of the pulse does not correspond to the known distribution laws of random variables.[4,7,8]

The results we obtained during the catheterization are synchronized with the PW graph (radial artery) that was obtained by a non-invasive method, with a single cycle of PPW having allotted characteristic points. Ten characteristic points of PW, which determine the primary amplitude-time parameters, have highly accurate characteristics due to use of effective mathematical algorithms.[8-10] This approach made it possible to determine reliable projection coincidences (Table 1) between characteristic points of PPW and the phases of CC.

The revealed projective coincidences allow us, at the initial stage, to produce an accurate marking of PPW according to the phases of CC and organ hemodynamics, obtaining reliable information on the hemodynamic parameters by a non-invasive way. The results of synchronization of central hemodynamics and PPW (radial artery - characteristic points from 1 to 10) revealed the presence of the following projection coincidences of the characteristic points of PW with the phases of CC: Point 1: a coincidence with the closing of MV; Point 2: with ZTEP for LA (med) - SS (min); Point 3: with ZTEP for CS (maximum) and SS (maximum); Point 4: with the opening of AV; Point 5: with the closing of PV; Point 6: with the opening of TV; Point 7: with the closing of AV; Point 8: with the opening of MV; Point 9: with ZTEP for LA and LV (diastole); Point 10: with the closing of MV.

Thus, the possibility of non-invasively extracting information on the factual state of cardiovascular hemodynamics in general is presented.

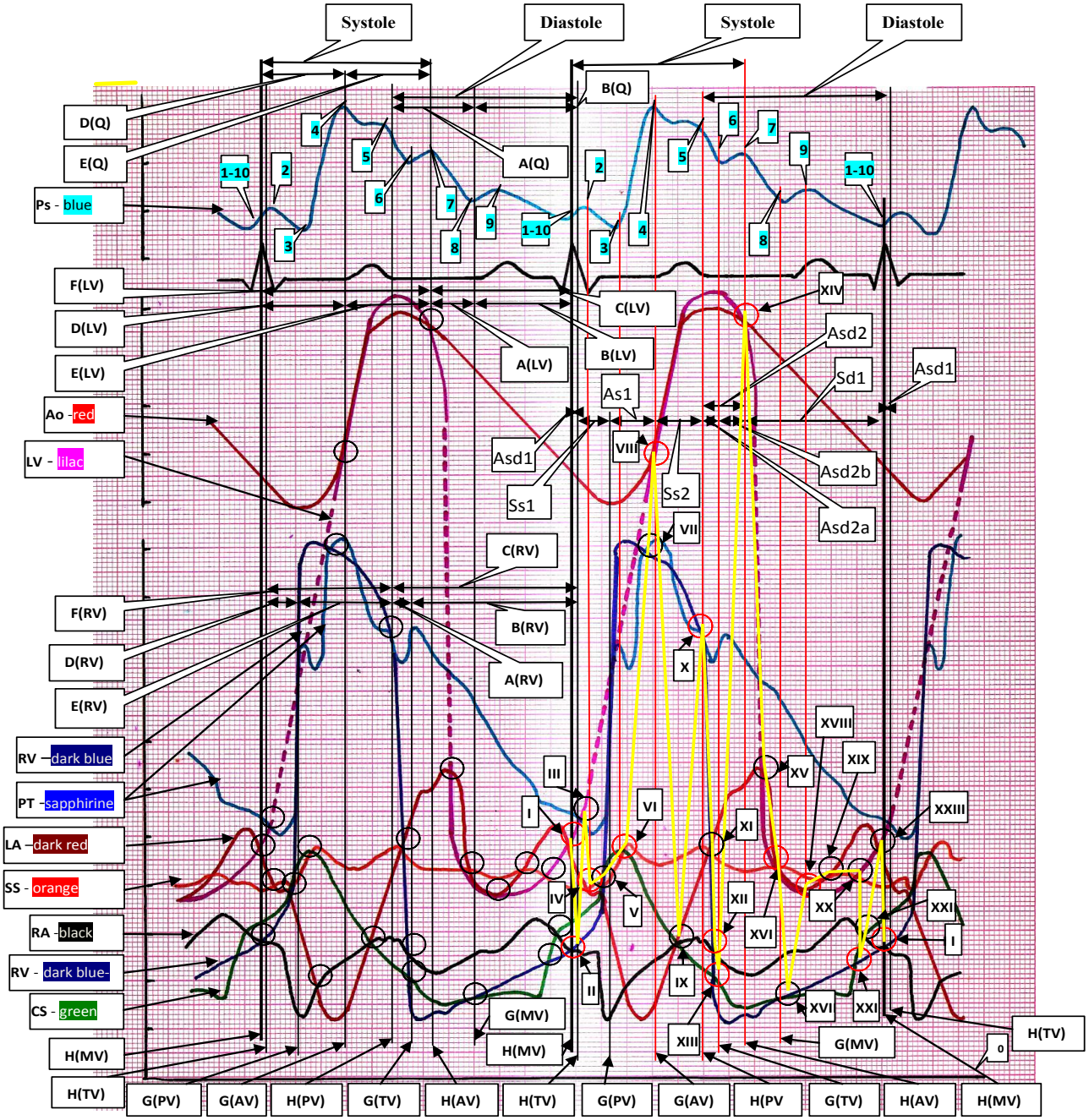

Table 1. Coincidences between characteristic points of PPW and the phases of CC in Norm

Coincidences of the characteristic points of PW (V.V. Boronoev) with the phases of CC:

1 – with the closing of MV
2– with ZTEP for LA (med) - SS (min)
3 – with ZTEP for CS (maximum) and SS (maximum)
4 – with the opening of AV
5 – with the closing of PV
6 – with the opening of TV
7 – with the closing of AV
8 – with the opening of MV
9 – with ZTEP for LA and LV (diastole)
10(1) – with the closing of MV
– Characteristic points of PW (V.V. Boronoev)

A(Q) – isometric ventricular relaxation
B(Q) – actual ventricular diastole
C(LV) – LV diastole
A(LV) – isometric relaxation of LV
B(LV) – actual LV diastole
C(RV) – RV diastole
A(RV) – isometric relaxation of RV
B(RV) – actual RV diastole
D(Q) – isometric ventricular contraction
E(Q) – actual ventricular systole
F(LV) – LV systole
E(LV) – actual ventricular systole
F(RV) – RV systole
D(RV) – RV isometric contraction period
E(RV) – actual RV systole
G(AV) – opening of AV
H(PV) – closing of PV
G(TV) – opening of TV
H(AV) – closing of AV
G(MV) – opening of MV
H(TV) – closing of TV
H(MV) – closing of MV
G(PV) – opening of PV
H(PV) – closing of TV
Asd1 – asynchronous period of ventricular systole-diastole -1
Ss1 – synchronization period of isometric ventricular contraction-1
As1 – asynchronous period of ventricular systole -1
Ss2 – synchronization of the actual ventricular systole -2
Asd2 – asynchronous period of ventricular systole-diastole -2
Asd2a – from the closing of PV to the opening of TV
Asd2b – from the opening of TV to the closing of AV
Ad1 – asynchronous period of ventricular diastole -1
Sd1 – period of synchronization of ventricular diastole-1
coincidence of invasive and non-invasive points (V.V.Boronoev)
matrix of pressure equalization points of venous block (from I to X)

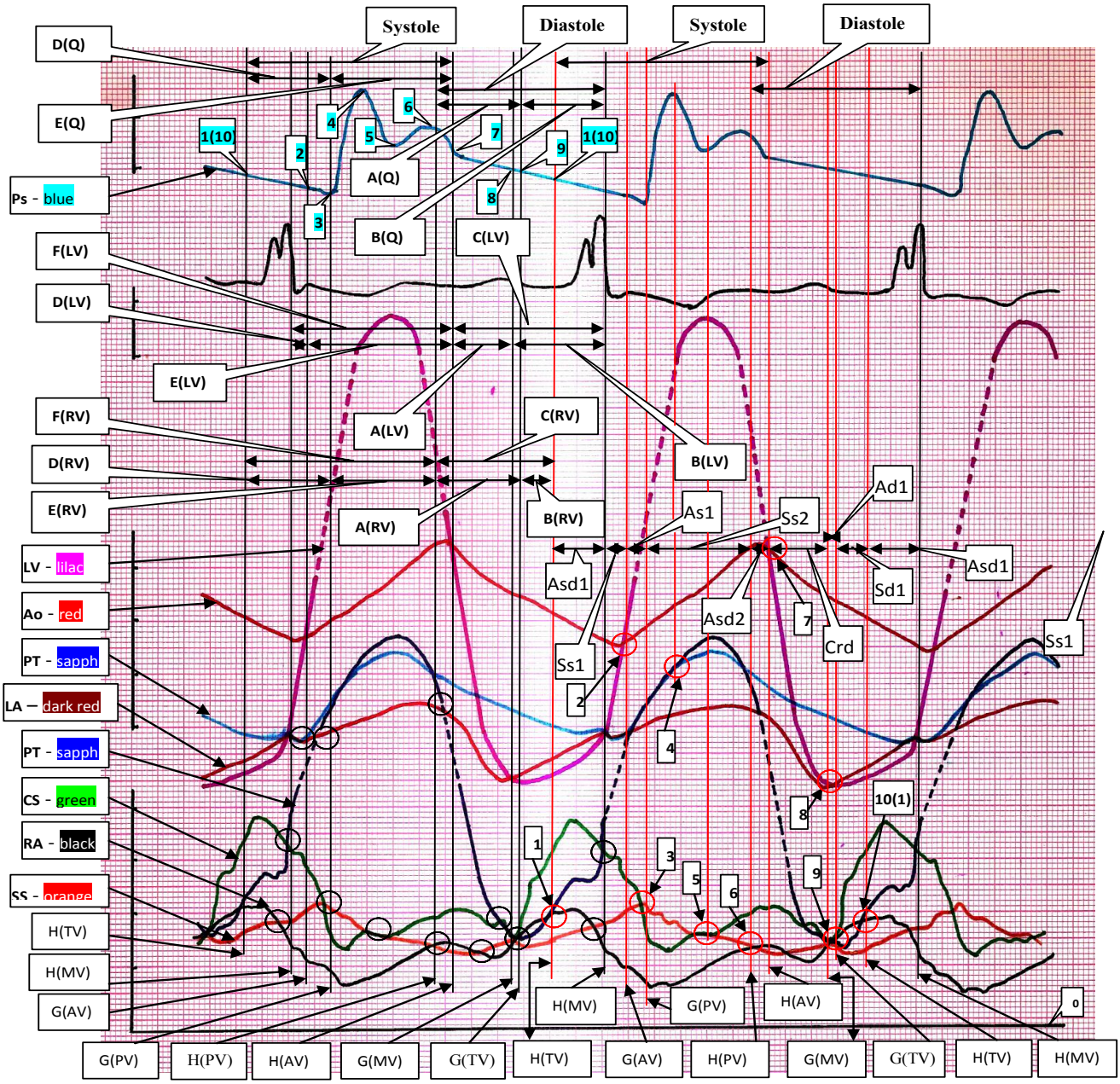

Table 2. Coincidences between characteristic points of PPW and the phases of CC in Patient S. with a mixed aortic valve disease

Coincidences of the characteristic points of PW (V.V. Boronoev) with the phases of CC:

1 – with the closing of TV
2– with the opening of AV
3 – with the opening of PV
4 – with ZTEP for PT and RV
5 – with ZTEP for CS (minimum) and SS (middle)
6 – with the closing of PV
7 – with the closing of AV
8 – with the opening of MV
9 – with ZTEP for RA, CS, PT, SS
10(1) – with the closing of TV
1(10) – Characteristic points of PW (V.V. Boronoev)

A(Q) – isometric ventricular relaxation
B(Q) – actual ventricular diastole
C(LV) – LV diastole
A(LV) – isometric relaxation of LV
B(LV) – actual LV diastole
C(RV) – RV diastole
A(RV) – isometric relaxation of RV
B(RV) – actual RV diastole
D(Q) – isometric ventricular contraction
E(Q) – actual ventricular systole
F(LV) – LV systole

D(LV) – LV isometric contraction period
E(LV) – actual LV systole
F(RV) – RV systole
D(RV) – RV isometric contraction period
E(RV) – actual RV systole
G(AV) – opening of AV
H(PV) – closing of PV
G(TV) – opening of TV
H(AV) – closing of AV
G(MV) – opening of MV
H(TV) – closing of TV
H(MV) – closing of MV
G(PV) – opening of PV

Asd1 – asynchronous period of ventricular systole-diastole -1
Ss1 – synchronization period of isometric ventricular contraction -1
As1 – asynchronous period of ventricular systole -1
Ss2 – synchronization of the actual ventricular systole -2
Asd2 – asynchronous period of ventricular systole-diastole -2
Crd– asynchronous period of isometric relaxation of the ventricles
Ad1 – asynchronous period of ventricular diastole -1
Cd1 – period of synchronization of ventricular diastole-1
– coincidence of invasive and non-invasive points (V.V.Boronoev)

Taking into account the stability of a single PW, we believe it is possible and expedient to carry out accurate projection marking of the whole set of synchronized hemodynamic curves throughout the entire graph of PPW. Full marking with precise binding of each segment of PW to the stages of specific wave and hemodynamic processes of the vascular bed as a whole will allow determining their correspondence at any time, using a non-invasive method of access. Detailed segmental marking of PW by the proposed method will allow creating an address cataloging the shapes and segments of PW for the norm (including under different loading regimes) and various types of clinical pathology based on objective data obtained by invasive methods. We believe that the key signs of targeted diagnostic interpretation will be, in addition to variations in the shape of PW itself, changes in the shape, duration, angularity and other characteristics of the corresponding segments of the pulse curve, which are the projection mapping of the hemodynamics of a particular vascular or organ process.

A similar study by catheterization was conducted in the group of patients (n=247) with acquired heart defects. Below are given the hemodynamic data of patient S., a 47-year-old man with a mixed aortic valve disease with prevalence of aortic valve stenosis, and stage 2 pulmonary hypertension (Table 2).

The defeat of the valvular apparatus of the heart with acquired defects leads to a loss of the sealing of the chambers of the heart in the phase of isometric contraction of the ventricles due to a disturbance in the closing ability of the valves, in combination with excessive pressure in the heart chambers (stenosis, insufficiency). The consequence is a disturbance in the controlling waveforms generated by the myocardium, as well as distortion of the temporal relationships of the phases and periods of CC (including changes in the topology and composition of the "pressure equalization zones"), leading to an imbalance and desynchronization of the regulation of hemodynamic and wave processes, both in the chambers of the heart .

Note the change in the shape of PW and the radical differences between the projection coincidences of the phases of CC and PPW (Table 2) at the characteristic points: Point 1: with the closing of TV; Point 2: with the opening of AV; Pont 3: with the opening of PV; Point 4: with ZTEP for PT and RV; Point 5: with ZTEP for CS (minimum) and SS (middle), Point 6: with the closing of PV; Pont 7: with the closing of AV; Point 8: with the opening of MV; Point 9: with ZTEP for RA, CS, PT, SS; Point 10: with the closing of TV.

A radical difference between the norm (Table 1) and aortic valve disease (Table 2) in the whole spectrum of coincidences of the key indexes of the CC phases with the characteristic points and projection segments of PPW will be examined in detail in the further works.

Conclusion

Synchronizing the graphic curves of the central, arterial, and venous pressure that were obtained by an invasive method during catheterization with ECG and a detailed single-pulse curve obtained by a non-invasive method on the radial artery, revealed the projection coincidence of the key points of PW with the sequence of key phases of CC in the norm.

The coincidence of the key points of PPW, considered as reference points, directly with the key points of cardiovascular hemodynamics and the peak values of ZTEPs, enables the targeted marking of the entire length of the radial pulse wave, where each anacrotic/dicrotic segment is considered as a projection element of the wave and hemodynamic processes of the vascular bed as a whole, both in the norm and under pathological conditions.

The exact attachment of each segment of PPW to a specific fragment of the wave/hemodynamic process of the vascular bed allows determining their relationships and correspondences in any time interval using non-invasive access methods, where the diagnostic set, in addition to PW itself, is the shape, duration, angularity and other characteristics of the corresponding segments of PW, which are the projection mapping of the hemodynamics of a particular vascular or organ process.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 182-185
http://dx.doi.org/10.21103/Article8(3)_OA2

ORIGINAL ARTICLE

Cardiology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Relationship Between Indices of Oxidative Stress, Endothelial Dysfunction and Chaperone Activity and the Severity of Coronary Atherosclerosis

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Abstract

The aim of this research was to study the relationship between the indices of oxidative stress, endothelial dysfunction and chaperone activity of proteins with the severity of coronary atherosclerosis. In patients with coronary heart disease, we found gender-related differences in the severity of coronary atherosclerosis. Significant differences in the indices of oxidative stress, endothelial dysfunction and chaperone activity were revealed depending on the severity of coronary atherosclerosis and the type of atherosclerotic lesion. The determination of studied parameters can serve as a good indicator of the severity of coronary atherosclerosis.**(International Journal of Biomedicine. 2018;8(3):182-185.)**

Key Words: coronary heart disease • endothelial dysfunction • oxidative modification of proteins • superoxide dismutase

Abbreviations

ADPH, aldehyde derivative of DNPH; **CHD**, coronary heart disease; **CAG**, coronary angiography; **DNPH**, 2.4-dinitrophenylhydrazine; **Hsp27**, heat shock protein 27; **OS**, oxidative stress; **KDPH**, ketone derivative of DNPH; **OMP**, oxidative modification of proteins; **PCC**, protein carbonyl content; **SOD**, superoxide dismutase.

Introduction

Although the complex mechanisms of the development of coronary atherosclerosis are not completely understood, recent advances have established a fundamental role for inflammation and oxidative stress in this process.[1-4] Oxidative modification of low-density lipoprotein has a central role in the initial phase of the atherosclerotic process. In CHD, a decrease in intracellular protection against reactive oxygen species, primarily due to a decrease in the level of SOD—the key enzyme of the antioxidant system—has been demonstrated by a number of researchers.[5] The imbalance between pro-oxidants and antioxidants leads to oxidative damage of proteins—an early indicator of the cell damage.[6,7] Oxidants induce the post-translational modification of proteins.[8] Peroxide treatment of rat cardiac myocytes rapidly induces phosphorylation of Hsp27, which increases the activity of Hsp27.[9] The activation of Hsp70 may play a role in protecting the cells against oxidative stress and inflammatory damage.[10] In addition, homocysteine (Hcy) is an established biomarker for endothelial dysfunction and vascular disease, and is linked to increased OS.[11]

The aim of this research was to study the relationship between the indices of oxidative stress, endothelial dysfunction and chaperone activity of proteins with the severity of coronary atherosclerosis.

Materials and Methods

We examined 93 CHD patients (33 women and 60 men, mean age of 61.8±8.1) who had coronary atherosclerosis of

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varying degrees, according to coronary angiography (CAG).

Exclusion criteria were myocardial infarction within previous 3 months, diabetes mellitus requiring insulin treatment, arterial hypertension (blood pressure >159/99 mmHg), hypotension (blood pressure <100/60 mmHg), atrial fibrillation and life-threatening ventricular arrhythmias, valvular heart disease, long time treatment with lipid-lowering drugs and ACE inhibitors, chronic heart failure (NYHA FC>II), chronic renal and hepatic failure.

All patients underwent the following examinations: assessment of traditional risk factors (high blood pressure, smoking, body mass index, diabetes), physical examination, clinical and biochemical laboratory methods, 12-lead ECG, echocardiography, Holter ECG monitoring, treadmill test, and coronary angiography. Blood samples were obtained in the morning after a 12h overnight fast. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), ALT, AST, CFK, apolipoprotein A (ApoA), apolipoprotein B (ApoB), high-sensitivity C-reactive protein (hsCRP), fibrinogen, ESR, WBC were determined in plasma using "Daytona» analyzer (RANDOX, Ireland).

CAG was performed by the Judkins technique using General Electric Innova 3100 (GE Healthcare, USA). In collegial analysis of CAG data, we determined the type of coronary blood supply and noted the number of affected coronary arteries, localization, and type of stenotic narrowing. To assess the degree of narrowing of vessels, a visual assessment was used with the following characteristics: normal coronary artery, changing contours of artery without determining the degree of stenosis, luminal stenosis as minimal (<25% stenosis), mild (25% to 49% stenosis), moderate (50% to 69% stenosis), severe (70% to 100% stenosis).[12] Finding a ≥70%, "severe" stenosis, was an indicator for revascularization. According to the degree of stenosis, 3 groups were formed: Group I included 22 patients with minimal-mild stenosis; Group II included 50 patients with moderate stenosis; Group III included 21 patients with severe stenosis. Morphologic characteristics of the lesion were evaluated by applying ACC/AHA morphology criteria[13]: Type A in 15 patients, Type B – in 57 patients, and Type C – in 21 patients. All patients were divided also into 3 groups according to the number of affected vessels: Group 1 included 22 patients (3 men and 19 women) with insignificant stenotic lesions; Group 2 included 41 patients (30 men and 11 women) with single- or two-vessel lesions; Group 3 included 30 patients (26 men and 4 women) with three-vessel or more multivessel lesions.

OMP was identified by PCC. Carbonyl groups formed from oxidation with 2.4-dinitrophenylhydrazine (DNPH) were estimated using the methods by Levine et al.[14] with modifications by Dubinina et al.[15] The assay is based on the spectrophotometric detection of the reaction between DNPH with protein carbonyl to form protein hydrazone. The optical density of 2,4-dinitrophenylhydrazones derivates was recorded on an SF-36 spectrophotometer. The optical density of aldehyde- and ketone derivatives of a neutral character was recorded at 356nm and 370nm, respectively (ADPHn and KDPHn). The optical density of aldehyde- and ketone derivatives of a basic character was recorded at 430nm and 530nm, respectively (ADPHb and KDPHb). The SOD activity was determined by the spectrophotometric method.

The serum level of L-Hcy was determined by EIA using «Axis-Shield» test kit. Chaperone activity was measured by monitoring the DTT-induced aggregation of insulin in the absence and presence of Hsp27.[16]

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Chicago, IL). Quantitative parameters are presented as Median (Me) and 25th and 75th percentiles as Inter Quartile Range (IQR). The Kruskal-Wallis H test was used to compare medians among 3 comparison groups. Spearman's correlation coefficient (r_s) was used to determine the strength and direction of association between two variables. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by the Voronezh State Medical University Ethics Committee. Written informed consent was obtained from all patients.

Results and Discussion

A statistical relationship between the sex and the number of affected vessels was determined: an insignificant lesion was more common in women, two-vessel lesions - in men (72.7%); the three-vessel or multivessel lesions were predominant in men (87.5%) (Figure 1). Comparison of the indices in the three groups revealed significant differences in the L-Hcy level (P=0.000), SOD activity (P=0.015), chaperone activity (P=0.011), blood levels of ADPHn (P=0.003) and KDPHn (P=0.028) (Table 1).

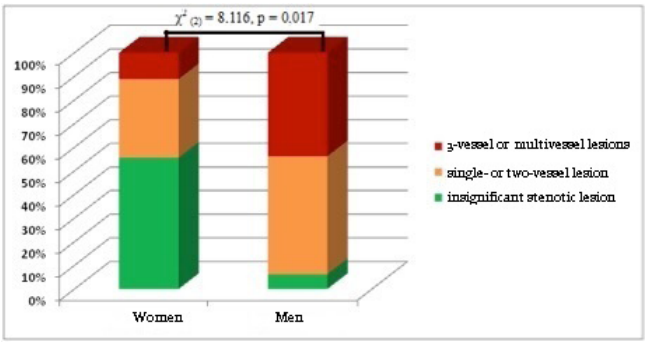

Fig. 1. *Statistical relationship between the sex and the number of affected vessels.*

The level of shaper activity was 78.8% [60.6%; 82.5%], 72.4% [67.4%; 75.3%] and 68.3% [60%; 68.3%] in Groups I, II, and III, respectively. At the same time, the level of L-Hcy was 9.6μmol/ml [8.1μmol/ml; 9.9μmol/ml], 10.2μmol/ml [9.8μmol/ml; 10.7μmol/ml] and 11.4 mol/ml [10.4μmol/ml; 12μmol/ml] in Groups I, II, and III, respectively. The highest SOD activity was found in Group I: 41.7% [39.1%; 47.3%]. We revealed a significant difference in this indicator among Groups I, II, and III (P=0.003). When evaluating PCC, a significant difference was established between these groups in levels of ADPHn (P=0.001) and KDPHn (P=0.025). The levels of ADPHb and KDPHb were without significant differences.

Table 1.

Biomarkers of CHD and the number of affected vessels

Variable	Group 1 (n=22)	Group 2 (n=41)	Group 3 (n=30)	*P*-value
L-Hcy, μmol/ml	9.58 8.11; 9.97]	10.43 [10.10; 11.39]	12.3 [11.61; 12.49]	0.000
SOD acivity, %	41.7 [39.0; 47.4]	35.2 [32.8; 36.0]	32.3 [30.7; 39.2]	0.015
Chaperone activity, %	78.8 [60.6; 82.5]	68.3 [66.7; 76.9]	60.0 [55.4; 68.2]	0.011
ADPHn, IU/mg	22.3 [21.5; 23.3]	24.8 [23.5; 25.7]	27.0 [24.8; 27.9]	0.003
KDPHn, IU/mg	19.9 [18.3; 20.8]	21.0 [20.3; 22.3]	23.5 [20.8; 25.0]	0.028
ADPHb, IU/mg	10.7 [9.2; 11.8]	11.3 [10.9; 11.8]	10.8 [9.6; 12.1]	0.493
KDPHb, IU/mg	6.2 [2.4; 9.2]	6.8 [6.6; 8.8]	8.8 [7.2; 9.5]	0.234

Chaperone activity depended on the type of morphologic characteristics of the lesion (*P*=0.002). Thus, the lowest activity was observed in patients with Type C. Similar changes were detected for SOD activity; a significant difference (*P*=0.004) was also revealed between the groups with Type A, B, and C. When assessing the level of L-Hcy and PCC, the opposite tendency was identified: the more complicated the atherosclerotic plaque, the higher the studied parameter levels. At the same time, significant differences were found in the level of L-Hcy (*P*=0.000), ADPHn (*P*=0.05) and KDPHn (*P*=0.001).

Correlation analysis revealed the relationships between the number of affected arteries and the blood levels of L-Hcy (r_s=0.843, *P*=0.000), ADPHn (r_s=0.671, *P*=0.002), KDPHn (r_s=0.544, *P*=0.005), SOD activity (r_s=-0.545, *P*=0.005), and chaperone activity (r_s= -0.616, *P*=0.001); the correlations with the levels of ADPHb and KDPHb were weak and not significant (r_s=-0.076, *P*=0.717 and r_s=0.309, *P*=0.132, respectively). The same patterns were found in the group of patients with severe stenosis (Group III).

Conclusion

In CHD patients, we found gender-related differences in the severity of coronary atherosclerosis. Significant differences in the indices of OS, endothelial dysfunction and chaperone activity were revealed depending on the severity of coronary atherosclerosis and the type of atherosclerotic lesion. The determination of studied parameters can serve as a good indicator of the severity of coronary atherosclerosis.

Competing interests

The authors declare that they have no competing interests.

Sources of Funding

This work was partially supported by the Council on Grants of the President of the Russian Federation for State Support of Young Scientists and Leading Scientific Schools (Grant MK-552.2018.7).

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International Journal of Biomedicine 8(3) (2018) 186-191
http://dx.doi.org/10.21103/Article8(3)_OA3

ORIGINAL ARTICLE

Psychiatry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Association of the *HTR2A* T102C SNP with Weight Gain and Changes in Biochemical Markers in Patients Receiving Antipsychotics

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Abstract

The purpose of our research was to study the association of the *HTR2A* T102C (rs6313) SNP with anthropometric and biochemical markers in patients treated with typical and atypical antipsychotics in monotherapy mode.

***Materials and methods*:** One hundred and seventeen white inpatients (95 men and 22 women) with F2 disorders (ICD-10, 1995) were enrolled in the study. All patients were divided into two groups by the antipsychotic class with which they were treated (Group 1 included 40 patients treated with typical antipsychotics; Group 2 included 77 patients treated with atypical antipsychotics) and two subgroups by weight change criteria during the study (Subgroup 1 included patients with weight change >6%; Subgroup 2 included patients with weight change <6%). The following examinations were performed: physical examination, anthropometric measurements (BMI. WC, TC), clinical examination, blood test, and genotyping for the *HTR2A* T102C (rs6313) SNP.

Results: There were no statistically significant differences in the distribution of genotypes of the *HTR2A* T102C (rs6313) SNP between Group 1 and Group 2 (P>0.05). Kruskal-Wallis one-way analysis of variance between subgroups showed statistically significant differences between carbamide levels in the second visit in Group 2 (P=0.02). We showed statistically significant differences between TT and CT genotypes of the *HTR2A* T102C SNP: carbamide level was greater in TT carriers (P=0.02). The strength of associations and risks between alleles of the *HTR2A* T102C SNP and antipsychotic-induced weight change were as follows: OR_C=0.49; CI_C [0.25; 0.95]; RR_C=0.58 CI_C [0.35; 0.97]; OR_T=2.03; CI_T [1.05; 3.94]; RR_T=1.7 CI_T [1.02; 2.81].

Conclusion: Our results of the pilot pharmacogenetic studies show an association of the T allele carriage of the *HTR2A* T102C (rs6313) SNP with risk of antipsychotic-induced weight gain. The continuation of this study and an increase in the sample size will allow establishing valid pharmacogenetic markers for the risk of antipsychotic-induced weight gain. **(International Journal of Biomedicine. 2018;8(3):186-191.)**

Key Words: antipsychotics • single nucleotide polymorphism • serotonin receptor 2A • *HTR2A* gene • weight gain

Abbreviations

FPG, fasting plasma glucose; **FGAs**, first-generation antipsychotics; **HWE**, Hardy-Weinberg equilibrium; **HDL-C**, high-density lipoprotein cholesterol; **LDL-C**, low-density lipoprotein cholesterol; **MetS**, metabolic syndrome; **SGAs**, second-generation antipsychotics; **SNP**, single nucleotide polymorphism; **TC**, total cholesterol; **VLDL-C**, very-low-density lipoprotein cholesterol.

Introduction

Dysfunction of the dopamine system has been known to underlie the pathophysiology of schizophrenia since the 1960s. FGAs, especially high potency drugs such as haloperidol, mainly bind to D2 receptors.[1-3] All SGAs tightly bind to serotonin (5-hydroxytryptamine, 5-HT) receptor 2A relative to the dopamine D2 receptor, and this

was once thought to be one of the defining characteristics of "atypicality" of SGAs.[1] In general, atypical agents have an enhanced 5-HT2A/D2 affinity ratio and that helps explain why typical and atypical agents may have different clinical effects.[4] The atypical antipsychotics generally have additional affinities for a variety of neurotransmitter receptor subtypes (serotonergic, dopaminergic, histaminergic, adrenergic, and muscarinic acetylcholine receptor).[5] FGAs are characterized by extrapyramidal symptoms, and hyperprolactinemia and, to a lesser extent, metabolic disorders; SGAs are more associated with weight gain, the appearance of type 2 diabetes, cardiovascular diseases, and the development of MetS.[6,7] Results of numerous studies show that antipsychotic induced weight gain occurs in 12-16 weeks.[8] It was found that patients with psychotic disorders are more likely to suffer from obesity than the general population.[9] Epidemiological studies have shown that patients with schizophrenia are 2.5 times more likely to die from cardiovascular complications and their estimated life span is 20% less than the general population.[10] The receptor antagonism at the central level of regulation, caused by taking antipsychotics, provokes an increased appetite and reduced possibility of feeling sated.[11]

5-HT2A is one of the most abundantly expressed serotonin receptors in the brain, with high levels in the cerebral cortical areas, hippocampus, nucleus accumbens, and caudate nucleus.[10] This receptor belongs to G-protein-coupled receptors and is the primary excitatory receptor of serotonin, mainly acting at post-synaptic neurons. The 5-HT2A receptor gene (*HTR2A*) is located on chromosome 13(13q14.2). The expression of *HTR2A* is regulated by several functional polymorphisms,[13,14] among which T102C (rs6313) is the most studied SNP in the gene. Compared with the T allele, the C allele leads to lower receptor expressions[13] and lower receptor binding potentials,[15] and therefore reduces excitation at post-synaptic neurons.[16] Significant association between the *HTR2A* T102C (rs6313) SNP and antipsychotic weight gain was found during treatment with olanzapine and risperidone in Japanese and Chinese populations.[17]

The purpose of our research was to study the association of the *HTR2A* T102C (rs6313) SNP with anthropometric and biochemical markers in patients treated with typical and atypical antipsychotics in monotherapy mode.

Materials and Methods

Participants

One hundred and seventeen white inpatients (95 men and 22 women) with ICD-10 Diagnosis Codes F20 (92/79%), F20.2 (1/0.85%), F20.6 (3/2.6%), F20+F10.2 (2/1.7%), F21.8 (2/1.7%), F22.8 (3/2.6%), F23 (6/5.1%), F23.1 (3/2.6%), and F25.1(4/3.4%) were enrolled in the study. Mean age of the disease onset was 24.56±1.95 years; mean age of the first medical help was 26.5±1.65 years; mean age of the first antipsychotic therapy was 25.7±1.7 years. The period of participation in the study was 8.36±1.13 weeks. Drugs taken by the patients are presented in Table 1.

All patients were divided into two groups (Table 2) by the antipsychotic class with which they were treated (Group 1 included 40 patients treated with typical antipsychotics; Group 2 included 77 patients treated with atypical antipsychotics) and two subgroups (Table 3) by weight change criteria during the study (Subgroup 1 included patients with weight change >6%; Subgroup 2 included patients with weight change <6%).

Table 1

Drugs taken by the patients in the study

Typical antipsychotics		Atypical antipsychotics	
Drug	Frequency (%)	Drug	Frequency (%)
Haloperidol	28 (70)	Olanzapine	15 (19.5)
Zuclopenthixol	6 (15)	Risperidone	17 (22.1)
Triphtazinum	6 (15)	Quetiapine	12 (15.6)
		Asenapine	5 (6.5)
		Clozapine	11 (14.2)
		Paliperidone	6 (7.8)
		Aripiprazole	3 (3.9)
		Sertindole	5 (6.5)
		Sulpiride	1 (1.3)
		Amisulpride	1 (1.3)
		Aminasine	1 (1.3)

Table 2

The distribution of patients by the antipsychotic drugs

Patients	Group 1		Group 2	
	n	%	n	%
Total	40	34.1	77	65.9
Male	29	72.5	66	85.7
Female	11	27.5	11	14.3

Table 3.

The distribution of patients by weight change criteria during the study

Variable	Group 1		Group 2		Total	P- value
	Subgroup 1	Subgroup 2	Subgroup 1	Subgroup 2		
Sample size	8	28	17	46	99	0.63
Median age, yrs	33.4 (24.9;41.09)	34.1 (29.9;38.3)	29.0 (24.9;33.1)	35.1 (32.1;38.1)		0.25
Gender (M/F)	5/3	20/8	16/1	39/7	99	0.11
Median period of participation, weeks	5.6 (4.1;7.1)	7.2 (5.9;8.5)	8.4 (6.2;10.4)	9.2 (6.8;11.6)		0.84
Intake of benzodiazepine in anamnesis (Yes/No)	3/3	9/13	7/7	20/9	71	0.24
Smoking (Yes/No)	5/3	16/7	8/8	20/18	85	0.54

Study design

The research consisted of two visits: the first during enrollment in the study, the second when the observation was completed. All patients signed an informed consent document before enrolling in the project. The following examinations were performed: physical examination, anthropometric measurements (BMI. WC, TC), clinical examination, blood test (ALT, AST, FPG, VLDL-C, LDL-C, HDL-C, TC, triglycerides (TG), total protein, albumin, creatinine, uric acid, carbamide) and molecular-genetic evaluation. Genomic DNA was isolated from peripheral leukocytes with "Hemolytic" reagent (InterLabService, Russian Federation) for pre-processing of whole peripheral and umbilical blood and with e*xtraction* kit Ribo-prep (InterLabService, Russian Federation).Genotyping for the *HTR2A* T102C (rs6313) SNP was performed using real-time PCR by the RotorGene 6000 (Quagen, Germany) with an HTR2A kit according to the manufacturer's protocol (Syntol, tge Russian Federation).

Statistical Analysis

Descriptive statistics were used to summarize the data. Shapiro - Wilk test was used for normality test. Chi-square and Fisher's exact tests were used to determine the association between categorical measure including allele and genotype. T test or paired T test were used for comparison between two groups with a normal distribution of the quantitative characteristic. Wilcox test or paired Wilcox test were used as nonparametric alternative. Analysis of variance and Tukey post hoc test were used for comparison between 3 groups with a normal distribution of the quantitative characteristic and homogeneous dispersion (established by Levene test). Kruskal - Wallis one-way analysis of variance and Dunn post hoc test with Bonferroni adjustment were nonparametric alternative. Correlation was measured with Spearman rank correlation coefficient. The strength of the associations was expressed as odds ratio (OR) with 95% confidence interval (CI) and 95% credible interval (CrI), relative risk was expressed as risk ratio (RR). Binomial logistic model was also performed. The quality of the model (Area Under the Curve (AUC) specificity and sensitivity) was measured by receiver operating characteristic (ROC) analysis. For all tests, a probability value of $P<0.05$ was considered statistically significant. Statistical analysis was performed by R programming language with IDE Rstudio. LePAC. Three exact probability tests for departure from HWE due to heterozygote excess, heterozygote deficit and omnibus probability test were carried out using GENEPOP (v. 4.7.0)

Results and Discussion

There were no statistically significant differences in the distribution of genotypes of the *HTR2A* T102C (rs6313) SNP between Group 1 and Group 2 ($P>0.05$) (Table 4). It was not possible to reject HWE because of heterozygote excess ($P=0.02$). Dynamics of the biochemical markers is shown in Tables 5 and 6 (*P* value for differences between the first and second visits in whole group).

Kruskal-Wallis one-way analysis of variance between subgroups showed statistically significant differences between carbamide levels in the second visit in Group 2 ($P=0.02$) (Tables 5 and 6). A Dunn post hoc test with Bonferroni adjustment showed statistically significant differences between TT and CT genotypes of the *HTR2A* T102C (rs6313) SNP: carbamide level was greater in TT carriers ($P=0.02$).

Table 4.

Genotype distribution in the groups

Group	Genotype			Allele	
	CC	CT	TT	T	C
Group 1	6 (5.12%)	28 (23.93%)	6 (5.12%)	40 (17.0%)	40 (17.0%)
Group 2	11 (9.4%)	49 (41.9%)	17(14.52%)	83 (36.0)	71 (30.0%)
Fisher exact test	$P = 0.70$				

Table 5.

Summarize data in Group 1, Me (Q_{25};Q_{75})

Variable		Genotype			P- value
		CC	CT	TT	
Weight, kg	FV	60.8(59.2;79.9)	68.5(62.2;81.8)	79(66.2;86.6)	0.7
	SV	60.0(56;83)	71(61;80)	77.4(70.7;86.6)	
FPG, mmol/L	FV	5.17(5.11;5.24)	4.96(4.66;5.48)	5.61(5.12;6.1)	0.5
	SV	5.61(4.87;5.89)	4.84(4.57;5.19)	4.89(4.68;5.31)	
TC, mmol/L	FV	3.98(3.69;4.18)	4.84(4.37;5.26)	4.42(4.06;4.60)	0.6
	SV	3.18(3.06;4.28)	4.51(4.24;5.20)	4.47(3.75;4.57)	
TG, mmol/L	FV	0.68(0.63;1.06)	1.47(1.02;1.90)	1.59(1.19;1.74)	0.1
	SV	0.73(0.69;1.00)	1.23(0.95;1.55)	1.52(1.49;1.58)	
VLDL-C, mmol/L	FV	0.31(0.28;0.48)	0.53(0.45;0.71)	0.62(0.55;0.68)	0.2
	SV	0.33(0.31;0.45)	0.54(0.35;0.58)	0.46(0.54;0.62)	
LDL-C, mmol/L	FV	2.30(2.22;3.10)	2.90(2.55;3.57)	2.66(2.43;3.20)	0.1
	SV	1.65(1.59;2.63)	2.74(2.48;3.62)	2.85(1.92;3.12)	
HDL-C, mmol/L	FV	1.20(0.95;1.46)	1.14(0.9;1.24)	0.77(1.01;1.16)	0.6
	SV	1.20(1.07;1.20)	1.10(0.94;1.22)	0.98(0.75;1.06)	
ALT, u/L	FV	16(14;20)	24.0(18.4;40.6)	17.5(13;30.17	0.03
	SV	14(9;18)	25.0(18.3;28.6)	15.85(12.75;28.1)	
AST, u/L	FV	26(22;29)	31.0(22.7;42.8)	20.95(18.7;44.1)	0.004
	SV	17(16;19)	23.3(18.7;30.2)	20.8(18.15;23.07)	
Albumin, g/L	FV	51.0(48.0;51.1)	47.0(44.9;48.6)	48.8(46.87;50.5)	0.84
	SV	48(45.5;53.5)	47.0(44.5;49.8)	51.0(48.27;52.75)	
Total protein, g/L	FV	77(76;82)	73.0(69.75;76.9)	73.8(72.4;75.57)	0.3
	SV	73(71;75)	71.0(68.7;75.2)	74.55(71.52;75.07)	
Carba-mide, mmol/L	FV	3.80(2.80;3.90)	3.45(3.11;3.91)	3.99(3.38;4.39)	0.4
	SV	3.00(2.60;4.00)	3.74(2.95;4.23)	3.79(3.45;4.13)	

FV – the first visit, SV – the second visit

Table 6

Summarize data in Group 2, Me (Q_{25};Q_{75})

Variable		Genotype			P- value
		CC	CT	TT	
Weight, kg	FV	72.1(69.4;84)	69.3(62.0;81.1)	73(62;77)	0.004
	SV	74.0(71.2;84.5)	72.5(62.5;82.0)	75.5(67.3;77.9)	
FPG, mmol/L	FV	5.69(5.48;5.84)	5.30(4.87;5.68)	5.40(5.05;5.60)	0.01
	SV	5.18(4.85;5.31)	5.14(4.66;5.5)	4.88 4.47;5.27)	
TC, mmol/L	FV	4.33(3.91;5.93)	4.75(4.04;5.74)	4.44(3.66;6.19)	0.99
	SV	4.50(4.13;5.18)	4.82(4.20;5.48)	4.86(3.64;5.42)	
TG, mmol/L	FV	1.39(1.16;1.54)	1.39(1.07;1.95)	1.361.04;2.14)	0.1
	SV	1.82(1.17;2.02)	1.57(0.96;2.05)	1.5(1.07;2.52)	
VLDL-C, mmol/L	FV	0.53(0.45;0.54)	0.5(0.41;0.61)	0.49(0.34;0.69)	0.1
	SV	0.53(0.40;0.67)	0.52(0.35;0.73)	0.59(0.43;1.57)	
LDL-C, mmol/L	FV	2.56(2.10;2.98)	2.95(2.37;3.58)	2.55(2.01;3.65)	0.8
	SV	3.04(2.46;3.44)	2.93(2.67;3.60)	2.99(2.50;3.39)	
HDL-C, mmol/L	FV	1.30(1.00;1.56)	1.19(0.97;1.33)	1.33(1.24;1.40)	0.5
	SV	0.96(0.91;1.05)	1.15(0.95;1.32)	1.26(1.07;1.40)	
ALT, u/L	FV	32.45(19.8; 53.07)	22.4(16.0;30.3)	25.2(17.7;31.0)	0.8
	SV	31.20(19.50;37.55)	22.55(14.47;32.25)	23.0(17.52;30.25)	
AST, u/L	FV	38.0(27.77;45.05)	28.5(21.0;35.2)	27.0(23.8;31.0)	0.4
	SV	26.0(18.25;33.5)	24.5(21.75;35.55)	21.0(19.5;30.65)	
Albumin, g/L	FV	50.2(46.6;50.9)	48.4(46.0;50.5)	47.0(46.45;49.40)	0.2
	SV	47.1(45.95;48.1)	48.6(45.4;50.7)	50.1(48.07;50.5)	
Total protein, g/L	FV	75.6(73.2;76.07)	73.5(70.0;77.0)	72.55(70.02;79.00)	0.7
	SV	77.0(72.05;77.50)	72.8(69.37;77.25)	71.85(69.55;74.75)	
Carba-mide, mmol/L	FV	4.29 (3.45;5.17)	3.90 (3.35;4.30)	3.80 (3.40;4.1)	0.42
	SV	4.0 (3.74;4.21)	3.8 (3.48;4.40)	4.75 (4.35;5.17)	

FV – the first visit, SV – the second visit

Triglyceride levels in the second visit were correlated with LDL-C (P=0.9), HDL-C (r=0.8), and VLDL-C (r=0.9) levels in the first visit, and with glucose (r=0.84) and VLDL-C (r=0.9) levels in the second visit; TC levels in the second visit were correlated with LDL-C levels in the second visit (r=0.91).

The strength of associations and risks between alleles of the *HTR2A* T102C SNP and antipsychotic-induced weight change were as follows: OR_C=0.49; CI_C [0.25; 0.95]; RR_C=0.58 CI_C [0.35; 0.97]; OR_T=2.03; CI_T [1.05; 3.94]; RR_T=1.7 CI_T [1.02; 2.81]. In Bayesian statistics: OR_C=0.499. CrI_C=[0.256; 0.951]; OR_T=2.003; CrI_T=[1.052; 3.904]. Fisher exact test: P =0.04.

We also performed a binomial logistic regression (Table 7), where the dependent variable was the binomial factor (weight change >6% during research, Yes/No), and predictors were genotypes of the *HTR2A* T102C SNP and antipsychotics (Haloperidol, Olanzapine, Risperidone, Quetiapine) and their daily dosage. The binomial logistic model was described by ROC analysis (Figure 1). Cutoff for specificity and sensitivity of the logistic model was 0.53. At this level, specificity of the model was 0.80 and sensitivity of the model was 0.80.

Table 7.

Binomial logistic regression coefficients

Variable	Estimate	Standard Error	z value	Pr (>|z|)
Intercept	3.4518	2.7262	1.266	0.20
rs6313 CT	2.6672	1.7791	1.499	0.13
rs6313 TT	4.8865	2.4338	2.008	0.04 *
Olanzapine	- 2.7532	5.2068	- 0.529	0.59
Risperidone	26.7116	4941.9003	0.005	0.99
Quetiapine	- 4.8765	3.5435	- 1.376	0.16
Dosage (in all)	- 0.6655	0.3143	- 2.118	0.03 *
Dosage of Olanzapine	0.3208	0.5887	0.545	0.58
Dosage of Risperidone	-7.9641	1235.4747	-0.006	0.99
Dosage of Quetiapine	0.6660	0.3149	2.115	0.03 *
*Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1* *Null deviance: 46.070 on 33 degrees of freedom* *Residual deviance: 28.018 on 24 degrees of freedom* *AIC: 48.018*				

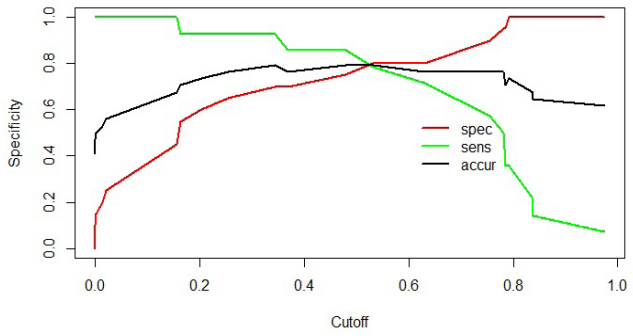

Fig. 1. *ROC analysis curve: AUC = 0.88; Concordance = 0.86.*

HWE was not observed in the prospective group, but it was observed (P=0.34) in the population group (n=229 patients) with same inclusion criteria (except monotherapy mod). Our assumption is that heterozygotes carriers of the *HTR2A* T102C (rs6313) SNP are more stable in monotherapy and needed changes in therapy less often. We are going to increase the prospective sample size to get more information. Received results showed that different classes (FGAs, SGAs) provided different changes in biochemical markers and weight. Atypical antipsychotics led to statistically significant differences in body weight and glucose concentration, which are considered as pre-diabetic changes and could be part of MetS. Mechanisms of these changes are widely

discussed. On the other hand, FGAs provided statistically significant differences in enzyme (AST, ALT) activity. We did not measure iso-enzymes of the hepatic fraction, so we cautiously put forward an assumption about hepatotoxicity of typical antipsychotics. TT genotype carriers of the *HTR2A* T102C (rs6313) SNP, who received atypical antipsychotics, had a higher concentration of carbamide. Carbamide is the chief nitrogenous end product and is dependent on protein intake. In another study, it was shown that the *HTR2A* T102C (rs6313) SNP affects food behavior and that TT carriers prefer high-protein food.[18]

Our study showed that the T allele carriers of the *HTR2A* T102C (rs6313) SNP have increased risk of antipsychotic-induced weight change. The intercept in our regression model consisted of the CC genotype of the *HTR2A* T102C (rs6313) SNP, haloperidol, and single dosage. Patients with the TT genotype of the *HTR2A* T102C (rs6313) SNP had significant differences in association with antipsychotic-induced weight change than CC carriers. Despite the synonymous substitution in this SNP, it can affect, by linkage, disequilibrium with the *HTR2A* 1438A/G SNP in the promoter region.[19] Also, changing Haloperidol to Quetiapine leads to higher OR of antipsychotic-induced weight change, with a positive d*ose-dependent* effect (mean dose of 318.75 mg/day). Similar results showed Brecher et al. (2007). Long-term treatment with quetiapine monotherapy was associated with moderate weight gain. Most weight gain occurs within the first 12 weeks of treatment and has no clear dose relationship.[20]

Conclusion

Our results of the pilot pharmacogenetic studies show an association of the T allele carriage of the *HTR2A* T102C (rs6313) SNP with risk of antipsychotic-induced weight gain. The continuation of this study and an increase in the sample size will allow establishing valid pharmacogenetic markers for the risk of antipsychotic-induced weight gain.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

We would like to express our sincere appreciation to Oleg V. Limankin, PhD, ScD, the head of St. Petersburg State Budgetary Healthcare Institution "P. P. Kashchenko Psychiatric Hospital №1" (St. Petersburg, the Russian Federation) and Farit G. Ziganshin, MD, the head of State autonomous healthcare institution “V. M. Bekhterev Republican clinical psychiatric hospital" of the Ministry of Health of Tatarstan Republic (Kazan, the Russian Federation). We also thank Dr. Nikita Khromov-Borisov (Federal State Budgetary Institution "V. A. Almazov Federal North-West Medical Research Centre", St. Petersburg, the Russian Federation) for valuable suggestions and helping in the statistical analysis.

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International Journal of Biomedicine 8(3) (2018) 192-196
http://dx.doi.org/10.21103/Article8(3)_OA4

ORIGINAL ARTICLE

Neurology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Pathogenetically Grounded Approach to the Treatment of Children who Underwent Perinatal CNS Lesions

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Abstract

The aim of this study was to evaluate, based on the analysis of neurobiochemical markers, the effectiveness of pathogenetically substantiated therapy for disorders of the psychomotor and physical development of children in the first year of life who underwent perinatal hypoxia.

Materials and Methods*:* The study included 419 patients (52% boys and 48% girls) aged from 1 to 6 months. The main group included 336 patients in the first year of life who received inpatient treatment for perinatal CNS damage of different degrees of severity. The main group was divided into two subgroups according to age: Group 1 (n=163) between the ages of 1 and 3 months and Group 2 (n=173) between the ages of 4 and 6 months. In accordance with the severity of the CNS lesion, the main group was also divided into 3 subgroups: mild degree (n=122), moderate degree (n=118), and severe degree (n=96). The control group included 83 apparently healthy children (n=43 between the ages of 1 to 3 months and n=40 between the ages of 4 to 6 months). The analysis of individual physical development of the children was carried out using Z scores (weight, age, head circumference) and centiles (*7* intervals ("corridors")) according to the WHO standard program WHO AnthroPlus, The concentrations of biochemical markers (L- Homocysteine, beta-NGF, S100 protein, angiotensin II) in the blood were evaluated in all children at admission, as a routine entry investigation. In accordance with a treatment regimen, the main group was also divided into 2 subgroups: subgroup A (n=170), patients who received therapy depending on a general clinical manifestation; and subgroup B (n=166), patients who received therapy depending on a dominant syndrome and variability of neurobiochemical markers**.**

Results*:* We found that Scheme B showed advantages for all studied neurobiochemical markers, with statistical significance for L-Hcy regardless of the age group. The positive dynamics were found in the ND severity against the background of Scheme B regardless of the age group and the degree of severity of the CNS lesion. Thus, the pronounced positive dynamics in the levels of neurotrophic and neurovascular markers of the CNS lesion in all age groups reflects the advantage of pathogenetic therapy. **(International Journal of Biomedicine. 2018;8(3):192-196.)**

Key Words: neurological deficit • nervous system • nerve growth factor • homocysteine

Abbreviations

AII, angiotensin II; **Hcy**, homocysteine; **CNS**, central nervous system; **ND**, neurological deficit; **NGF,** nerve growth factor; **SOCG**, Scale of the optimal course of gestation; **SOCD**, Scale of the optimal course of delivery.

Introduction

Despite the modern improvements in perinatal care with the introduction of various innovative treatment regimens for managing the revealed pathology in children during the first year of life, severe consequences of perinatal CNS lesions still persist at a high frequency.[1-3] According to the statistics of the Ministry of Health of Russia, since 2000 there has been more than a twofold increase in the rate of newborn encephalopathy, with perinatal hypoxia being the dominant factor in the formation of this pathology.[3,4] According to the Russian Federal State Statistics Service, for the last five years in the Central Federal District, the proportion of children in the first year of life with an officially registered diagnosis

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"consequences of perinatal injury" has grown annually by an average of 0.6% (i.e. several thousand new patients who require a profile diagnosis, observation, and appropriate treatment, both in the medical organization and at home). There is a need for an effective early diagnosis system to optimize the treatment of children who underwent perinatal hypoxia, taking into account the complex analysis of neuromarkers of the CNS lesion.[5-8] Modern standards for treatment of this condition are based on the syndromic approach, which is established during a neurological examination of the patient and, fundamentally, does not take into account the pathogenetic component in the development of a particular complex of neurological disorders.[9]

The microcirculatory insufficiency in childbirth and the antenatal period is the dominant link in the development of degenerative processes in CNS of newborns, which requires a more detailed analysis of the variability of neurovascular markers that reflect the formation of adaptation processes of the body.[3,5,10] The capillary endothelium of the brain is extremely sensitive to ischemic-hypoxic effects, and its pathology occupies leading positions in the formation of neurological disorders.[5,7] Homocysteine (Hcy) and angiotensin II (AII) are leading markers in the diagnosis of the pathomorphological state and function of the endothelium of the microcirculatory bed. They are released in high concentrations during damage to capillary structures, causing disruption of the passage of nerve impulses between neurons of the brain and myelination of the axonal structures of the white matter, that affects the metabolism of nerve cells and the regenerative potential of astrocytic glia, manifested by the variability in the blood levels of S100 protein and NGF.[6] An increase in the Hcy level is directly related to the activity of the enzyme cystathionine beta-synthase, which is involved in the metabolism of white substance myelin structures. Therefore, hyperhomocysteinemia is direct evidence of the impaired conduction and synergy of nerve impulses between neurons in brain structures.[7] An increase in the AII level is a direct consequence of hyperhomocysteinemia, which provokes a cytotoxic effect on the vascular endothelium, which in turn potentiates expression of prostacyclin derivatives, and which, through feedback, lead to an even greater spasm of the microcirculation network of CNS.[11]

The complexity of pathogenetic mechanisms involving the metabolism of neurobiochemical markers must be taken into account when choosing the optimal theraputic regimen for hypoxic-ischemic brain injury in order to stabilize microcirculation and myelin structures of the brain, and to reduce dysmetabolism and the consequences of the perinatal CNS lesions**.**

The aim of this study was to evaluate, based on the analysis of neurobiochemical markers, the effectiveness of pathogenetically substantiated therapy for disorders of the psychomotor and physical development of children in the first year of life who underwent perinatal hypoxia.

Materials and Methods

The study included 419 patients (52% boys and 48% girls) aged from 1 to 6 months. The main group included 336 patients in the first year of life who received inpatient treatment for perinatal CNS damage of different degrees of severity. The control group included 83 apparently healthy children (n=43 between the ages of 1 to 3 months and n=40 between the ages of 4 to 6 months).

Children in the control group passed standard clinical examinations in specified periods of observation at the stage of outpatient services. There were several obligatory criteria for patients to be included in the control group: absence of neurological symptoms, absence of a neurologist's supervision, and pharmacotherapy of neurological deviations during the first year of life.

The main group was divided into two subgroups according to age: Group 1 (n=163) between the ages of 1 and 3 months and Group 2 (n=173) between the ages of 4 and 6 months.

It should be noted that the age period of 1-3 months is characterized by a decrease in both neuronal loss and the severity of neurological disorders, which is especially important for the timely diagnosis of ND. In the age period of 4-6 months, there is an aggravation of neurodystrophic processes, a rupture of synaptic connections, and a disruption in the interaction of different areas of the brain. All of which determine a broader clinical picture of neurologic symptoms in this age group.

In accordance with the severity of the CNS lesion, the main group was also divided into 3 subgroups: mild degree (n=122), moderate degree (n=118), and severe degree (n=96).

To assess the risk factors and the dynamics of clinical manifestations of the perinatal CNS damage consequences, we evaluated the women's somatic health, reproductive and gynecological history, and peculiarities of the course and complications of pregnancy and childbirth, using a scale of the optimal course of gestation (SOCG) and a scale of the optimal course of delivery (SOCD).[11] To assess the severity of perinatal CNS damage, the Apgar score at birth was taken into account.[12] The consequences of the perinatal CNS damage according to its severity were established during a neurologic examination and evaluation of the children's neurological status with the subsequent formation of a list of dominant clinical symptoms.[9] The analysis of individual physical development of the children was carried out using Z scores (weight, age, head circumference) and centiles (*7* intervals ("corridors")) according to the WHO standard program WHO AnthroPlus,[13] which made it possible to assess the homogeneity of research groups on the studied criteria and markers.

The obtained Z-score data were correlated as follows: $0.672 \leq 0.676$ - "normal" physical development; $-1.28 \leq 0.671$ and $0.677 \leq 1.28$ - physical development "below average" and "above average," respectively; $1.89 \leq -1.29$ and $1.29 \leq 1.89$ -physical development "low" and "high,"; $z \leq -1{,}9$ and $1{,}9 \leq z$ - "very low" and "very high" physical development, respectively.[14]

The concentrations of biochemical markers in the blood were evaluated in all children at admission and after treatment. The serum level of L-Hcy was determined by EIA using «Axis-Shield» test kit. The serum level of the beta subunit of human NGF (beta-NGF) was measured by EIA (Beta-NGF,

«RayBio», Russia). S100 protein was evaluated by means of the ELISA method, with the Cobas e411 analyser **(**Roche Diagnostics GmbH, Germany) and reagents by BioKhimMak (Russia). The serum level of AII was determined by using EIA kits according to manufacturer instructions (BCM Diagnostics, Moscow, Russia).

In accordance with a treatment regimen, the main group was also divided into 2 subgroups: subgroup A (n=170), patients who received therapy depending on a general clinical manifestation (Scheme A); and subgroup B (n=166), patients who received therapy depending on a dominant syndrome and variability of neurobiochemical markers (Scheme B).

During treatment, we used therapeutic agents with various degrees of nootropic effect, vasoactive agents, folic acid, B vitamins. For correction of spasticity, drugs with muscle relaxant effect were used. For correction of increased intracranial pressure, we used diacarb, triampur and magnesium sulfate. Non-drug therapies in all treatment regimens included massage, physical therapy, physiotherapy

The study was approved by the Voronezh State Medical University Ethics Committee (Protocol No. 6 of October 19, 2013). Written informed consent was obtained from the child's parents.

Statistical analysis was performed using StatSoft Statistica v6.0. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

In the control group, SOCG was 77±3%, SOCD - 67±4%, Apgar score - 7±1, Z-score - 0±0.8CU, and ND severity - 29±1 points. The parameters of the physical and psychomotor states in children according to the age group and severity of the CNS lesion are presented in Table 1. The analysis showed that the severity of the CNS lesion was reliably associated with the degree of ND, the markers of the optimal course of pregnancy and childbirth, and that the state of the physical development of children reliably reflected the established severity of the CNS lesion (CNSL).

An analysis of the frequency of identified neurologic syndromes showed that, regardless of age, the incidence of neurologic syndromes increased with an increase in the severity of the CNS lesion. It should be noted that in Group 1, regardless of the degree of severity of the CNS lesion, the syndrome of motor disorders was dominant (58.4%) in combination with hyperexcitability syndrome (47.6%), to which the syndrome of posthypoxic ventricular dilatation (26.3%) was added at a severe degree of CNS damage. In Group 2, the syndrome of motor disorders (34.7%) and the syndrome of delayed psychomotor development (31.9%) were dominant.

Further, the blood levels of biochemical markers were assessed depending on the severity of the CNS lesion. In Group 1 patients with a mild to moderate degree of CNS damage, the values of the S100 protein significantly increased by 48% and 86%, respectively, compared to the control level (175.7±19.6 pg/ml). The severe degree of CNS damage was characterized by a decrease in the S100 protein level by ≈33% without statistical significance. In Group 2, regardless of the degree of severity of the CNS lesion, this marker had a consistently high level: by 42%-48% higher than the norm.

Table 1.

The physical and psychomotor states of the children and the severity of the CNS lesion

Age group	Variable	Mild degree of CNSL (1)	Moderate degree of CNSL (2)	Severe degree of CNSL (3)	Statistics
1-3 months	n	60	58	45	
	ND, points	25±2	19±2	12±1	F= 11.8583 P=0.0000 P_{1-2}=0.0453, P_{1-3}=0.0000 P_{2-3}=0.0273
	SOCG, %	63±4%	51±6%	34±4%	F= 8.3469P=0.0004 P_{1-3}=0.0002 P_{2-3}=0.0486
	SOCD, %	58±3%	44±3%	37±3%	F= 12.3615 P=0.0000 P_{1-2}=0.0023, P_{1-3}=0.0000
	Apgar score	6±1	5±1	4±1	F= 0.9392 P=0.3931
	Z-score, CU	0.9±0.2	1.4±0.1	1.9±0.1	F= 10.6572 P=0.0000 P_{1-2}=0.0392, P_{1-3}=0.0000
4-6 months	n	62	60	51	
	ND, points	22±1	17±2	10±2	F= 12.1910 P=0.0000 P_{1-3}=0.0000 P_{2-3}=0.0133
	SOCG, %	62±4%	50±3%	36±3%	F= 13.9444 P=0.0000 P_{1-2}=0.0316, P_{1-3}=0.0000 P_{2-3}=0.0147
	SOCD, %	57±2%	43±3%	34±3%	F= 18.6146 P=0.0000 P_{1-2}=0.0006, P_{1-3}=0.0000 P_{2-3}=0.0544
	Apgar score	5±1	5±1	4±1	F= 0.3425 P= 0.7105
	Z-score, CU	1.0±0.1	1.5±0.1	2.1±0.1	F= 29.1534 P=0.0000 P_{1-2}=0.0011, P_{1-3}=0.0000 P_{2-3}=0.0002

In Group 1, the level of NGF increased by 14% relative to normal indices at a mild degree of CNS damage, but at moderate and severe degree, this indicator decreased by 10% and 25%, respectively, compared to the control level. In Group 2, this marker statistically decreased only at a mild degree of CNS damage.

The level of L-Hcy exceeded the control values in both groups regardless of the degree of severity of the CNS lesion, with a maximum threefold increase in Group 2 patients with a severe degree of the CNS lesion. In Group 1, the AII level exceeded the control values regardless of the degree of severity of the CNS lesion, but in Group 2, it decreased with increasing severity of the CNS lesion.

The levels of the studied markers before the therapeutic interventions did not differ statistically in subgroups A and B. Dynamic changes in the levels of neurobiochemical markers and the degree of ND after treatment are presented in Table 2.

Table 2.

Dynamic changes in the levels of neurobiochemical markers and the degree of ND after treatment

Age group	Variable	Mild degree of CNSL		Moderate degree of CNSL		Severe degree of CNSL	
		Scheme A	Scheme B	Scheme A	Scheme B	Scheme A	Scheme B
1-3 months	n	30	29	30	29	24	21
	ND (score)	24±1	27±1*	20±1	23±1*	13±1	17±1*
	S-100 (pg/ml)	213.3±27.6	165.1±21.4	312.8±36.9	273.4±32.3	331.3±34.2	306.6±31.7
	NGF (pg/ml)	24.5±1.97	27.8±2.2	16.2±1.9	18.3±2.2	11.3±1.3	11.9±1.2
	L-Hcy (μmol/ml)	7.82±0.57	5.81±0.42*	9.19±0.76	6.11±0.43*	10.7±0.99	7.96±0.98
	AII (ng/ml)	0.116±0.017	0.101±0.019*	0.134±0.012	0.122±0.014	0.134±0.089	0.12±0.076
4-6 months	n	31	32	30	29	25	26
	ND (score)	21±1	24±1*	16±1	19±1*	11±1	14±1*
	S-100 (pg/ml)	299.6±25.1	267.1±22.4	281.5±18.1	260.6±16.7	178.1±20.7	165.7±19.8
	NGF (pg/ml)	17.9±2.9	19.8±3.19	11.1±1.23	11.4±1.27	8.1±1.37	8.6±1.49
	L-Hcy (μmol/ml)	10.9±1.12	7.13±0.19*	15.1±1.82	8.59±1.11*	22.9±2.6	14.9±1.71*
	AII (ng/ml)	0.132±0.016	0.125±0.012	0.139±0.021	0.114±0.017	0.11±0.03	0.086±0.021

** - P<0.05 (between Scheme A and Scheme B within the group).*

We found that Scheme B showed advantages for all studied neurobiochemical markers, with statistical significance for L-Hcy regardless of the age group. These data are potentially important, since there is serious under-recognition of neonatal and childhood stroke associated with elevated total homocysteine.[15-19] The positive dynamics were found in the ND severity against the background of Scheme B regardless of the age group and the degree of severity of the CNS lesion. Thus, the pronounced positive dynamics in the levels of neurotrophic and neurovascular markers of the CNS lesion in all age groups reflects the advantage of pathogenetic therapy. Thus, a pathogenetically grounded approach to the choice of therapy for perinatal CNS damage allows reducing the risks of developing severe consequences in the age dynamics.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 197-200
http://dx.doi.org/10.21103/Article8(3)_OA5

ORIGINAL ARTICLE

Endocrinology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Clinical, Neuroimaging and Histological Characteristics of Non-functioning Pituitary Adenoma in Patients with Growth Hormone Deficiency

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Abstract

The aim of this study was to determine the clinical and diagnostic markers of tumor aggressiveness that are predictive of tumor recurrence in patients with non-functioning pituitary adenoma (NFPA) and growth hormone deficiency.

A total of 87 patients with NFPA and growth hormone deficiency were enrolled in the study, including 31 patients after transsphenoidal hypophysectomy (postoperative follow-up from 1 to 3 years). The mean age of patients was 32.2±2.5 years. The search for clinical and diagnostic markers of NFPA aggressiveness that are predictive of tumor recurrence after transsphenoidal hypophysectomy revealed a direct correlation with such risk factors as the young age of the patient, large tumor size, asymmetry and deformation of the pituitary gland, signs of tumor invasion into surrounding tissues, arteries, and cavernous sinus, panhypopituitarism, and the small-cell and/or dark-cell chromophobic adenomas. Preliminary data, as well as a number of studies, confirm that predictors of pituitary tumor recurrence and markers of persistent disease activity still have to be identified in order to improve the long-term management of NFPA.**(International Journal of Biomedicine. 2018;8(3):197-200.)**

Key Words: non-functioning pituitary adenoma • transsphenoidal hypophysectomy • pituitary gland • growth hormone deficiency

Abbreviations

BMI, body mass index; **ESS,** empty sella syndrome; **GHD**, Growth hormone deficiency; **IGF-1**, insulin-like growth factor 1; **NFPA**, non-functioning pituitary adenoma; **PG**, the pituitary gland; **QL**, the quality of life; **TC**, thigh circumference; **TSHE**, transsphenoidal hypophysectomy; **WC**, waist circumference.

Introduction

Non-functioning pituitary adenomas (NFPAs) account for 14%–54% of pituitary adenomas and have a prevalence of 7–41.3/100,000 population.[1,2] Benign in origin and not provoking a hormonal hypersecretory syndrome, NFPAs are clinically challenging because they present at a late stage with local mass effects or hypopituitarism with GHD and disorders in sexual and reproductive functions. At the time of initial diagnosis, visual field defects are detected in 60%–80% of NFPA patients.[3,4]

Recurrence is one of the most troublesome clinical outcomes of NFPA. A previous meta-analysis[5] found that NFPA recurs most often between one and five years after surgery, and that the rate of recurrence decreases after 10 years. Unfortunately, until recently there has been no consensus

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on the prognostic predictors of NFPA recurrence. No single convincing prognostic factor for recurrence was identified in a meta-analysis study on NFPA.[5] In most studies, clinical factors of age, gender, tumor size and tumor invasion have had no predictive value for recurrence. Recently, many other factors have been introduced that influence the proliferation of pituitary adenomas, such as angiogenesis, apoptosis, Ki-67, growth factors, oncogenes, tumor suppressor genes and hormone receptors.[6-9]

The search for diagnostically significant markers of aggressiveness of NFPA in predicting the post-operation period (tumor recurrence, the need for re-operation or radiotherapy) remains an urgent problem.

The aim of this study was to determine the clinical and diagnostic markers of tumor aggressiveness that are predictive of tumor recurrence in patients with NFPA and GHD.

Materials and Methods

We analyzed the data of 87 NFPA patients (44 women and 43 men) with GHD, including 31 patients after TSHE (postoperative follow-up from 1 to 3 years). The mean age of patients was 32.2±2.5 years.

Methods of investigation included: 1) general clinical examination, assessment of neurological status, anthropometry (height, weight, TC, WT, BMI); 2) instrumental methods of examination (visual field perimetry, colour vision, fundus oculi, visual acuity, ECG, CT/MRI of sella turcica and adrenal glands, ultrasound of reproductive organs); 3) determination of blood hormones (GH, IGF-1, LH, FSH, PRL, TTG, ACTH, testosterone, estradiol, progesterone, cortisol) in RIA using "Gamma-12" and "Strantg 300," and 4) histological examination of postoperative specimens; 5) assessment of QL by QoLAGHD questionnaire (Quality of Life Adults with growth Hormone deficiency, KIMS Study Questionnaire).

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Chicago, IL).

The study was approved by the Republican Specialized Scientific-Practical Medical Center of Endocrinology Ethics Committee. Written informed consent was obtained from each patient.

Results

The age-gender distribution of NFPA patients is presented in Table 1. Thirty-one NFPA patients after TSHE were divided into 3 groups depending on the histological characteristics (Table 2).

Table 1.

The age-gender distribution of NFPA patients

Age, years	Number of men	Number of women
18 – 29	10	12
30-44	12	9
45-59	13	16
60-74	7	3
≥75	2	3

Table 2.

Groups of patients depending on the histological characteristics of NFPA

Group 1		Group 2		Group 3	
n	%	n	%	n	%
24	77.5	6	19.3	1	3.2%
Total: 31					

As can be seen from Table 2, the number of patients (Group 1) with large-cell chromophobic adenoma (24/77.5%) were the most prevalent. Small-cell NFPAs were identified in 6(19.3%) patients (Group 2). We observed a giant recurrent malignant dark-cell adenoma with brain metastases in only one case (3.2%) in a teenage girl (Group 3).

Clinical manifestations of NFPA in the form of endocrine and neurologic disorders were detected in 41.5% and 32.8% of patients, respectively. Symptoms of neuro-ophthalmic disorders were detected in 25.7% of patients.

In women, NFPA was accompanied by obesity, primary and secondary hypothyroidism, secondary hypogonadism, the syndrome of persistent galactorrhea/amenorrhea (symptomatic and idiopathic forms), diabetes insipidus, and ESS. In men, NFPA was accompanied by obesity, primary and secondary hypothyroidism, secondary hypogonadism, diabetes insipidus, and gynecomastia.

According to an MRI of the brain and PG, endosellar tumors were detected in 15(48.4%) patients, endo-extrasellar tumors (mainly with suprasellar growth) - in 16(51.6%) patients. In MRI, a soft tissue structure of NFPA was diagnosed in 16(52%) patients and a cystic structure in 11(35.5%) patients. The structure of NFPA was represented by a hemorrhagic component in 4(12.9%) cases, and both cystic and hemorrhagic components were found in 2(6.4%) of them. A pituitary microadenoma (<1cm) was identified in 18(58.1%) patients, a pituitary macroadenoma (>2cm) in 12(38.7%) patients and a giant pituitary adenoma in 1(3.2%) patient.

The next step of our research was to look for the most significant aggressiveness markers that play a predictive role in patients with remission and tumor recurrence after TSHE. Further, we determined the significance of the differences between such parameters as the number of patients during the remission period and the number of relapses after TSHE, with different parameters taken into account. The results of multifactorial analysis showed that there were many such markers (Figure 1).

After analyzing the data on the frequency of remissions and NFPA recurrence, a correlation relationship between different parameters and the frequency of recurrences was selectively studied.

Further, we studied risk factors (markers of NFPA aggressiveness) for the probability of NFPA recurrence in the postoperative period. Table 3 shows the predictive power of aggressiveness markers, depending on various indicators. Thus, the developed scale of aggressiveness markers of NFPA allows identifying factors according to three degrees, in view of which it is possible to plan a set of measures for preventing tumor recurrence.

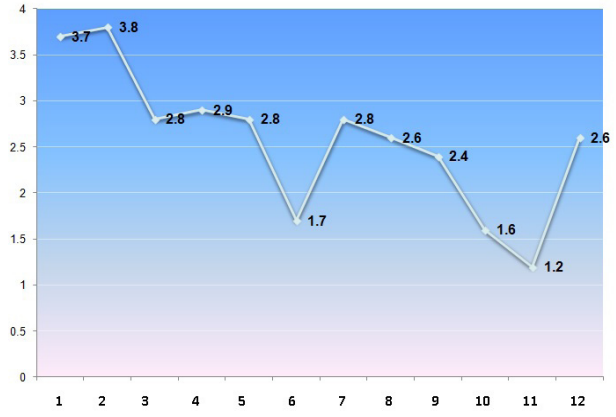

Fig. 1.

1 - small-cell NFPA; 2 - adenoma size; 3 - hypopituitarism; 4 - tumor invasion; 5- tumor hemorrhage; 6 - rapidly progressive disease course; 7 - age; 8 - severity of disease; 9 - disease duration; 10 - asymmetry of PG; 11 - skull trauma; 12 - GHD

Table 3.

Probability of NFPA recurrence in the postoperative period

Predictive power	RR	Rusk factors
Very high	>3.0	Young age of the patient; large tumor size, asymmetry and deformation of PG; signs of tumor invasion into surrounding tissues, arteries, and cavernous sinus; panhypopituitarism; the small-cell and/or dark-cell chromophobic adenomas.
High	2.0-3.0	Disease duration; age; rapidly progressive disease course; high blood cholesterol; MRI tumor hemorrhage.
Moderate	1.5-2.0	Skull trauma; GHD; adenoma size, high IGF-1 levels.

Approximately half of the patients with NFPA have a residual tumor after surgery.[10-14] To date, there is no reliable marker to predict tumor regrowth after surgery. Several large series of studies evaluating postoperative tumor recurrence and regrowth have shown that the presence of residual tumors and/or follow-up duration appear to be the two major determinants of recurrence and regrowth.[13-16] Determining prognostic markers of NFPA aggressiveness has a large clinical value. Comparative analysis of the results obtained revealed a direct correlation with such risk factors as the young age of the patient, large tumor size, asymmetry and deformation of PG, signs of tumor invasion into surrounding tissues, arteries, and cavernous sinus, panhypopituitarism, and the small-cell and/or dark-cell chromophobic adenomas. It should be emphasized that stereotactic tumor biopsy is effective in predicting NFPA recurrence in the postoperative period. Our preliminary data, as well as a number of studies, confirm that predictors of pituitary tumor recurrence and markers of persistent disease activity still have to be identified in order to improve the long-term management of NFPA.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 201-205
http://dx.doi.org/10.21103/Article8(3)_OA6

ORIGINAL ARTICLE

Endocrinology

INTERNATIONAL JOURNAL OF BIOMEDICINE

The rs738409 (I148M) Variant of the *PNPLA3* Gene and Type 2 Diabetes in Yakutia

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Abstract

The purpose of our research was to study the association of the *PNPLA3* SNP rs738409 (C>G) with type 2 diabetes (T2D) in the Yakuts. The frequency distribution of alleles and genotypes of the *PNPLA3* SNP rs738409 was in accordance with HWE. There were no statistically significant differences in the distribution of alleles and genotypes of the *PNPLA3* SNP rs738409 between T2D patients and non-T2D patients ($P>0.05$); the G allele and homozygous GG genotype prevailed in both groups. In T2D patients, a high frequency of the G allele (74.1%) was found, with a predominance of the GG genotype (58.5%). We also found that the mutant allele frequency is higher than in the studied populations of the world. Further studies with larger sample size are required to achieve sufficient statistical power to detect the association of the *PNPLA3* SNP rs738409 with the development of T2D in Yakut patients. **(International Journal of Biomedicine. 2018;8(3):201-205.)**

Key Words: type 2 diabetes • gene polymorphism • non-alcoholic fatty liver disease • Yakut population

Abbreviations

ALD, alcoholic liver disease; **DM**, diabetes mellitus; **CHC**, chronic hepatitis C; **CVD**, cardiovascular diseases; **HCC**, hepatocellular carcinoma; **NASH**, non-alcoholic steatohepatitis; **NAFLD**, non-alcoholic fatty liver disease; **PNPLA3**, patatin like phospholipase domain containing 3; **SNP**, single nucleotide polymorphism; **T1D**, type 1 diabetes; **T2D**, type 2 diabetes.

Introduction

The North is a multicomponent extreme factor for humans and has a multifaceted negative impact on the human body by a specific production and environmental component, causing a number of changes in the metabolism and functional activity of all body systems and changing a body's need for energy, food and biologically active food components.

The indigenous population of the North is characterized by a protein-lipid type of nutrition, which contributes to the formation of a "polar metabolic type." This type of nutrition is characterized by a high content of protein in the daily diet (15% and more), fat (35% and more), carbohydrates (50% and less), in contrast to the "European type," in which carbohydrates predominate. In the diet of a person living in the Far North, the energy role of carbohydrates is reduced and the role of fats, and to a lesser extent of proteins, is increased, thus forming the so-called "polar metabolic type." In the indigenous inhabitants of the North, the energy metabolism switches from carbohydrate type to fatty type, lipid metabolism increases due to food sources of fat, and lipid metabolism increases due to

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food sources of fat, that is, rapid "combustion" of exogenous and not endogenous fat. This is why aborigines can consume more meat and fat (the Eskimo, for example, can eat 6-8 kg of meat per day). Therefore, the indigenous people of the North, engaged in traditional northern types of management, rarely suffer from CVD, in contrast to sedentary aborigines living in settlements and students in boarding schools, who are forced to consume large amounts of carbohydrates. Thus, increasing globalization has led to a change in nutrition of the indigenous inhabitants of the northern regions and an increase in metabolic diseases and T2D.[1]

Epidemiological data indicate the frequent combination of T2D and NAFLD characterized by an accumulation of lipids both in the hepatocytes themselves and in the intercellular space.[2] T2D patients are characterized by insulin resistance, and often have obesity, dyslipidemia and increased activity of hepatic enzymes. For these patients, there is a tendency to accumulate fat in the liver, which causes a higher risk of developing severe liver pathology compared to patients without T2D.[3]

Studies in recent years prove the hereditary mechanisms of the development of NAFLD. A genome-wide association study of a multiethnic population found that a single rs738409 C>G polymorphism of the *PNPLA3* gene, which encodes I148M, is strongly associated with hepatic fat content and conferred susceptibility to NAFLD.[4,5]

PNPLA3 is expressed in the liver and adipose tissue and has acyl hydrolase activity.[6] The PNPLA3 protein exhibits lipase activity against triglycerides and acylglycerol transacetylase activity, and its expression is highly responsive in energy mobilization and the storage of lipid droplets.[7] In humans, the *PNPLA3* I148M mutation has been shown to influence not only intrahepatic remodeling but also reduces very low density lipoproteins secretion.[8]

Recently, a study showed that carriers of the rs738409[G] allele have lower de novo lipogenesis as compared to non-carriers due to a reduction in liver SREBP1c mRNA levels.[9] The reduction in hepatic de novo lipogenesis may be interpreted as a compensatory effect of hepatic fat increase.

The rs738409 polymorphism has been associated with the loss of the protein's hydrolyzing function and with the hepatic triglyceride accumulation.[6] The rs738409 polymorphism is strongly associated with steatosis, fibrosis/cirrhosis in various liver diseases with different etiologies (NAFLD, ALD, CHC, HCC).[10-15]

The purpose of our research was to study the association of the *PNPLA3* SNP rs738409 (C>G) with T2D in the Yakuts.

Materials and Methods

Molecular genetic studies were conducted in the molecular genetics department at YSC CMP. DNA samples were obtained from the YSC CMP biomaterial collection ("Genome of Yakutia", registration No. USU_507512).

The study included 106 patients (79 women and 27 men) of the Yakut nationality aged between 31 to 82 years (mean age, 60.7±0.42 yrs) with T2D (Group 1). The comparison group consisted of 72 healthy volunteers (24 men and 48 women aged between 19 and 55 years, mean age of 28.2±0.49 yrs) (Group 2). All participants in the study were of Yakut ethnicity and lived in the territory of the Republic of Sakha (Yakutia) [the RS(Y)]. Exclusion criteria were the following: chronic viral hepatitis, autoimmune hepatitis, primary biliary cholangitis, primary sclerosing cholangitis, hereditary hemochromatosis, Wilson-Konovalov disease, alcohol abuse (>30 g/l).

Genomic DNA was extracted from peripheral blood were conducted using the phenol-chloroform standard method. After DNA extraction, the samples were subjected to a PCR-RFLP reaction to analyze the rs738409 polymorphism of the *PNPLA3* gene where the homozygous wild type CC genotype (200 and 133 bp), the heterozygous genotype is CG (333, 200 and 133 bp) and the homozygous mutant-type GG genotype (333 bp). The following primers were used: F: 5'-TGGGCCTGAAGTCCGAGGGT-3' and R: 5'-CCGACACCAGTGCCCTGCAG-3' (Biotech Industry Ltd, Moscow, Russia). The reaction mixture (25 µL) contained 13 µl of ddH2O, 2.5 µl 10xPCR buffer, 2.5 µl 25 mM MgCl2 , 2.5 µl 2.5 mM dNTP Mix, 1,5 µl (10pkmol/ µl) of each oligonucleotide primer, 0.3 ul (1.5 units.) "hotstart" Taq-polymerase and 3 µl of DNA. PCR amplification was carried out in the MJ Mini Gradient Thermal Cycler (Bio-Rad). Thermal Cycling Conditions were as follows: 95 °C for 5 min, and then 37 cycles at 94°C for 30 sec, at 66°C for 30 sec, and at 72 °C for 40 sec and a final elongation at 72°C for 5 minutes.

To determine the rs738409 (C>G) polymorphism genotype, the restriction endonuclease BstF5 I (SibEnzyme, Novosibirsk, Russia) was used (at 65 for 16 hours). The products of the restriction enzyme digestion were subjected to electrophoresis in a 1.5% agarose gel in a horizontal electrophoretic tank containing TBE buffer (1X concentration) with a constant current of 120 V for 1 hour. The resulting bands were visualized under ultraviolet (UV) light using a gel documentation system (Vilber Lourmat, France) (Figure 1). The images were recorded to digital files.

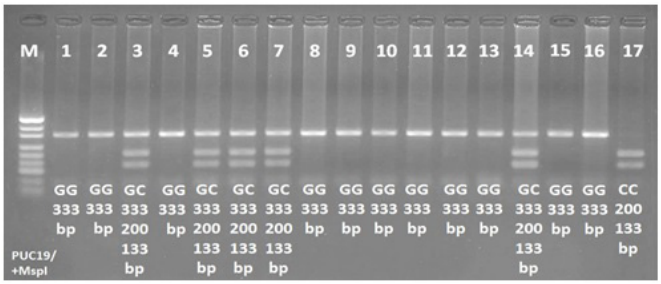

Fig. 1. *Electropherogram representing PCR amplification product of the PNPLA3 SNP rs738409 (C>G)*

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). The chi-square test was used to determine the deviation from Hardy-Weinberg equilibrium (HWE) and the differences in genotypes and alleles between groups. The expected heterozygosity (He) was calculated using the Nei (1987) estimator. A probability value of $P<0.05$ was considered statistically significant.

The study was approved by our regional ethics committee. Written informed consent was obtained from all patients.

Table 1.

Prevalence and incidence of endocrine pathology per 1,000 population in RS(Y)

Endocrine pathology	Prevalence of disease per 1000 population						Incidence of disease per 1,000 population					
	2006	2008	2010	2012	2014	2016	2006	2008	2010	2012	2014	2016
Endocrine system diseases	80.3	82.4	82.04	87.2	89.0	89.3	12.8	14.5	15.6	14.7	14.9	15.8
DM (total)	20.4	22.6	30.88	30.41	32.3	33.9	2.6	2.9	3.2	3.8	3.9	4.0
T1D	1.83	1.83	2.4	0.77	0.80	0.85	0.1	0.2	0.2	0.1	0.1	0.1
T2D	19.1	20.3	28.0	29.2	28.8	29.45	2.4	2.8	3.1	3.2	3.4	3.6
Thyroid diseases	26.8	27.8	27.8	29.2	28.8	28.6	6.8	7.8	7.8	9.2	8.8	8.2
Hypothyroidism	9.2	8.8	9.46	9.6	9.8	9.4	1.12	1.15	1.20	1.25	1.33	1.38
Thyrotoxicosis	6.5	6.4	6.8	6.6	6.9	7.2	0.6	0.7	0.8	0.8	0.93	0.97
Obesity	20.3	22.3	28.7	29.9	38.8	39.45	2.3	2.7	3.4	3.7	3.9	4.2

Results and Discussion

Currently in Yakutia, the morbidity incidence caused by endocrine pathology tends to increase, primarily due to the growth of diabetes and obesity (Table 1). In RS(Y), the total morbidity rate caused by endocrine pathology is 89.3 per 1000 population and the level of newly diagnosed endocrine pathology is 15.8 per 1000 population. On average, about 8% of the population of the RS(Y) are on prophylactic medical examination for endocrine pathology. In Aldan (industrial area) and Allayhovsky (arctic region) districts, these indicators are higher than the average values for the republic

The prevalence of DM in RS(Y), according to the Online Diabetes Registry for the period from 2013 to 2016, amounted to 15%, with an annual increase of 3.5%-6.85%. In DM patients, the need for medical assistance increases with the deterioration of their condition and the occurrence of complications. The prevalence of vascular complications of DM remains high, including diabetic retinopathy, diabetic nephropathy, and cardiovascular complications. In the RS(Y), the proportion of complications of DM is (according to data at the end of 2016) 35.57% for T1D and 30.2% for T2D. The average life expectancy of T1D patients is 47.5 years for men and 45.67 years for women; in T2D - 63.59 for men and 67.54 years for women. Mortality from DM in the RS(Y) is 24.17 per 100,000 population.

In clinical practice, the high risk of coexistence of NAFLD and DM should be considered. The prevalence of NAFLD in the general population of Western countries is 20-30% and among obese adults it is 80%-90%.[16] The prevalence of NAFLD is remarkably increased in patients with T2D, ranging from 30% to 75% according to age, ethnicity, the study population and the diagnostic tools used.[17]

NAFLD is defined as either excessive fat accumulation in the liver with more than 5% of hepatocytes containing visible intracellular triglycerides or steatosis affecting at least 5% of the liver volume or weight in patients consuming less than 30g of alcohol per day for men and less than 20 g of alcohol per day for women.[18-20]

The frequency distribution of alleles and genotypes of the *PNPLA3* SNP rs738409 was in accordance with HWE. There were no statistically significant differences in the distribution of alleles and genotypes of the *PNPLA3* SNP rs738409 (C>G) between Group 1 and Group 2 (P>0.05); the G allele and homozygous GG genotype prevailed in both groups. Population-genetic analysis among the Yakuts on the *PNPLA3* SNP rs738409 (C>G) showed that the level of observed heterozygosity (Ho) in T2D patients was 0.311; in healthy individuals Ho=0.319. The level of the expected heterozygosity (He) in T2D patients was 0.387, in healthy individuals He=0.395. The genotype frequencies of the *PNPLA3* rs738409 *(*C>G) SNP are presented in Table 2.

According to the 1000 Genomes Project data, the frequency of the *PNPLA3* (rs738409) G allele in different populations is characterized by heterogeneity.

Table 2.

Genotype and allele distribution of the PNPLA3 SNP rs738409 in the groups

Group		Genotype, %			Allele		χ^2	H_o	H_e	P
		CC	GC	GG	C	G				
Group 1	Obs.	10.38	31.13	58.49	0.259	0.741	4.123	0.311	0.387	0.05
	Exp.	6.71	38.38	54.91						
Group 2	Obs.	11.11	31.94	56.94	0.271	0.729	2.632	0.319	0.395	0.105
	Exp.	7.34	39.50	53.17						

The frequency of G allele of the *PNPLA3* SNP rs738409 in T2D patients and healthy volunteers of Yakut nationality prevails in comparison with other populations. Thus, in T2D patients, a high frequency of the G allele (74.1%) was found, with a predominance of the GG genotype (58.5%). In study by JM Patit et al., the frequencies of G and C alleles of the PNPLA3 SNP rs738409 in T2D patients of the France's population were 29.6 and 70.4%, respectively.[21] According to A. J. Cox, the lowest frequency of the G allele (13.7%) with the GG genotype frequency of 1.5% was found in African American patients with T2D.[22]

C. Huang et al. showed that the *PNPLA3* genetic variants were associated with advanced liver fibrosis in diabetic patients only, but not in non-diabetic patients. The PNPLA3 gene was the most important predictive factor of bridging fibrosis in diabetic patients, using the recessive model (OR: 4.53, CI: 1.356–15.106, P=0.014) or the dominant model (OR: 2.20, CI: 1.026–4.734, P=0.04). Compared to non-diabetic patients, patients with the diabetes/GG genotype were more likely to have advanced liver fibrosis (OR: 8.79, CI: 2.889–26.719, P<0.001), followed by those with diabetes/non-GG genotype (OR: 1.55, CI: 1.048–2.286, P=0.03).[23]

In study by R. Posadas-Sánchez et al., the I148M/*PNPLA3* (rs738409) polymorphism was associated with the presence of premature coronary artery disease in T2DM patients and with some cardiometabolic parameters.[24]

However, a number of studies indicate that there is no straightforward association between PNPLA3 and insulin action or glucose homeostasis metabolism.[25,26]

As noted by many domestic and foreign researchers, carriers of the *PNPLA3* G allele are more susceptible to liver diseases (NAFLD, NASH) with a high risk of developing cirrhosis and HCC.[4] Patients with T2D and NAFLD have a higher risk of CVD, as well as mortality, due to the depletion of hepatic glycogen stores and a decrease in the reserve regulation capacity of glucose homeostasis with the accelerated development of vascular complications. At the same time, fatty hepatosis, regardless of the cause, can contribute to high insulin levels due to reduced insulin clearance.[21]

Undoubtedly, further studies with larger sample size are required to achieve sufficient statistical power to detect the association of the *PNPLA3 SNP* rs738409 SNP with the development of T2D in Yakut patients.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 206-212
http://dx.doi.org/10.21103/Article8(3)_OA7

ORIGINAL ARTICLE

Obstetrics and Gynecology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Results of Fetal Ultrasound Imaging and Doppler Ultrasound Study in Pregnant Women with Extragenital Pathology

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Abstract

The aim of this research was to study the parameters of fetal ultrasound imaging and Doppler ultrasound study in pregnant women with extragenital diseases (EGDs) during the treatment regimes with and without hyperbaric oxygen therapy (HBOT).

Materials and Methods: A total of 235 pregnant women were examined prospectively at 5 to 40 weeks of gestation. The main group included 191 women with EGDs (anemia, arterial hypertension, *c*hronic pyelonephritis); the control group included 44 women with physiological pregnancy without EGDs. Evaluation of treatment efficacy was based on data from clinical and laboratory findings before treatment and after its completion. The following hardware methods of research were performed: ultrasonography, fetometry, dopplerometric study of fetoplacental complex.

Results: Based on data obtained from this study, the following findings were made:

- In the early stages of gestation, there were no disturbances in fetoplacental blood circulation.
- Starting the 19th week of pregnancy, there is a significant increase in the uterine artery resistive index in pregnant women with arterial hypertension.
- In women with a high perinatal risk on the background of the studied EGDs, the third trimester of pregnancy, despite the ongoing conventional treatment, is characterized by persistent impairment in fetoplacental blood circulation.
- The inclusion of HBOT in complex therapy in the early stages of pregnancy in women with a high perinatal risk allows leveling out the inevitable disturbances in fetoplacental blood circulation on the background of the studied EGDs. **(International Journal of Biomedicine. 2018;8(3):206-212.)**

Key Words: pregnancy • anemia • arterial hypertension • chronic pyelonephritis • hyperbaric oxygen therapy

Abbreviations

AH, arterial hypertension; **BPD**, biparietal diameter; **CRL**, crown-rump length; **ChP**, *c*hronic pyelonephritis; **CT,** conventional treatment; **EGDs**, extragenital diseases; **FPC**, fetoplacental complex; **FL**, femur length; **HBO**, hyperbaric oxygen; **HBOT**, **HBO** therapy; **IUGR**, intrauterine growth retardation; **MCA**, fetal middle cerebral artery; **NT**, nuchal translucency; **NBL**, nasal bone length; **PR**, perinatal risk; **PI**, placental insufficiency; **PB**, premature birth; **RCH**, retrochorial hematoma; **RA,** radial artery; **RI**, resistive index; **SA**, spiral artery; **TAD**, transverse abdominal diameter; **TCD**, transverse cerebellar diameter; **UmA**, umbilical artery; **UtA**, uterine artery.

Introduction

In recent years, extragenital diseases (EGDs) have occupied a leading position (28%) in the structure of causes of maternal mortality in developed countries and Russia (23%).[1] The onset and development of pregnancy against the background of such EGDs as anemia, AH, and ChP, occurs in conditions of angiopathy, primarily of the uterus vessels.[2] Angiopathy contributes to the unavoidable development of placental insufficiency during all EGDs.[3]

Anemia in pregnancy is a major public health and economic problem worldwide and contributes to both maternal and fetal morbidity and mortality.[4-6] Anemia, even in early pregnancy has been associated with adverse pregnancy outcome.[7] Clinical manifestations include fetal growth restriction, preterm delivery, low birth weight,[8] impaired lactation, poor maternal/infant behavioural interactions, post partum depression and increased fetal and neonatal mortality.[5,6]

Pyelonephritis in pregnancy confers a high risk of maternal complications and preterm birth.[9] Serious morbidity associated with pyelonephritis in pregnancy is common. Sepsis and septic shock occur secondary to pyelonephritis more frequently than secondary to any other infectious process during pregnancy.[10] Acute respiratory distress syndrome complicates approximately 1–8.5% of pyelonephritis cases.[11, 12]

High blood pressure during pregnancy poses various risks, including decreased blood flow to the placenta, placental abruption, intrauterine growth restriction, premature delivery, preeclampsia, and Cesarean delivery.

Since the late 70s, some authors in Russia have tried to treat both acute hypoxia in labor and fetal growth restriction secondary to placental insufficiency with HBO.[13-15] HBO has been used for threatened abortion, fetal hypoxia, toxemias of pregnancy, and diabetes in pregnant women. The research found that HBO was invaluable in improving both placental blood flow and O_2 diffusion at the cellular level.[16] XM Xiao and colleagues reported that HBOT could reduce the values of systolic/diastolic ratio and the pulse index in umbilical arteries of late-onset fetal growth restriction patients, could improve the uteroplacental microcirculation and neonatal birth weight. HBOT is an effective method for the treatment of late-onset fetal growth restriction.[17]

The effects of HBO are based on the gas laws, and the physiological and biochemical effects of hyperoxia. HBO has complex effects on immunity, oxygen transport and hemodynamics. The positive therapeutic effects come from a reduction in hypoxia and edema.[18] Infections, injury, and disease increase tissue demands for oxygen while such problems as anemia, toxins and hemorrhage can decrease the body's ability to transport oxygen via hemoglobin. Delivery of 100% oxygen under pressure allows plasma to carry much more oxygen and reduces the importance of hemoglobin-based delivery.[19] 100% oxygen dissolved in plasma can be delivered from capillaries to tissues at least three times farther than delivered when carried by hemoglobin alone.[19,20]

When hemoglobin drops to critical levels to disallow proper oxygen delivery, hyperbaric oxygen therapy may be used as bridge therapy to emergently supply oxygen. To address severe anemia in trauma or illness, the future may well afford the use of hyperbaric oxygen therapy in the military far-forward, in pre-hospital EMS settings, in trauma center emergency departments, in operative and recovery units, and in intensive care units of hospitals.[21]

HBO has been shown to be a valuable adjunct to the medical and surgical treatment of various infections. Host defense mechanisms against infection are impaired by hypoxia and oxygen has an adjunct effect with antibiotics.[22-24]

HBO restores the bactericidal capacity of leukocytes in hypoxic wounds by increasing tissue oxygen tensions.[25] HBO has been observed to activate many mechanisms, including accelerated macrophage infiltration[26] and improved antibactericidal capacity.[27]

Hyperbaric oxygen (HBO) has been found to be useful in the management of various cardiovascular disorders.[28] The effectiveness of HBOT on selected divers suffering hypertension was shown in the pilot study by Minthara Benny. [29] Lopez-Calderon and colleagues showed that HBO therapy reduced BP and decreased vascular reactivity to angiotensin II in the coronary arteries of hypertensive rats. These changes were associated with a decrease in the expression of AT1Rs.[30]

The value of hyperbaric oxygen is now well appreciated in human medicine and accepted as treatment for many indications.[31] HBO has shown a therapeutic effect when used for various pathologies.[28] The use of HBO during pregnancy has been shown to be safe.[32-35]

The aim of this research was to study the parameters of fetal ultrasound imaging and Doppler ultrasound study in pregnant women with EGDs during the treatment regimes with and without HBOT.

Materials and Methods

The study was performed in Municipal clinical hospital №29 named after N.E. Bauman (clinical base of RUDN University). The study was conducted in accordance with ethical principles of the Declaration of Helsinki.

A total of 235 pregnant women were examined prospectively at 5 to 40 weeks of gestation. Depending on the presence of EGDs, the women were divided into 2 groups. The main group included 191 women with EGDs; the control group included 44 women with physiological pregnancy without EGDs.

Depending on the nature of EGDs, the main group was divided into three groups: Group 1 comprised 88 pregnant women with anemia (code ICD-X: O99.0 - anemia complicating pregnancy, childbirth and the puerperium), Group 2 comprised 50 pregnant women with AH (code ICD-X: O10 - pre-existing hypertension complicating pregnancy, childbirth and the puerperium), and Group 3 comprised 53 pregnant women with ChP (code ICD-X: O23.0 - kidney infection in pregnancy). In turn, the three groups were divided into subgroups, depending on the nature of the therapy: conventional treatment (CT) or CT+HBOT. All examined women with EGD belonged to a group with high perinatal risk (PR). We identified the degree of PR based on the scale developed by O.G. Frolova and E.I. Nikolaeva (1981) and modified in by V.E. Radzinsky et al.(2003). According to this modified scale, there is three level of PR: low risk (<15points), moderate risk (from 15 to 20 points), and high risk (≥25 points).

Inclusion criteria were singleton pregnancy, high PR, and voluntary informed consent to HBOT.

Exclusion criteria were cancer, multiple pregnancies, signs of placental abruption and the contraindications to the use of HBOT (an untreated pneumothorax (absolute contraindication) and relative contraindications (a history of epilepsy, viral infectios, pacemaker, claustrophobia, etc.).

Evaluation of treatment efficacy was based on data from clinical and laboratory findings before treatment and after its completion. The following hardware methods of research were performed: ultrasonography, fetometry, dopplerometric study of fetoplacental complex.

Ninety-eight women of the main group received the HBOT sessions (excess air pressure 1.3-1.5 atm in a pressure chamber) in addition to CT. We used Monoplace Hyperbaric Oxygen Therapy Chamber BLKS-307-«Khrunichev» (Russia), equipped with air-conditioning 54–58A and designed to conduct sessions in a high-pressure oxygen environment. The mode is one excess atmosphere.[36] The course includes 5–7 daily sessions lasting 40 minutes each. The first course was carried out in 6–9 weeks, the second in 16–18 weeks, and the third in 24–28 weeks of pregnancy.

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Continuous variables were presented as mean (M) ± standard error of the mean (SEM). T test was used for comparison between two groups with a normal distribution of the quantitative characteristic. Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare means of 2 and 3 or more groups of variables not normally distributed. Group comparisons with respect to categorical variables are performed using chi-square tests. Multiple comparisons were performed with one-way ANOVA and post-hoc Tukey HSD test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

The analysis of ultrasound data in the first trimester in patients with a high PR revealed no significant differences in the main fetometric parameters (CRL, NT, NBL) depending on EGDs. However, a number of characteristic changes were detected during the ultrasound scanning (Table 1).

RCH was not found in the control group compared to the main group, except for the CT+HBOT subgroups. Thus, in pregnant women with AH, RCH was detected significantly more often ($P<0.05$) compared to those with anemia and ChP (24% vs. 14.3% and 15.4%, respectively). Ultrasonic signs of chorionitis were diagnosed significantly more often in Group 3 (30.8%), while this symptom was not detected in the control group and Group 2 ($P<0.05$). Pregnant women of Group 1 showed signs of chorionitis in 5.7% of cases; there were no significant differences between subgroups. Reduction of amniotic fluid volume and fetal egg size was diagnosed in two (4.5%) women of the control group, whereas this symptom was significantly more frequent ($P<0.05$) in the main group (Table 1).

Reduced fetal egg size and changes in the yolk sac (cystic changes, decrease in size) were detected more often ($P<0.05$) in women of the main group. In Group 1, this symptom was diagnosed in 29.5% of cases, which was significantly more often compared to the control group and Groups 3 and 2 (4.5%, 13.2% and 20%, respectively).

Fetometry data

Fetometry in 20-22 gestational weeks showed a statistically significant decrease ($P<0.05$) in BPD, TAD and FL in Group 1 against the background of CT in comparison with CT+HBOT and the control group (Table 2). In other subgroups, no significant differences were found. However, there was a tendency to increased sizes of BPD, TAD, FL and TCD in the CT+HBOT subgroups compared to the CT subgroups.

Thus, it can be assumed that a decrease in the size of BPD, TAD and FL in the period of 20-22 weeks of gestation in the group of women with anemia who received CT is the most important reason for justifying the timing for HBOT.

We found significant differences in a number of analyzed fetometric parameters in 29-32 gestational weeks (Table 3). Significant decreases ($P<0.05$) in BPD, TAD and FL were found in all pregnant women with a high PR against the background of EGDs in comparison with the control group.

Table 1.

Ultrasonic data in the first trimester of pregnancy

Patient Groups			RCH	Chorionitis	RAFV + RFES	RFES and changes in the yolk sac
Main Group	Group 1	CT (n=42)	6 (14.3%) *P=0.0431	2 (4.8%)	11 (26.2%) *P=0.0048	13 (30.9%) *P=0.0011
		CT+HBOT (n=46)	0	3 (6.5%)	11 (23.9%) *P=0.0048	13 (28.3%) *P=0.0011
	Group 2	CT (n=25)	6 (24.0%) *P=0.0165 **P=0.0385	0	5 (20.0%) *P=0.0766	5 (20.0%) *P=0.0766
		CT+HBOT (n=25)	0	0	5 (20.0%) *P=0.0766	5 (20.0%) *P=0.0766
	Group 3	CT (n=26)	4 (15.4%) *P=0.0819	8 (30.8%) *P=0.0155	9 (34.6%) *P=0.008	4 (15.4%)
		CT+HBOT (n=27)	0	9 (33.3%) *P=0.01	9 (33.3%) *P=0.01	3 (11.1%)
Control group (n=44)			0	0	2 (4.5%)	2 (4.5%)
Total (n=235)			16 (6.8%)	22 (9.3%)	52 (22.1%)	45 (19.1%)

*RAFV - reduced amniotic fluid volume; RFES- reduced fetal egg size *- statistically significant differences between the subgroup and control group; **- statistically significant differences between two subgroups*

However, in the CT+HBOT subgroups, the values of BPD, TAD and FL were significantly greater (P<0.05) compared to the CT subgroups.

Table 2.

Fetometry data in 20-22 gestational weeks, mm

Patient Groups			BPD	TAD	FL	TCD
Main group	Group 1	CT (n=42)	50.6±0.8 *P<0.001 **P<0.001	47.3±0.9 *P<0.001 **P<0.001	37.3±0.9 *P<0.001 **P=0.004	21.8±0.4 *P<0.001 **P=0.013
		CT+HBOT (n=46)	52.2±0.7 *P=0.086	49.9±0.4 *P=0.031	39.3±0.8	22.4±0.4 *P=0.093
	Group 2	CT (n=25)	51.6±0.9 *P=0.046	49.3±0.9 *P=0.056	38.3±0.7 *P=0.021	22.1±0.3 *P=0.038
		CT+HBOT (n=25)	53.0±0.5	51.0±0.6	39.4±0.5	22.8±0.3
	Group 3	CT (n=26)	50.7±0.8 *P=0.002	47.3±0.9 *P<0.001 **P=0.046	37.6±0.8 *P=0.005	22.0±0.5 *P=0.027
		CT+HBOT (n=27)	51.7±0.8	50.3±0.5	38.9±0.9 *P=0.032	23.0±0.3
Control group (n=44)			53.6±0.4	51.3+0.5	40.1±0.3	23.6±0.3
Total (n=235)			51.9±0.7	49.4±1.8	38.7±0.7	22.5±0.3

**- statistically significant differences between the subgroup and control group; **- statistically significant differences between two subgroups*

Table 3.

Fetometry data in 29-32 gestational weeks, mm

Patient Groups			BPD	TAD	FL	TCD
Main group	Group 1	CT (n=42)	69.3±0.9 *P<0.001 **P<0.001	72.3±0.9 *P<0.001 **P<0.001	51.1±0.4 *P<0.001 **P<0.001	32.3±0.7 *P<0.001 **P<0.001
		CT+HBOT (n=46)	75.3±0.6 *P<0.001	80.9±0.4 *P<0.001	56.6±0.5 *P<0.001	35.5±0.7 *P=0.084
	Group 2	CT (n=25)	72.3±0.7 *P<0.001 **P<0.001	80.3±0.8 *P<0.001 **P<0.001	50.9±0.4 *P<0.001 **P<0.001	36.0±0.7 **P=0.006
		CT+HBOT (n=25)	77.4±0.8 *P=0.003	84.0±0.6	57.6±0.4 *P=0.037	38.0±0.3
	Group 3	CT (n=26)	68.9±0.9 *P<0.001 **P<0.001	73.3±0.9 *P<0.001 **P<0.001	51.9±0.5 *P<0.001 **P<0.001	33.0±0.8 *P<0.001 **P<0.001
		CT+HBOT (n=27)	76.2±0.8 *P=0.029	79.5±0.5* *P<0.001	57.0±0.4 *P=0.005	36.1±0.7
Control group (n=44)			79.5±0.3	85.3±0.3	60.0±0.2	37.3±0.2
Total (n=235)			74.1±0.7	79.3±0.6	55.0±0.4	35.4±0.5

**- statistically significant differences between the subgroup and control group; **- statistically significant differences between two subgroups*

The lagged size of TCD was found in the CT subgroups of Groups 1 and 3, while in the CT+HBOT subgroups, TCD did not differ significantly from the control group.

According to the data of fetometry, IUGR was detected in all women of the main group with a high PR (Table 4).

Table 4.

IUGR frequency in pregnant women with a high PR

Patient Groups			IUGR, degree			Total
			I	II	III	
Main group	Group 1	CT (n=42)	8 (19.0%) *P=0.036	3 (7.1%)	0	11 (26.2%) *P=0.048
		CT+HBOT (n=46)	6 (13.0%)	0	0	6 (13.0%)
	Group 2	CT (n=25)	3 (12.0%)	2 (8.0%)	0	5 (20.0%) *P=0.076
		CT+HBOT (n=25)	3 (12.0%)	0	0	3 (12.0%)
	Group 3	CT (n=26)	3 (11.5%)	2 (7.7%)	0	5 (19.2%) *P=0.083
		CT+HBOT (n=27)	3 (11.1%)	0	0	3 (11.1%)
Control group (n=44)			2 (4.5%)	0	0	2 (4.5%)
Total (n=235)			25 (10.6%)	7 (2.9%)	0	36 (15.3%)

**- statistically significant differences between the subgroup and control group; **- statistically significant differences between two subgroups*

In the control group, IUGR (only degree I) was diagnosed only in 4.5%. At the same time, the total frequency of IUGR was significantly higher in the CT subgroups compared to the CT+HBOT subgroups (>2.0-fold increase). In addition, in the CT+HBOT subgroup of Group 1, we found IUGR of degree I compared to degree I and II (7.1%) in the CT subgroup. Similar features were observed in subgroups of Group 3. The inclusion of HBOT in complex therapy in Group 2 contributed to a significant decrease (P<0.05) in IUGR frequency (a 1.7-fold decrease) in the CT+HBOT subgroup.

Dopplerometric study of FPC

The leading pathogenetic mechanism for the formation of primary PI in women with EGDs is hemodynamic disturbances in the vascular bed of the uterus. Hemodynamic disorders occurring in the placenta originate in the early gestation period and are aggravated with different EGDs. Abnormal uterine artery Doppler findings have shown a significant correlation with the risk of adverse perinatal outcomes such as small for gestational age and admission to Neonatal Intensive Care Units (NICU).[3] Zemel et al., has demonstrated that changes occur in the maternal circulation as early as the first trimester in women who develop pre-eclampsia and IUGR.[5]

A Doppler blood flow study in 6-9 gestational weeks did not reveal significant differences in RI of the uterine, radial and spiral arteries (Table 5) depending on the nature of EGDs and the therapy mode. In addition, there were no significant differences between the main group and the control group.

Doppler assessment of blood flow in UtA and UmA in 19-22 gestational weeks showed (Table 6) that, in general, in pregnant women with anemia and ChP, regardless of the nature of the therapy mode, RI of UtA and UmA did not differ significantly from the values of the control group. Significant changes were observed in women with AH: a significant increase in RI of UtA in the CT subgroup was found in

comparison with the CT+HBOT subgroup, the control group, and Groups 1 and 3, regardless of the mode of therapy.

Table 5.

A Doppler blood flow study in pregnant women with a high PR (6-9 gestational weeks)

Patient Groups			RI		
			UtA	RA	SA
Main group	Group 1	CT (n=42)	0.66±0.003	0.66±0.004	0.51±0.006 *P=0.018
		CT+HBOT (n=46)	0.68±0.004	0.66±0.004	0.52±0.007
	Group 2	CT (n=25)	0.71±0.004 *P<0.001	0.69±0.004 *P=0.002	0.55±0.007
		CT+HBOT n=25)	0.69±0.004	0.67±0.004	0.56±0.007 *P=0.004
	Group 3	CT (n=26)	0.67±0.002	0.65±0.009	0.54±0.006
		CT+HBOT (n=27)	0.68±0.004	0.66±0.004	0.54±0.007
Control group (n=44)			0.68±0.004	0.66±0.004	0.54±0.007
Total (n=235)			0.66±0.003	0.66±0.004	0.53±0.006

**- statistically significant differences between the subgroup and control group*

Table 6.

Doppler assessment of blood flow in UtA and UmA in 19-22 gestational weeks

Patient Groups			RI	
			UtA	UmA
Main group	Group 1	CT (n=42)	0.54±0.003	0.78±0.005 *P=0.014
		CT+HBOT (n=46)	0.50±0.004	0.75±0.005
	Group 2	CT (n=25)	0.67±0.004 *P<0.001 **P<0.001	0.78±0.003 *P=0.007
		CT+HBOT (n=25)	0.54±0.002	0.76±0.004
	Group 3	CT (n=26)	0.53±0.002	0.70±0.006 *P=0.007
		CT+HBOT (n=27)	0.52±0.004	0.73±0.004
Control group (n=44)			0.52±0.004	0.74±0.004
Total (n=235)			0.64±0.003	0.73±0.005

- statistically significant differences between the subgroup and control group;- statistically significant differences between two subgroups*

Changes in maternal-fetal circulation in 29-32 gestational weeks are shown in Table 7. In Group 1, a significant decrease in RI of UtA was detected in the CT subgroup, whereas in the CT+HBOT subgroup this indicator did not differ from the control values. In the CT subgroup, against a background of reduced RI of UtA, we found a significant increase (P<0.05) in RI of UmA and MCA compared to the control group and Group 3. At the same time, in the CT+HBOT subgroup, we found normalization in RI of MCA during 29-32 gestational weeks.

In Group 3, there were no significant blood-flow disturbances in MCA, but a significant increase in RI of UtA and UmA in the CT subgroup was diagnosed compared to the control group and the CT+HBOT subgroup.

Table 7.

Doppler assessment of maternal-fetal circulation in 29-32 gestational weeks

Patient Groups			RI		
			UtA	UmA	MCA
Main group	Group 1	CT (n=42)	0.34±0.003 *P<0.001 **P<0.001	0.76±0.002 *P<0.001 **P<0.001	0.74±0.002 *P<0.001 **P<0.001
		CT+HBOT (n=46)	0.43±0.002	0.65±0.003	0.70±0.002
	Group 2	CT (n=25)	0.67±0.003 *P<0.001 **P<0.001	0.77±0.002 *P<0.001 **P<0.001	0.75±0.002 *P<0.001 **P<0.001
		CT+HBOT (n=25)	0.50±0.002	0.68±0.004	0.71±0.002
	Group 3	CT (n=26)	0.53±0.002 *P<0.001 **P<0.001	0.70±0.002 *P<0.001	0.70±0.002
		CT+HBOT (n=27)	0.44±0.003	0.66±0.002	0.70±0.002
Control group (n=44)			0.45±0.002	0.64±0.002	0.69±0.002
Total (n=235)			0.40±0.002	0.69±0.002	0.75±0.002

- statistically significant differences between the subgroup and control group;- statistically significant differences between two subgroups*

In Group 2, a significant increase in RI of UtA, UmA and MCA was diagnosed in the ST subgroup (P<0.05). At the same time, in the CT+HBOT subgroup, we found normalization of maternal-fetal circulation, although the tendency for an increase in RI was traced.

In conclusion, HBOT in complex therapy in women with a high PR contributed to a significant decrease in IUGR frequency. In all patients of the CT+HBOT subgroups, we found IUGR only of degree I compared to degrees I (12%) and II (8%) in the CT subgroups. Thus, HBOT in complex therapy allows improving the function of FPC and reducing the incidence of PI in pregnant women with a high PR against the background of EGDs.

Analysis of ultrasound changes in the first trimester of pregnancy showed that such signs as "reduced fetal egg size" and "changes in the yolk sac" in the early stages of gestation are highly correlated with IUGR (r=0.67, P<0.01). Out of 26 women with anemia and with reduced fetal egg size and changes in the yolk sac, IUGR was diagnosed in 65.3%, in 7(77.8%) out of 9 women with ChP, and in 8(80%) out of 10 women with AH. Thus, ultrasound examination of women with EGDs in early stages of pregnancy allows identifying a high-risk group for premature birth.

Based on the data presented, we can conclude the following:

- In the early stages of gestation, there were no disturbances in fetoplacental blood circulation.
- Starting the 19th week of pregnancy, there is a significant increase in RI of UtA in pregnant women with AH.
- In women with a high PR on the background of the studied EGDs, the third trimester of pregnancy, despite the ongoing CT, is characterized by persistent impairment in fetoplacental blood circulation.
- The inclusion of HBOT in complex therapy in the early stages of pregnancy in women with a high PR allows leveling out the inevitable disturbances in fetoplacental blood circulation on the background of the studied EGDs.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

The publication was prepared with the support of the "RUDN University Program 5-100."

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International Journal of Biomedicine 8(3) (2018) 213-216
http://dx.doi.org/10.21103/Article8(3)_OA8

ORIGINAL ARTICLE

Otolaryngology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Cytokine Gene Polymorphisms in Chronic Adenoiditis

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Abstract

The aim of our research was to study the multiphase response in a system of pro-inflammatory and anti-inflammatory cytokines due to the additive contribution of homozygous and heterozygous genotypes for the polymorphic allelic variants of the interleukin-1β (*IL-1β*) and interleukin-4 (*IL-4*) genes in patients with chronic adenoiditis.

Materials and Methods: The study included 388 children with chronic adenoiditis. Associations between the *IL1B* gene (rs1143634) (C+3954T) SNP and the *IL-4* gene (rs2243250) (C-589T) SNP and the clinical manifestations and clinical outcome of chronic adenoiditis were investigated. Genotyping for the studied SNPs was performed using real-time PCR. The study of genotype-associated cytokine production in accordance with the level of concentration of IL-1β, IL-4 in blood serum with the method of solidphase EIA using horseradish peroxidase as an indicating enzyme was carried out.

Results*:* The presence of homozygous or heterozygous genotypes of the studied SNPs of the *IL-1β* and *IL-4* genes was characterized with genetically determined cytokine-production forming the phenotypical polymorphism. The conducted research into congenital immunity factors with an assessment of genetically determined cytokine production has revealed 5 options of the cytokine response and their corresponding frequencies. We extrapolated the results on clinical and functional outcomes of chronic adenoiditis, which allowed us to identify non-randomness in the nature of chronic adenoiditis as a multifactorial disease.

Conclusion: The obtained data are evidence of the phenotypic-genetic heterogeneity of chronic adenoiditis. **(International Journal of Biomedicine. 2018;8(3):213-216.)**

Key Words: chronic adenoiditis • interleukin-1β • interleukin-4 • single nucleotide polymorphism

Introduction

The multifactorial character of chronic adenoiditis (CA) is based on the complex interactions of genetic and environmental factors.[1] Non-specificity of clinical manifestations does not allow forecasting the course of the disease. However, there is a preventive approach to the prognosis, favorable or not favorable, that considers the efficacy of conservative therapy and risk of operative intervention.[2,3] The approach studies disorders in the physiologic immune processes in CA in children, which has been convincingly shown on the example of other nosological entities.[4]

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The aim of our research was to study the multiphase response in a system of pro-inflammatory and anti-inflammatory cytokines due to the additive contribution of homozygous and heterozygous genotypes for the polymorphic allelic variants of the interleukin-1β (*IL-1β*) and interleukin-4 (*IL-4*) genes in CA patients.

Materials and Methods

The study included 388 children with CA. The CA diagnosis was made on the basis of anamnesis data and clinical, epidemiological, endoscopic, radiological, and immunological data.

The inclusion criteria were the following:

- Children with CA (of both sexes, aged between 3 and 10)

diagnosed by ENT specialist, allergist, and pediatrician
- Residence in Krasnoyarsk city from birth
- Caucasians
- Remission for concomitant diseases
- Lack of care in the previous month
- Patient's ability to perform the procedure protocol

Exclusion criteria were the following:
- Children less than 3 years and over 10 years of age
- Children of a race other than Caucasian
- Exacerbation of concomitant diseases
- Acute respiratory viral infection
- Use of drugs that can affect the results of the study
-Violation of the procedure protocol

Clinical examination included an analysis of the complaints, historical data with exact duration of the disease and causes resulting in the development of CA, including causes of hereditary predisposition. In addition, we estimated the conceptual, subjective, and objective signs: the beginning and duration of the disease, the child's age at the moment the diagnosis was verified, the severity of the course of the disease, frequency and duration of CA manifestations, character of complications, the degree of pharyngeal tonsil hypertrophy, allergic manifestations, the degree of elevation of temperature in CA and/or acute viral respiratory infection, and spread of hypertrophy of cervical lymph nodes.

In the present study, we investigate associations between the *IL-1β* gene *(*rs1143634) (C+3954T) SNP and the *IL-4* gene (rs2243250) (C-589T) SNP and the clinical manifestations and clinical outcome of CA. Molecular-genetic studies were carried out in 317(81.7%) children with CA.

Genotyping for the studied SNPs was performed using real-time PCR followed by amplification with the use of oligonucleotides labeled with fluorescent agents complimentary to the part of PCR-product (*TaqMan* technique) of a sick child's blood sample DNA, with automatic detection. Each experiment contained a negative control in which DNA-matrix for PCR was substituted for distilled water. Amplification was performed in 50 µl volume containing 300 ng of DNA, 0.1µl of primer containing 16-25 pairs of nucleotides (Applied Biosystems, USA). The applied structural designs of primers for describing gene characteristics are presented in accordance with the data of the National Center for Biotechnology Information (rs – reference SNP) according to the protocol attached to the primer[4]:

1) For the *IL-1β* gene (rs1143634) (C+3954T)
CTCCACCTTTCAGAACCTATCTTCTT [C/T]
GACACATGGGATAACGAGGCTTATG
2) For the *IL-4* gene (rs2243250) (C-589T)
AACACCTAAACTTGGGAGAACATTGT [C/T]
CCCCAGTGCTGGGGTAGGAGAGTCT

PCR was performed in the amplifier Rotor-Gene-6000.

We carried out the study of genotype-associated cytokine production in accordance with the level of concentration of IL-1β, IL-4 in blood serum with the method of solidphase EIA using horseradish peroxidase as an indicating enzyme on the base of the certified Regional Laboratory and Diagnostic Center of Immunochemical Methods of Research of Krasnoyarsk cit. For this purpose, we used the certified IL-1β/IL-4 test-systems (BioChemMackDiagnostics, Russia).

Each of the three possible variants of genotypes was associated with production of coded cytokine.

Statistical analysis was performed using IBM SPSS Statistics V22.0 (SPSS Inc., Chi-cago, IL, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Continuous variables were presented as mean(M)±standard error of the mean (SEM) and as median (interquartile range [IQR]). Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Kruskal - Wallis one-way analysis of variance and Dunn post hoc test with Bonferroni adjustment were nonparametric alternative. Group comparisons with respect to categorical variables are performed using chi-square tests. Three exact probability tests for departure from HWE due to heterozygote excess, heterozygote deficit and omnibus probability test were carried out using GENEPOP (v. 4.7.0) A probability value of $P<0.05$ was considered statistically significant.

The study was carried out as a part of the ENT-department research "Translational otorhinolaryngology" (State registration No. 01201001212). Written informed consent was obtained from the child's parents.

Results and Discussion

The presence of homozygous or heterozygous genotypes of the studied SNPs of the *IL-1β* and *IL-4* genes was characterized with genetically determined cytokine-production forming the phenotypical polymorphism (Table 1).

Table1.

Associations between the IL-1β gene (C+3954T) SNP and the IL-4 gene (C-589T) SNP and cytokine production in CA patients (n=317)

SNP	Genotype		Concentration of cytokines, pg/ml Me [Q_{25}; Q_{75}]	$P \leq 0.05$
IL-1β gene (C+3954T)	CC	1	199.40 [190.28; 216.43]	P_{1-2}; P_{1-3} P_{2-3}
	CT	2	158.35 [66.88; 177.99]	
	TT	3	129.80 [113.50; 155.74]	
IL-4 gene (C-589T)	CC	1	139.40 [134.16; 149.30]	P_{1-2}; P_{1-3} P_{2-3}
	CT	2	187.70 [141.10; 200.50]	
	TT	3	216.54 [206.40; 247.20]	

To understand the multiphase response in the system of opposite cytokines in CA, we proceeded from estimating the presence of balance or imbalance of genotype variants. By 'balance' we mean the degree of equilibrium between opposed variants in a healthy subject for adequate functioning of cytokine system.

The most balanced was a heterozygous carrier, which was comprehensively presented in the population taken as a whole and allowed the individuals to adjust to environmental conditions. We found combined carriers of heterozygous genotypes of the *IL-1β* and *IL-4* SNPs in only 6.3±1.4% of cases in 20 CA children (Figure 1 A). This result could relate to

a balanced pro-inflammatory and anti-inflammatory cytokine response within the age reference range, which agreed with the results of laboratory studies of cytokine production.

Slightly more often, we registered disharmonious balance in pro-inflammatory and anti-inflammatory cytokine response, this being named 'high'. It consisted in homozygous genotype (CC)—a highly producing variant of pro-inflammatory cytokine IL-1β, causing elevated gene expression and production of this interleukin—combining with homozygous genotype (TT)—a highly producing variant of anti-inflammatory cytokine IL-4, which was accompanied by elevated gene expression and production of anti-inflammatory IL-4. Herewith, a certain balance between pro-inflammatory and anti-inflammatory responses could be achieved. However, from the point of view of polygenic interaction and mutual influence of other cytokine gene mutations participating in this response, such a balance should be rightly considered disharmonious because it is on the high limit of the reference range of the organism's reactivity. In so doing, a combination of highly producing variants of other pro-inflammatory cytokines, for example, IL-6, IL-8, etc., would cause a quick derangement of unstable disharmonious balance, a sharp increase of pro-inflammatory response, and a recurrent or severe course of CA as well. On the other hand, the same influence on the state of disharmonious genetic balance could be exerted by exogenous environmental factors. Disharmonious balance (high) was seen in 13.6±1.9% of cases in 43 CA children (Figure 1B).

The second kind of disharmonious balance on polymorph allele variants consisted in homozygous genotype (TT)—a low producing variant of cytokine IL-1β, causing down-regulation of gene expression and reducing production of this interleukin—combining with homozygous genotype (CC)—a low-producing variant of anti-inflammatory cytokine IL-4, causing the reduction of gene expression and production of IL-4.

In this case, the balance was also achieved but on the low limit of the reference range of reactivity, and it was disharmonious due to the polygenic influence of other pro-inflammatory and anti-inflammatory cytokines and the influence of exogenous environmental factors. The second variant of disharmonious balance of cytokine response was seen significantly less frequently in comparison with the first variant of disharmonious balance—in 25 children (7.9±1.5 %) (Figure 1C).

Deranged balance with increased pro-inflammatory response and decreased anti-inflammatory response was considered as an imbalance and occurred in the group under study statistically more often, in 35.0±2.7% of cases (n=111). This imbalance consisted in a combination of CC genotype of the *IL-1β* (C+3954T) SNP and CC genotype of the *IL-4* (C-589T) SNP (Figure 1D).

A contraversive variant of imbalance meant a prevalence of anti-inflammatory response and a decrease in pro-inflammatory response. The imbalance consisted in combined carriership of TT genotype of the *IL-1β* (C+3954T) SNP and TT genotype of the IL-4 (C-589T) SNP (Figure 1E).

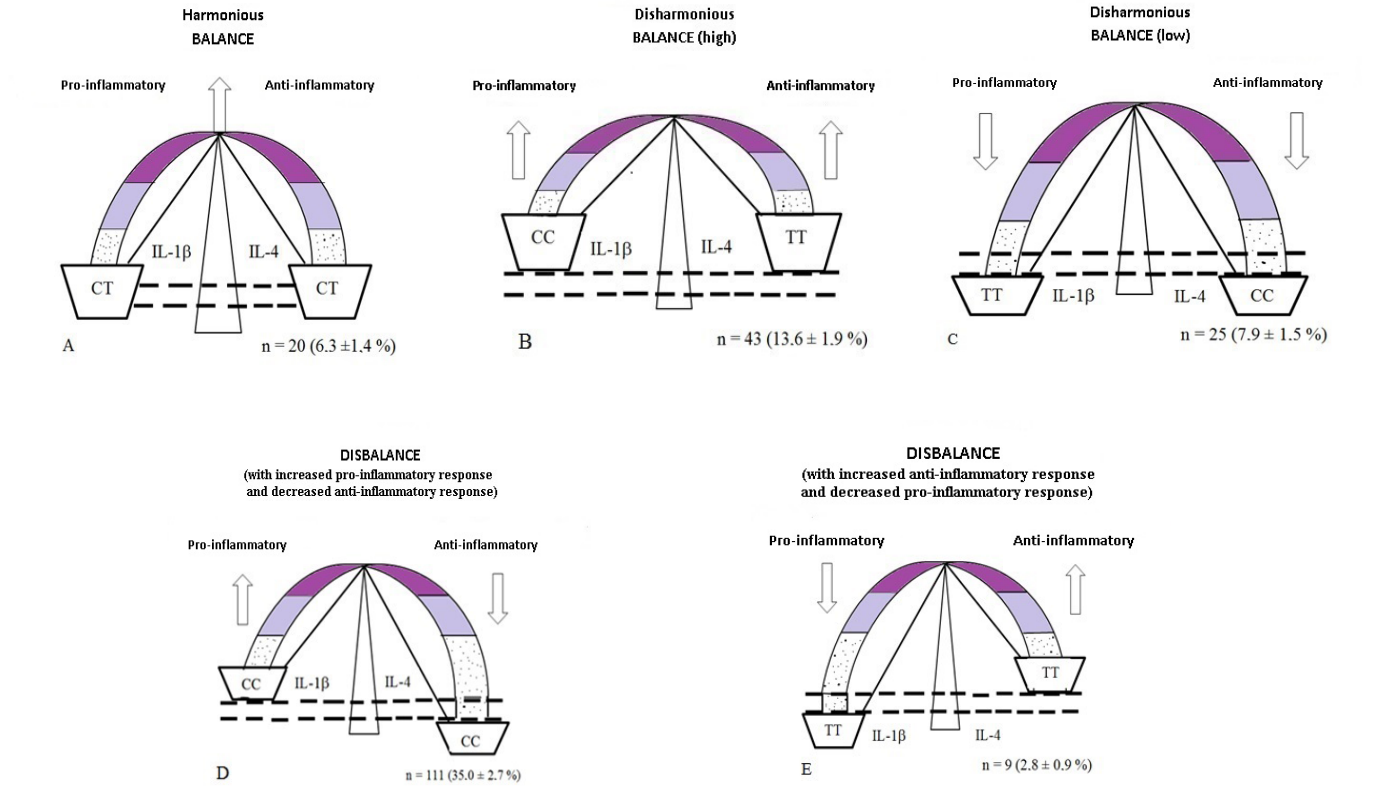

***Fig. 1**. Genetic and phenotypic balance of the state of cytokine response in CA children*

Risk level: -low risk, -moderate risk, -high risk; dotted line – a reference range of permissible cytokine response (within normal limits)

This variant was found in 2.8±0.9% of cases (n=9). Overall, the illustration visually demonstrates the genetic heterogeneity of immune cytokine response in CA in children. Also possible are intermediate combinations of homozygous and heterozygous variants of genetic and phenotypic imbalance of status of pro-inflammatory and anti-inflammatory cytokine response in CA. In this way, we should stress that the percentage of children with CA with an inappropriate balance was 93.7%, which was within the confidence interval.

Conclusion

In conclusion, the conducted research into congenital immunity factors with an assessment of genetically determined cytokine production has revealed 5 options of the cytokine response and their corresponding frequencies. We extrapolated the results on clinical and functional outcomes of chronic adenoiditis, which allowed us to identify non-randomness in the nature of chronic adenoiditis as a multifactorial disease.

Thus, modern advances in molecular and clinical genetics are evidence of the phenotypic-genetic heterogeneity of CA. Thorough analysis of cytokine gene functioning revealed their role and place in the diagnosis of multifactorial disease, in particular, CA in children, and in applied otorhinolaryngology, which previously remained outside the clinical interpretation and integration into existing practice.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 217-219
http://dx.doi.org/10.21103/Article8(3)_OA9

ORIGINAL ARTICLE

Infectious Diseases and Hepatology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Chronic Triple Infection with Hepatitis B, C, and D Viruses in the Republic of Sakha (Yakutia)

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Abstract

The purpose of this work was to study the features of the clinical course of mixed infections with hepatitis B+C+D viruses in the Republic of Sakha (Yakutia) (RS(Y)).

Materials and Methods: The incidences of these infections were studied in the infectious disease department of the Yakutsk City Clinical Hospital. A total of 74 patients with chronic infection with hepatitis B, C, and D viruses were analyzed. The following markers of HBV (HBsAg, HBeAg, anti-HBcIgG, HBV DNA), HCV (anti-HCV) and HDV (anti-HDV, HDV RNA) were detected.

Results: According to PCR (n=35), HCV-RNA was detected in 29(82.8%) patients. In 65.8% of cases, HCV-RNA replication was observed in the absence of HDV-DNA. Mono-replication of HBV (HBV-DNA+, HCV-RNA-) was detected in 17.1% patients, mono-replication of HCV (HBV-DNA-, HCV-RNA+) in 65.7% patients and mixed replication of viruses C, D and/or G (HBV-DNA-, HCV-RNA+, HDV-RNA+/HGV-RNA+) in 17.1% patients. The comparison of biochemical parameters of patients with chronic mixed hepatitis showed that more expressed changes are observed with the mixed replication than with the mono-replicative form of hepatitis.(**International Journal of Biomedicine. 2018;8(3):217-219.)**

Key Words: chronic mixed hepatitis • hepatitis B virus • hepatitis C virus • hepatitis D virus

Abbreviations

CVH, chronic viral hepatitis**; CHB**, chronic hepatitis B; **CHC,** chronic hepatitis C**; HBV,** hepatitis B virus; **HCV**, hepatitis C virus; **HDV**, hepatitis D virus; **HGV**, hepatitis G virus.

Introduction

HCV, HBV, and HDV share parallel routes of transmission; because of this, dual or triple viral infection can occur in a proportion of patients at the same time. In 1991-1995, only 2.6% of adult patients with acute viral hepatitis had a mixed etiology of the disease, but in recent years, multiple hepatitis co-infection reached 13.8%-16.8%.[1-6] The proportion of patients with multiple hepatitis co-infections depends on the environmental and clinical setting.[7] The Republic of Sakha (Yakutia) (RS(Y)) is one of the regions of Russia that is unfavorable for the prevalence of viral hepatitis B, C and D, as well as their adverse outcomes—cirrhosis and primary liver cancer.[8] According to the electronic register "Chronic viral hepatitis in Yakutia," 14,791 people are registered and 4.3% of them have chronic triple infection with hepatitis B, C, and D viruses.

The purpose of this work was to study the features of the clinical course of mixed infections with hepatitis B+C+D viruses in RS(Y).

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Materials and Methods

We studied the data of the incidences of these infections in the infectious disease department of the Yakutsk City Clinical Hospital. The following examinations were performed: physical examination, as well as clinical, biochemical, serological (ELISA) and molecular (PCR) evaluations. The following markers of HBV (HBsAg, HBeAg, anti-HBcIgG, HBV DNA), HCV (anti-HCV) and HDV (anti-HDV, HDV RNA) were detected.

Statistical analysis was performed using the statistical software «Statistica» (v8.0, StatSoft, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as means and SEMs for continuous variables. Categorical variables were analyzed using the Chi-square test with the Yates' correction. Comparisons between three groups were performed with the one-way ANOVA with Tukey's post-hoc test. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

A total of 74 patients (59.5% male and 40.5% female) with chronic triple infection with hepatitis B, C, and D viruses were analyzed. Nineteen (25.7%) of them were in the cirrhotic stage. The distribution of patients based on degree of activity of CVH (n=55) was as follows: chronic viral hepatitis with minimal activity was registered in 18.2% of patients, with moderate and severe activity in 23.6% and 58.3% of patients, respectively.

Most of the patients (68.9%) were residents of Yakutsk. Chronic mixed hepatitis was registered in patients from 15 districts. The largest number of patients was registered in the central and western zones of RS(Y) (Namsky, Nyurbinsky and Khangalas districts). Chronic mixed hepatitis was observed in indigenous people in 59.4% of cases, among non-indigenous people in 40.6%. A prevalence of young people under 29(41.9%) was noted.

During our study of the epidemiological anamnesis, we found that in 36.5% of patients there was an indication of previously endured acute B hepatitis. A history of parenteral manipulations, including operations, dental manipulations and various other parenteral interventions, was noted in 67.5% of patients. In 20.3% of cases, the development of the disease was linked with family contact. In 5.4% of cases, hepatitis occurred after intravenous drug use. About 6.8% of patients had association with sexually transmitted diseases, which suggests the possibility of the sexual way of transmission due to absence of other factors of possible infection with hepatitis B. Alcohol abuse was noted in 7(9.4%) patients, while the proportion of people with chronic hepatitis in the cirrhotic stage was 21%.

Molecular diagnostics was performed on 35 patients. HCV-RNA was detected in 29(82.8%) patients. In 65.8% of cases, HCV-RNA replication was observed in the absence of HDV-DNA. Suppression of HBV replication by HCV in acutely or chronically infected patients is well-described phenomenon. In vivo study in chimpanzees showed that acute HCV superinfection in chronic HBV infection resulted in marked reduction in the titer of serum HBsAg.[9,10] In clinical studies, the inhibition of HBV replication by HCV was also observed.[11,12] The mechanisms accounting for the suppression of HCV on HBV were investigated by Shih et al.[13] Their findings suggest that HCV may directly interfere with HBV replication and furthermore identified the HCV core protein as a repressor of HBV production.

HBV-DNA was detected in 6(17.1%) patients, HDV-RNA in 4(11.4%) patients, and HGV-RNA in 1(2.8%) patient. Chronic hepatitis with severe and moderate activity was diagnosed in all patients. HCV-RNA was not found in 6(17.1%) patients, and 29(82.9%) patients had no HBV-DNA replication.

Patients were divided by replicative activity into 3 groups: Group 1 included 6(17.1%) patients with mono-replication of HBV (HBV-DNA+, HCV-RNA-), Group 2 included 23(65.7%) patients with mono-replication of HCV (HBV-DNA-, HCV-RNA+), and Group 3 included 6(17.1%) patients with the observed mixed replication of viruses C, D and/or G (HBV-DNA-, HCV-RNA+, HDV-RNA+/HGV-RNA+).

HBsAg was detected in 27(77.1%) patients, including 83.3%, 86.9% and 33.3% in Groups 1, 2 and 3, respectively. The diagnosis of CHB was confirmed by the detection of antibodies to HBeAg in 20% of cases, total antibodies to HBcAg in 74.3% of cases and only in one patient (2.8%) by PCR. Antibodies to HCV were detected in 30(85.7%) patients by ELISA; in the other 5(14.3%) patients, diagnosis was confirmed by PCR. CHD was verified in 5(14.3%) patients by ELISA, and HDV-RNA in these cases was detected in 4(80%) patients.

Depending on the replicative activity of HBV and HCV, we studied clinical symptoms (Table 1). The main complaint of patients was an asthenic syndrome, manifested by unmotivated weakness and fatigue. The second place complaint was a dyspeptic syndrome with the greatest frequency in patients with mixed replication of hepatitis viruses. Jaundice and splenomegaly was observed mostly in patients with replication of HCV and the mixed replicative form of chronic hepatitis. Hepatomegaly was detected in 73.9%, 50.0% and 33.3% of patients in Groups 2, 3 and 1, respectively.

Table 1.

Clinical symptoms depending on the replicative activity of HBV and HCV

Symptoms and syndromes	Group 1		Group 2		Group 3	
	n	%	n	%	n	%
Asthenic syndrome	5	83.3	23	100	6	100
Dyspeptic syndrome	3	50.0	18	78.3	6	100
Hemorrhagic syndrome	1	16.7	9	39.1	4	66.7
Pain	1	16.7	14	60.9	3	50.0
Jaundice	-	-	7	30.4	3	50.0
Hepatomegaly	2	33.3	17	73.9	3	50.0
Splenomegaly	-	-	5	21.7	4	66.7
Arthralgia	1	16.7	12	52.2	3	50.0
Telangiectasia	1	16.7	10	43.5	5	83.3

The frequency of extrahepatic manifestations was most often found in patients of Group 3, then in Group 2. In patients of Group 1, we found equal frequencies (16.7%) of arthralgia and telangiectasia. Aminotransferase activity was the highest in Group 3 (4.6±0.54mmol/l) and the lowest in Group 1 (0.72±0.4 mmol/l) (Table 2). An increase in the serum level of bilirubin up to 45.0±36.2mmol/l was also observed in Group 3. The comparison of biochemical parameters of patients with chronic mixed hepatitis showed that more expressed changes are observed with the mixed replication than with the mono-replicative form of hepatitis.

Table 2.

Biochemical markers depending on the replicative activity of HBV and HCV

Variable	Group 1	Group 2	Group 3	*P*-value
ALT, mmol/L	0.72±0.4	2.34±0.4	4.65±0.54	0.002
Total bilirubin, mmol/L	15.6±5.4	36.6±32.2	45.0±36.2	>0.05
Total protein, g/L	82.7±1.7	77.2±4.3	79.3±6.2	>0.05
Albumin, g/L	46.4±1.2	46.2±3.6	40.0±1.3	>0.05
Platelets, 10^9g/L	240.8±20.5	247.7±50.1	237.8±58.8	>0.05
PTI, %	88.9±6.9	85.9±9.3	83.3±5.9	>0.05

In conclusion, the level of incidence of CVH in RS(Y) remains on a high level. The features of the mixed hepatitis have revealed that the most significant clinical and biochemical changes are common for mixed replication of viruses B and C. For objective assessment of the situation of viral hepatitis and its outcomes in RS(Y), we recommend including the registration of mixed hepatitis in the unified electronic "Chronic viral hepatitis in RS(Y)" register, as well as official registration of mixed forms of hepatitis in the Federal Service for Supervision of Consumer Rights Protection and Human Well-Being are recommended.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 220-223
http://dx.doi.org/10.21103/Article8(3)_OA10

ORIGINAL ARTICLE

Toxicology

INTERNATIONAL JOURNAL OF BIOMEDICINE

Experimental Study of Deltamethrin-Induced Nephrotoxicity in the Rat Model

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Abstract

The purpose of this study was to determine the nephrotoxic effect of deltamethrin in experimental animals at a dose of 43.5mg/kg *(*1/2 LD50).

Materials and Methods: For the experiment, 48 male Wistar rats with a body weight of 240±10 g were divided into 4 groups of 12 animals each. Groups 1 and 3 were control groups, which were administered a physiological solution intragastrically. The animals in Groups 2 and 4 received a single dose (43.5 mg/kg) of the synthetic pyrethroid deltamethrin, which corresponds to 1/2 LD50. Rats were withdrawn from the experiment in two stages: 1) rats in Groups 1 and 2 – one day after the deltamethrin administration; 2) rats in Groups 3 and 4 – 3 days after the deltamethrin administration. Biochemical and pathomorphological changes in the kidneys were evaluated. The evaluation criteria were the content of pyruvate, inorganic phosphate, and glutathione (GSH) and the activity of glutathione peroxidase activity (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in the kidneys. Histological preparations of kidney tissue were studied.

Results: The single administration of a toxic dose of deltamethrin caused a decrease in body weight of rats, an increase in kidney weight, and the accumulation of pyruvate and inorganic phosphate in the kidneys. A decrease in the GSH content was accompanied by an increase in the activity of GPx, GR and GST. One day after the experiment, in the convoluted tubules, epithelial cells with blurred contours of the boundaries were enlarged; and the granularity of the cytoplasm containing vacuoles was expressed. The nuclei of epithelial cells had different sizes; some of them were in a state of pycnosis. In the organ parenchyma, large and small blood vessels full of blood were visible. Three days after the intoxication, these symptoms became more pronounced. In the intertubular connective tissue, hemorrhages and leukocyte infiltrates were detected.

Conclusion: The study confirms the nephrotoxic effect of a single toxic dose (43.5 mg/kg [1/2 LD50]) of deltamethrin. Pathomorphological changes in the kidneys are accompanied by the disturbances in energy metabolism and activation of the glutathione antioxidant system with the development of glutathione deficiency.(**International Journal of Biomedicine. 2018;8(3):220-223.)**

Key Words: pesticides • pyrethroids • deltamethrin • kidney • antioxidant system

Abbreviations

BW, body weight; **GPx**, glutathione peroxidase; **GR**, glutathione reductase; **GST**, glutathione-S-transferase; **GSH**, glutathione; **KW**, kidney weight; **KMI**, kidney mass index.

Introduction

Synthetic pyrethroids are used extensively as insecticides and acaricides in agriculture and veterinary medicine, acting as neurotoxic agents. Among pyrethroids, deltamethrin is one of the most popular and widely used insecticides in the world.[1] It is used in agriculture and veterinary medicine for the destruction of pests of plants and ectoparasites of animals.[2] Deltamethrin plays a key role in controlling malaria vectors and preventing the spread of diseases carried by ticks.[3-5] This pesticide is highly toxic to aquatic life, particularly fish, and therefore must be used with extreme caution around water. Deltamethrin[1,6,7] is highly

toxic to humans and other mammals.In recent years, however, a number of studies have been published demonstrating pathologically proven motor neuron death in people after acute, massive ingestion of pesticides containing pyrethroids.[8-10] The effect of deltamethrin on different organs and systems has also been described.[11-14] In this connection, there is an increasing interest in studying its toxicity for animals and humans.

The purpose of this study was to determine the nephrotoxic effect of deltamethrin in experimental animals at a dose of 43.5 mg/kg *(*1/2 LD50).

Materials and Methods

For the experiment, 48 male Wistar rats with a body weight of 240±10 g were divided into 4 groups of 12 animals each. Groups 1 and 3 were control groups, which were administered a physiological solution intragastrically. The animals in Groups 2 and 4 received a single dose (43.5 mg/kg) of the synthetic pyrethroid deltamethrin, which corresponds to 1/2 LD50. Rats were withdrawn from the experiment in two stages: 1) rats in Groups 1 and 2 – one day after the deltamethrin administration; 2) rats in Groups 3 and 4 – 3 days after the deltamethrin administration.

In the course of the experiment, the preparative form of deltamethrin was used under the trade name "Butox 50" (Intervet Productions SA, France). 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.

At the final stage of the experiment, the rats were weighed. Kidneys of the animals were removed and weighed. The right kidneys were homogenized at 0-2°C and biochemical markers were determined in the resulting homogenate. The content of total protein, pyruvate and inorganic phosphate was determined by unified methods. The GSH level was determined according to Rousar et al.[15] the activity of GPx and GR according to Vlasova et al.[16], and GST according to Habig and Jakoby.[17]

For histological examination, the pieces of kidney tissue (the middle third of the left kidney) from each rat were fixed in a 4% neutral solution of formaldehyde, dehydrated in spirits with increasing concentration, then poured into paraffin. On a rotary microtome, tissue sections of 5 μm thick were made and stained with hematoxylin and eosin. 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 a decrease in BW of rats as a result of exposure to a toxic dose of deltamethrin (Table 1). This change was observed both in the first and third days of the experiment. An opposite trend was observed in the change in the mass of the kidneys of experimental animals. A decrease in BW and an increase in the mass of the kidneys led to an increase in KMI (Table 1).

Table 1.

BW of rats and KMI after after a single deltamethrin administration at a dose of 1/2 LD50

Group	BW, g	KW, g	KMI, g/100 g
Day 1			
Group 1	240 (239-248)	0.740 (0.700-0.817)	0.304 (0.288-0.341)
Group 2	237 (231-241) *P*=0.0223	0.905 (0.878-1.070) *P*=0.0003	0.393 (0.369-0.456) *P*=0.0001
Day 3			
Group 3	244 (240-248)	0.704 (0.654-0.776)	0.291 (0.272-0.314)
Group 4	234 (233-242) *P*=0.0050	0.995 (0.950-1.14) *P*=0.0003	0.420 (0.403-0.492) *P*=0.0002

The single administration of a toxic dose of deltamethrin caused the accumulation of pyruvate and inorganic phosphate in the kidneys (Table 2). A day after the beginning of the experiment, the median value for pyruvate and inorganic phosphate increased by 29.7% and 39.7%, on the third day - by 24.1% and 116%, respectively, compared to the control.

Much attention in the studies of pesticide-related intoxications is devoted to determining the antioxidant system parameters.[11,12,18] Of greatest interest, in our opinion, is the study of GSH, since GSH not only enters into battle with free radicals and products of lipoperoxidation, but also participates in the reactions of conjugation of synthetic pyrethroids.[19,20] In our experiment, there was a decrease in the content of GSH in the kidneys of rats subjected to a single deltamethrin administration (Table 2).

Table 2.

The content of pyruvate, inorganic phosphate (Pi), and GSH in the kidneys of rats after after a single deltamethrin administration at a dose of 1/2 LD50

Group	Pyruvate, μmol/g	Pi, μmol/g	GSH, nmol/mg protein
Day 1			
Group 1	0.431 (0.410-0.462)	5.56 (4.19-7.32)	30.8 (24.9-32.2)
Group 2	0.559 (0.490-0.601) *P*=0.0032	7.77 (6.25-9.24) *P*=0.0209	18.8 (17.6-24.3) *P*=0.0005
Day 3			
Group 3	0.440 (0.407-0.510)	4.56 (3.58-7.06)	29.1 (26.0-33.1)
Group 4	0.546 (0.479-0.620) *P*=0.0056	9.83 (8.17-12.18) *P*=0.0002	20.7 (18.8-26.2) *P*=0.0015

A decrease in the GSH content in the kidney was accompanied by activation of enzymes of its metabolism (Table 3). GPx activity increased by 73.4% and 42.2% on the first and third days of the experiment, respectively. GR activity increased by 82.9% and 81.5% on the first day and the third day, respectively.

Table 3.

The activity of glutathione-dependent enzymes in the kidneys of rats after a single deltamethrin administration at a dose of 1/2 LD50

Group	GPx, U/mg protein	GR, U/mg protein	GST, U/mg protein
Day 1			
Group 1	504 (446-647)	299 (252-368)	373 (345-474)
Group 2	874 (598-958) *P*=0.0043	547 (452-634) *P*=0.0001	726 (635-793) *P*<0.0001
Day 3			
Group 3	578 (465-610)	248 (230-349)	414 (352-455)
Group 4	822 (674-923) *P*=0.0001	450 (354-564) *P*=0.0010	607 (573-672) *P*=0.0001

Although the total GST activity in the kidneys is lower than in the liver, renal GST also promotes the formation of conjugates with degradation products of deltamethrin. A single deltamethrin administration at a dose of 1/2 LD50 led to an increase in GST activity in kidneys by 94.6% after the first day of the experiment. On the third day of the experiment, the GST activity remained quite high (Table 3).

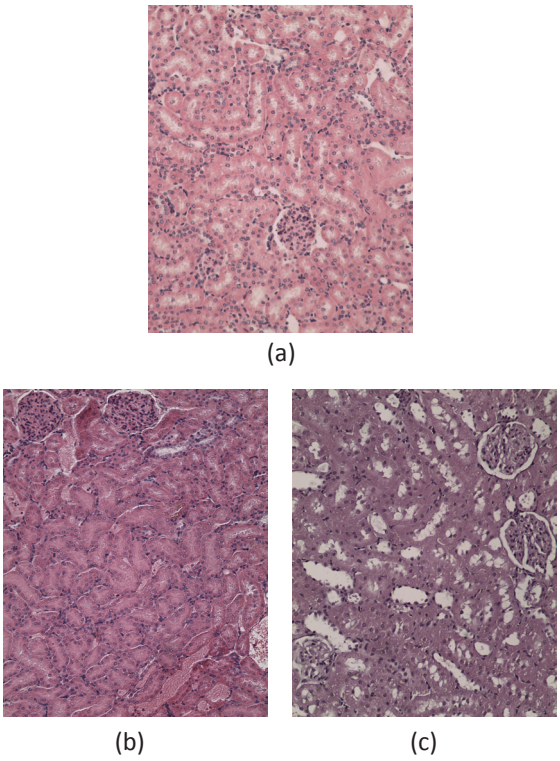

Fig. 1.

Light microscopy of the kidneys of rats. Control (a), 24 hours (b) and 72 hours (c) after a single deltamethrin administration at a dose of 1/2 LD50 (43.5 mg/kg). Hematoxylin Eosin staining.
(a) The size of the glomeruli is unchanged, small subcapsular spaces, epithelium of the renal tubules without damage.
(b) The granularity of the cytoplasm of renal tubule epithelial cells, narrowing of lumen of proximal tubules, congestive hyperemia.
(c) Cytoplasmic vacuoles in renal tubule epithelial cells.

During the histological examination, we found the following changes (Figure 1): One day after the experiment, in the convoluted tubules, epithelial cells with blurred contours of the boundaries were enlarged; and the granularity of the cytoplasm containing vacuoles was expressed. The nuclei of epithelial cells had different sizes; some of them were in a state of pycnosis. In individual cells, the diameter of the nuclei was increased in comparison with the control. Some vascular glomeruli were hyperemic. In the organ parenchyma, large and small blood vessels full of blood were visible. Three days after the intoxication, these symptoms became more pronounced. Degenerative processes in the epithelial cells of tubules increased; therefore, the number of fragments of desquamated cells increased in the tubular lumen, and the lumen of the tubules was narrowed. The intensity of vacuolization of the cytoplasm on different sites of histological specimens was expressed in different degrees. In the intertubular connective tissue, hemorrhages and leukocyte infiltrates were detected. On the control histological specimens, various sections of the renal tubules and glomeruli were observed, surrounded by a capsule, without pathological changes.

Discussion

An increase in the mass of the kidneys may indicate the active inclusion of these organs in clearing and removing deltamethrin and its metabolites from the bodies of rats. To activate metabolism and neutralize deltamethrin in the kidneys, a large amount of energy is needed. However, the accumulation of pyruvate, noted above, indicates a disturbance of aerobic oxidation, probably due to inhibition of the tricarboxylic acid cycle. This assumption is confirmed by an increase in the concentration of inorganic phosphate in the kidneys of rats in the early stages of acute poisoning with deltamethrin. The source of a large number of phosphoric acid residues can be the active catabolism of the purine and pyrimidine mono-, di- and triphosphates.[21]

The increased burden on the glutathione system of the kidneys after deltamethrin administration promotes the activation of enzymes of its metabolism, probably due to activation of the antioxidant response element (ARE).[22,23] ARE induces the synthesis of GP and GR, which function in close cooperation and contribute to providing the glutathione redox cycle. In itself, the use of GSH in these two reactions does not lead to GSH deficiency. However, there are a number of enzymes that extract GSH from this cycle and irreversibly attach GSH or its individual amino acids to various substrates, in particular, to deltamethrin decay products. Such enzymes include GST; we noted its activation in the kidneys of rats after acute poisoning. GST, along with GP and GR, is an enzyme whose gene is regulated by ARE.[22]

After the action of GST and associated enzymes, GSH breaks down into constituent amino acids. For the re-synthesis of GSH, the energy of ATP is required, the availability of which is limited by the inhibition of the cycle of tricarboxylic acids and the mitochondrial respiratory chain. This can contribute to the development of GSH deficiency and ultimately disrupt the functioning of the glutathione system of the kidneys.

The described biochemical changes also have morphological manifestations. Our study demonstrates that under the simulated conditions, free radical processes are activated. Active oxygen metabolites can be produced by both kidney cells and immune system cells.[24,25] The reactive oxygen species themselves are capable of provoking and stimulating the development of destructive processes.[26]

Moreover, lesions can occur in different parts of nephrons (glomeruli, renal tubules, vessels) and induce necrosis or apoptosis.[27]

Thus, our study confirms the nephrotoxic effect of a single toxic dose (43.5 mg/kg [1/2LD50]) of deltamethrin. Pathomorphological changes in the kidneys are accompanied by the disturbances in energy metabolism and activation of the glutathione antioxidant system with the development of glutathione deficiency.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 224-227
http://dx.doi.org/10.21103/Article8(3)_OA11

ORIGINAL ARTICLE

Women's Health

INTERNATIONAL JOURNAL OF BIOMEDICINE

Prognostic Significance of Anthropometric and Bioimpedance Parameters of Yakut Women for Birth of Newborns with High Body Weight

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Abstract

The aim of this study was to identify the prognostically significant anthropometric and bioimpedance indicators for the birth of a child with a high BW in Yakut women.

The study included 220 women of Yakut nationality with physiological pregnancy and childbirth who were examined on the third and fourth days after delivery. The parameters of anthropometry and bioimpedancemetry in parturient women and the anthropometric indices of newborns were investigated. Anthropometric measurements were carried out according to the method of V.V. Bunak. Body composition was assessed based on bioimpedance analysis using the ABC-01 MEDASS device (Medass, Russia). The conducted research revealed that the anthropometric and bioimpedance indices of Yakut women in the postpartum period (3-4 days after delivery) were significantly different from general population indicators. The parameters of body weight and body mass index of the puerperal women were significantly higher, and phase angle and Xc50 - significantly lower. PCA revealed anthropometric and bioimpedance indices predicting a high birth weight. The obtained data in combination with other indicators can be used to predict the birth of a child with a high birth weight in Yakut women. **(International Journal of Biomedicine. 2018;8(3):224-227.)**

Key Words: anthropometry • bioimpedance • puerperal women • fetal macrosomia

Abbreviations

BIA, bioimpedance analysis; **BW**, body weight; **BMI**, body mass index; **BC**, buttock circumference; **BL**, body length; **CC**, chest circumference; **CE**, conjugata externa; **DS**, distantia spinarum; **DC**, distantia cristarum; **DT**, distantia trochanterica; **HC**, head circumference; **HBW**, high birth weight; **PhA**, phase angle; **TCD**, transverse chest diameter; **TBW**, total body water; **VEF**, volume of extracellular fluid; **VCF**, volume of cellular fluid; **WC**, waist circumference.

Introduction

Fetal macrosomia (FM) can be caused by many factors, including genetic predisposition, elevated BMI of the women, overdue pregnancy, iatrogenic causes (use of a number of drugs), presence of diabetes mellitus, obesity, and others.[1] Prenatal diagnosis of FM is of great importance in obstetric practice to determine the tactics of labor, prevent maternal and perinatal morbidity and mortality, and assess the long-term negative consequences for the health of the mother and newborn.[2-8]

Identifying available methods for FM diagnosis is an important practical task.[9-10] One such method is anthropometry, which is an accessible and non-invasive approach.[11] In the scientific literature there are data on the dependence of the anthropometric indicators of the newborn on the constitution, height and size of the mother's pelvis. The definition of a mother's BMI is also of great importance for predicting the morphofunctional parameters of a newborn.

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The aim of this study was to identify the prognostically significant anthropometric and bioimpedance indicators for the birth of a child with a high BW in Yakut women.

Materials and Methods

The study included 220 women of Yakut nationality with physiological pregnancy and childbirth who were examined on the third and fourth days after delivery. We investigated the parameters of anthropometry and bioimpedancemetry in parturient women and the anthropometric indices of newborns. All examined women (all primiparous) without chronic diseases and complications of the pregnancy and childbirth were born and permanently resided in the city of Yakutsk and the central regions of Yakutia. The mean age of the examined women was 26.0 [23.0; 31.0] years.

The ethnicity of the study participants was determined based on personal data. The parameters of 220 newborns (birth weight, BL, CC, and HC) were collected from neonatal records. The examined women were students, employees, workers and homemakers.

Anthropometric measurements were carried out according to the method of V.V. Bunak.[12] BL was measured using a Martin metal anthropometer with an accuracy of 0.1cm. BW was measured without clothing using medical scales with an accuracy of 50 g. The circumference dimensions of waist and buttocks were determined using centimetric tape. P*elvic* (pelvis major) measurements (distantia spinarum, distantia cristarum, distantia throchanterica, and conjugata externa) were performed with a large caliber compass with an accuracy of 1 mm. The accuracy of the instruments used was verified after every 100 measurements, using a special calibration block.

Body composition was assessed based on BIA using the ABC-01 MEDASS device (Medass, Russia). The principle of operation of this device is based on the relationship between the electrical resistance of the body tissues measured at different frequencies and the volume of the fluid compartments of the body. The accuracy of impedance measurements was 2%. Bioimpedancemetry was performed by the tetrapolar method using a sinusoidal current with a constant frequency of 50 kHz with no more than 1mA in the range of measured impedance values up to 1000 Ohm. Recording was performed for 5min after fixing 4 paired silver-silver chloride (Ag-AgCl) electrodes (Schiller Biotabs) (current and potential) on the wrist and shin (on the right side). The distance between injecting and reading electrodes was 5 cm. The impedancemeter and computer were used to measure and calculate the main fluid volumes of the body (TBW, VEF, VCF), as well as the lean body weight and body fat mass. The subjects were asked not to move when the instrument was measuring the bioelectrical impedance. The data processing software developed by the device manufacturer (Medass, Russia) was used for all calculations. The accuracy of the device used was verified after every 50 measurements, using a Dummy Simulator.

BIA uses body resistance (R) and reactance (Xc) to a flow of alternating electrical current to determine impedance and estimates body composition parameters from regression equations derived against a reference method. Phase angle (PhA) was calculated (at 50 kHz) as the arctangent of the ratio of Xc to R (converted to degrees) with values for the majority of people lying between 3° to 15°.[13] O. Selberg and D. Selberg [14] suggest classifying phase angles greater than 5.4° as normal, in the range 4.4°–5.4° as borderline, and less than 4.4 as abnormal. 6.6 (5.4-7.8)°. According to R. Baumgartner et al.,[15] the mean phase angle for women is 6.3 (4.9-7.7)°.

Statistical analysis was performed using statistical software package SPSS version 17.0 (SPSS Inc, Chicago, IL). The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution were presented as mean (standard deviation [SD]); non-normal variables were reported as median (interquartile range [IQR]) (Me[25-75]). Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Mann-Whitney U test was used to compare means of 2 groups of variables not normally distributed. PCA (Principal Component Analysis) was used to determine which anthropometric and bioimpedance indicators explained the majority of the observed variations in HBW. The risk coefficients were determined by Bayesian method in modification by Genkin-Gubler for studied indicators.[16] A value of $P<0.05$ was considered significant.

Results and Discussion

Among the study participants, 12 anthropometric and 21 bioimpedance parameters were evaluated. By PCA, from among the measured parameters, 7 were selected as predictors of a HBW. There were 5 anthropometric parameters (BMI, TCD, CC, BC, DT) and 2 bioimpedance parameters (PhA, Xc50).

BMI was calculated according to length and body weight, which averaged 159.0 [156.0; 165.0] cm and 66.7 [59.7; 73.5] kg, respectively. The BMI values of the examined women were 26.20 [23.20; 28.40] kg/m^2. In accordance with BMI, normal weight was found in 39.1% of cases. Excess body weight was determined in 47.3% of women and obesity in 13.6% of cases. We did not find women who were underweight.

The values of TCD, CC and BC were 26.0 [25.0; 27.0] cm, 90.0 [87.0; 94.0] cm, and 98.0 [93.0; 104.0] cm, respectively. Pelvic measurements revealed that DS, DC, DT, and CE were 26.0 [25.0; 28.0] cm, 28.5 [27.0; 30.0] cm, 32.0 [31.0; 34.0] cm, and 21.0 [20.0; 23.0] cm, respectively.

BIA determined that PhA was 5.9 [5.5; 6.2]° and Xc50 – 60.8 [53.2; 67.7] Ohm.

The indicators of the physical development of newborns were as follows: BL– 52.0 [50.0; 54.0] cm, BW – 3,520.0 [3,000.0; 3,750.0] g, CC - 34.0 [33.0; 34.0] cm, and HC – 35.0 [34.0; 35.0] cm.

It should be noted that parameters of BW and BMI in parturient women significantly differed from parameters of the general Yakut female population of the same age group. According to the anatomical and anthropological studies (population research) conducted in the region, BW and BMI of Yakut women (aged between 21 and 35 years) in a non-pregnant state were 59.0±0.6 kg and 23.2±0.2 kg/m^2, respectively.[17] BMI and BW of women in the postpartum period were significantly higher, which is the physiological

norm because during pregnancy BW increases due to an increase in the uterus, mammary glands, fatty tissue, etc.[18]

Bioimpedance parameters (PhA, Xc50) also had significant differences from general population indicators. Significantly lower values of Xc50 in puerperal women are probably associated with physiological hydration during pregnancy and in the postpartum period. Thus, TBW in these women was significantly higher than in women in a non-pregnant state: 31.3 [30.0; 34.8] kg vs. 28.2 [26.4; 30.5] kg (P<0.05).[19] The magnitude of PA in puerperal women was also significantly lower, but was within the normal range.

Pelvic (pelvis major) dimensions allow predicting the favorable course of the delivery. Bony pelvis dimensions of all the women we examined corresponded to the standards adopted in obstetrics.[20]

Based on the centile analysis, it was established that for the group of Yakut women that we examined, the mass of the newborn from 3,000 g to 3,750 g was considered normal. The birth weights <3,000 g and >3,750 g were considered as a low birth weight and a high birth weight, respectively.

The most important morphometric and bioimpedance parameters that have prognostic significance for the birth of a child with a high weight (Table 1), have been identified using PCA. Two components were selected. The first component had a calculated weight of 54.828% and included 5 anthropometric parameters. The most significant parameter was BMI, which had a factor load of 0.914. This component included also TDC, CB, CC (factor load from 0.824 to 0.875). Of all the measured pelvic dimensions, DT with a factor load of 0.693 was significant. The second component had a calculated weight of 25.090. It included 2 bioimpedance indices: PhA and Xc50. The listed morphometric and bioimpedancec indicators have prognostic value for FM in Yakut women.

Table 1.

The morphometric and bioimpedance parameters, which have prognostic significance for HBW (PCA)

Component	Eigenvalues		Variable	Factor loadings
	percentage of variance	cumulative percentage		
Component 1	54.828	54.828	BMI	0.914
			TCD	0.875
			BC	0.867
			CC	0.824
			DT	0.693
Component 2	25.090	79.918	PhA	0.984
			Xc50	0.934

On the next stage, we constructed a prognostic table for determining the risk of high birth weight (Table 2). For all the selected parameters, the corresponding prognostic coefficients were computed. The prognostic coefficients with the sign "+" testified in favor of HBW, and with the sign "-" - the opposite.

Prenatal prognosis of the risk of high birth weight was calculated by converting the parameters of the 7 prognostic signs into prognostic factors in accordance with Table 2: the higher the sum of the prognostic factors - the greater the risk of HBW.

Table 2.

Prognostic table for determining the risk of high birth weight

№	Prognostic criteria	Scale	Prognostic coefficients
1	BMI, kg/m^2	1) <18.5	0
		2) 18.5-25.0	-5
		3) 25.0-30.0	+3
		4) >30.0	+2
2	TCD, cm	1) <25.0	-4
		2) 25.0-27.0	-2
		3) >27.0	-6
3	BC, cm	1) <94.0	-3
		2) 94.0 -104.0	0
		3) >104.0	+1
4	CC, cm	1) <86,5	-11
		2) 86,5 – 94,0	0
		3) >94,0	-5
5	DT, cm	1) <30.5	- 2
		2) 30.5 – 34.0	0
		3) >34.0	+5
6	PhA	1) <5.5	+5
		2) 5.5 – 6.5	-2
		3) >6.5	+4
7	Xc50, Ohm	1) <53.6	-3
		2) 53.6 – 69.2	-1
		3) >69.2	+3

The next stage consisted of checking and evaluating the effectiveness of the developed method. The results of a survey of 25 puerperal women and their newborns were analyzed. The expert evaluation showed a reliable prognosis in 23(92%) women.

The conducted research revealed that the anthropometric and bioimpedance indices of Yakut women in the postpartum period (3-4 days after delivery) were significantly different from general population indicators. The parameters of BW and BMI of the puerperal women were significantly higher, and PA and Xc - significantly lower. PCA revealed 7 parameters predicting a HBW: five anthropometric parameters (BMI, TCD, CC, BC, and DT) and two bioimpedance parameters (PhA and Xc50). The obtained data in combination with other indicators can be used to predict the birth of a child with a HBW in Yakut women.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 228-231
http://dx.doi.org/10.21103/Article8(3)_OA12

ORIGINAL ARTICLE

Epidemiology/Population

INTERNATIONAL JOURNAL OF BIOMEDICINE

Cold Trauma in the Structure of External Causes of Mortality and Disability in the Republic of Sakha (Yakutia)

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Abstract

The purpose of this study was to analyze the data of official medical statistics on mortality and disability in the Republic of Sakha (Yakutia) (RS(Y)) as a result of exposure to excessively low natural temperatures. For the retrospective epidemiological analysis, we used the data of the official statistics of RS(Y) for 2011-2015, data on the disability of the Federal State Institution "Main Bureau of Medical and Social Expertise of RS(Y)" for 2014-2016, and data of the burn department of the Center for Emergency Medical Care of Yakutsk for 2014-2016. In Yakutia, during 2011-2015, 662 people died in road accidents, and 812 died as a result of exposure to excessive natural cold; the mortality rates were 13.8 and 17.0 per 100,000 population, respectively. In the structure of external causes of mortality, the share of cold trauma in 2015 was 10.4% (15.1 per 100,000 population), which put cold trauma in the third rank after suicide and murders, having displaced road accidents in fourth place (5.6%, 7.8 per 100,000 population). The disability rate due to frostbites was 1.19 per 100,000 population in 2014 and 1.73 per 100,000 population in 2015 and 2016. (**International Journal of Biomedicine. 2018;8(3):228-231.)**

Key Words: excessive natural cold • hypothermia • frostbites • disability rate mortality rate

Introduction

The threat to public health (disability, premature mortality) posed by exposure to low natural temperatures is one of the poorly understood health problems. The data in scientific publications testify to the relevance of this issue on a global scale. Thus, in the USA for the period between 2003 and 2013, 13,419 deaths from hypothermia were recorded—0.3-0.5 deaths per 100,000 population. The share of males was 67%; there is also an age-specific feature: for persons over 65 years this coefficient was 1.8-1.1 deaths per 100,000 population. [(1)] In Sweden, mortality as a result of hypothermia, frostbite and hypothermia in cold water is 3.4, 1.5 and 0.8 cases per 100,000 population, respectively. The mortality rate for severe hypothermia varies from 12% to 80%, according to foreign literature, and depends on the age, predisposing factors, and the causes and timing of the initiation of treatment.[(2)] This problem is especially acute in Russia, whose vast territories are located in the northern latitudes. In the Amur Region, in a specialized department for treatment of victims with thermal lesions, patients with cold trauma (CT) account for 12%-19% (60-90 people per year). The marginal rate of hospitalization of patients with CT in the Chita region is 1.9 cases per 10,000 population per year.[(3)]

The Republic of Sakha (Yakutia) (RS(Y)) 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 1km^2. In RS(Y), the cold season lasts 7 months a year with an average winter temperature of -35 to -40°C. In such conditions, CT occupies a special place in the structure of causes of mortality from external causes, being an acute medical and social problem.

It should be noted that the medical and demographic situation in RS(Y) is characterized by a high level of premature mortality from preventable causes, especially accidents, injuries and poisonings. During the period between 1990 and 2014, in the structure of the causes of mortality, external causes consistently occupied the second ranked place after diseases of

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the circulatory system. Between 2010 and 2015, the mortality rate from external causes decreased by 30.3% (from 195.3 to 136.2 per 100,000 population); but different dynamics were noted in the types of external causes. For example, along with a significant reduction in the level of violent deaths (murders, suicides), there has been an increase in the death rate from accidental alcohol poisoning.[4]

Official statistics attribute the causes of mortality from external causes mainly to road accidents, violence, accidental alcohol poisoning, accidental drowning, accidental falls, and so on. CT is taken into account among the "other" reasons, and therefore the true picture of the scale of the disaster, at least for RS(Y), remained hidden.

The purpose of this study was to analyze the data of official medical statistics on mortality and disability in RS(Y) as a result of exposure to excessively low natural temperatures from 2011 to 2015.

Materials and Methods

For the retrospective epidemiological analysis, we used the data of the official statistics of RS(Y) for 2011-2015, data on the disability of the Federal State Institution "Main Bureau of Medical and Social Expertise of RS(Y)" for 2014-2016, and data of the burn department of the Republican Hospital #2—the Center for Emergency Medical Care of Yakutsk—for 2014-2016. For the study of mortality, Diagnosis Code X31 of ICD-10 (Exposure to excessive natural cold [EENC]) was selected; for the study of disability - Codes T33.2 (Superficial frostbite of thorax), T34 (Frostbite with tissue necrosis), T35 (Frostbite involving multiple body regions and unspecified frostbite), and T69.8 (Other specified effects of reduced temperature).

Results

Analysis of statistical data revealed that more people die from cold trauma every year in RS(Y) than in road accidents. In Yakutia, during 2011-2015, 662 people died in road accidents, and 812 died as a result of EENC; the mortality rates were 13.8 and 17.0 per 100,000 population, respectively (Table 1).

The largest share of deaths (80.3%) was made up by people of working age, including 78% by men aged between 16 and 59 and 22% by women aged between 16 and 54.

In general, the dynamics of the mortality rate from EENC has a positive tendency—a decrease by 35.1% during 2011-2015 from 20.4 to 15.1 per 100,000 population. However, in the structure of external causes of mortality, the share of CT in 2015 was 10.4% (15.1 per 100,000 population), which put CT in the third rank after suicide and murders, having displaced road accidents in fourth place (5.6%; 7.8 per 100,000 population).

According to data of the Center for Emergency Medical Care of Yakutsk, a specialized department for patients with thermal trauma receives about 200 EENC victims each year, and a combination of frostbite of limbs of different degrees with general cooling is about 10%. In 2014-2016, 513 patients (17.9 per 100,000 population) received specialized treatment for frostbite and general hypothermia, including 33 children (6.4%). Severe frostbites with tissue necrosis occurred in 35.0% (n=179) of all cases, including 5 children (1.0%). Due to the severity of the injury, 7 patients died; hospital mortality was 1.4% (Table 2); and 251(49%) patients suffered various surgical interventions for frostbite, including 187(36.5%) patients who underwent amputation of the extremities (Table 3).

Undoubtedly, the amputation of extremities is a serious bodily injury, resulting in a restriction of physical possibilities and a deterioration in the quality of life. At the same time, there was a discrepancy between the number of persons who underwent amputation of limbs and the number of persons recognized as disabled for this reason. For example, in 2014-2016, 40 people underwent amputation of the lower extremities at the level of the shins and feet and 9 people - amputation of the hand at the level of the forearm and hand (not counting the cases of finger amputation), but of these only 14 were recognized as disabled.

The disability rate due to frostbites was 1.19 per 100,000 population in 2014 and 1.73 per 100,000 population in 2015 and 2016. The most common cause of disability was amputation of frostbitten limbs (55.6%), including 66.7% amputation of hands and 33.3% of legs. According to experts, official statistics reflect, at best, only about half of people with disabilities in society, which is due to a number of circumstances. First, some of the persons recognized as disabled by the results of the examination in the expert commissions do not apply to the social protection authorities. Second, since disability accounting is oriented toward the source of pension provision, people with disabilities who receive other kinds of pensions (by age, loss of breadwinner, etc.) do not fall into the general statistics.

Table 1.

The number of deaths in RS(Y) as a result of road traffic accidents and CT in 2011-2015

Causes of mortality	2011		2012		2013		2014		2015		Total	
	n	per 100,000 population	n	per 100,000 population	n	per 100,000 population	n	per 100,000 population	n	per 100,000 population	n	per 100,000 population
Road accidents	157	16.4	134	14.0	156	16.3	140	14.6	75	7.8	662	13.8
CT	195	20.4	164	17.6	150	15.7	158	16.5	145	15.1	812	17.0

Table 2.

The number of patients who received treatment in the burn department of the Center for Emergency Medical Care of Yakutsk in 2014-2016

Nosological forms (Code)	2014		2015		2016		Total
	discharged after treatment	deaths	discharged after treatment	deaths	discharged after treatment	deaths	
T33.2-T69.8	156	5	179	1	171	1	513
including children under the age of 17	7	-	14	-	12	-	33
T34	57	-	64	-	58	-	179
including children under the age of 17	1	-	2	-	2	-	5
T69.8	21	-	17	-	19	-	57

Table 3.

Number of surgical interventions for frostbite in 2014-2016

Type of surgery	2014	2015	2016	Total
Amputation of the extremities, including:	52	67	68	187
- amputation at the level of the shins	2	7	7	16
- hand amputation	-	-	5	5
- amputation of the upper limb fingers	28	30	24	82
- amputation of lower limb fingers	19	24	13	56
- amputation at the level of the forearm	-	1	3	4
- foot amputation	3	5	16	24
Osteonecretomy	5	19	3	27
Autodermoplasty	12	13	3	28
Necroectomy	4	5	-	9
Total	73	104	74	251

Third, disability accounting is departmental; therefore, some people with disabilities who receive pensions in other departments (the Ministry of Defense, the Ministry of Internal Affairs, the Federal Security Service) are also not included in the overall statistics.

Thus, on the example of RS(Y), the severity of the problem of EENC for the territories of the Far North is undeniable. The problem, as well as other external causes of mortality, is not only medical but also social, as it goes far beyond the competence of the health system. Two major adverse factors are to be noted: the lack of self-protective behavior and the spread of alcoholism, which play a leading role, as in the case of mortality from road traffic accidents. In other words, CT is a preventable factor of premature mortality and disability of people of mostly young age groups. The unfavorable background is the vast territory of RS(Y), the remoteness of settlements, lack of roads, and lack of infrastructure and a stable satellite connection for the timely call of help. This problem is especially acute in the arctic regions of the republic, and requires a special system of state measures to find a solution.

It is clear that the clinical course and outcome of CT depend on the timely diagnosis, scope and adequacy of first aid and the subsequent basic therapy and rehabilitation. At the same time, many questions concerning diagnostics and treatment tactics are not sufficiently disclosed or are contradictory.[5] There are no unit recommendations or standards for treatment of victims with general cooling or frostbite. Many questions of management strategies to improve patients' treatment (preferable passive or active warming in hypothermia, extracorporeal methods, thrombolytic therapy), as well as timing for surgical treatment, are also unresolved.[3,6] All the

above-mentioned issues indicate the need to develop clinical guidelines for the diagnosis and treatment of CT, based on the data of modern scientific research and accumulated practical experience.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 232-234
http://dx.doi.org/10.21103/Article8(3)_OA13

ORIGINAL ARTICLE

Epidemiology/Population

INTERNATIONAL JOURNAL OF BIOMEDICINE

Metabolic Syndrome in Indigenous Minorities of the North of Yakutia

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Abstract

The aim of our research was to study the prevalence of MetS in the North of Yakutia. The study was conducted under expeditionary conditions in the northern regions of Yakutia in the places where the indigenous peoples of the North live. . A total of 686 people aged between 20 and 70 were examined in 4 districts: Anabarsky (Anabar), Nizhnekolymsky (Lower Kolyma), Verkhnekolymsky (Upper Kolyma), and Tomponsky (Tompo). In all regions, there was a high prevalence of hypertension, with the highest frequency in the Anabarsky District. The greatest frequency of MetS was found in the Evenks (56.7%) and the lowest in the Chukchi (20%). Women had higher MetS frequency than men, and differences between men and women are statistically significant, with the highest frequency in Evenks women. **(International Journal of Biomedicine. 2018;8(3):232-234.)**

Key Words: indigenous minorities • abdominal obesity • arterial hypertension • metabolic syndrome

Abbreviations

AO, abdominal obesity; **AH**, arterial hypertension; **BMI**, body mass index; **CVD**, cardiovascular disease; **FPG**, fasting plasma glucose; **HDL-C**, high-density lipoprotein cholesterol; **IGT**, impaired glucose tolerance; **LDL-C**, low-density cholesterol; **MetS**, metabolic syndrome; **OGTT**, oral glucose tolerance test; **TG**, triglycerides; **WC**, waist circumference.

Introduction

Cardiovascular disease (CVD) is one of the leading causes of mortality in the Republic of Sakha (Yakutia) (RS(Y)), as it is across Russia. According to the Federal State Statistics Service, the CVD morbidity rate of the population remained on the same level from 2013 to 2015, and the mortality decreased slightly by 0.9%, making the mortality rate 45.4%.[(1)] Despite the tendency to a reduction in the CVD mortality rate (403.7 per 100,000 population in 2013, 406.5 per 100,000 population in 2014, and 386.7 per 100,000 population in 2015), the ischemic heart disease mortality rate (152.3 per 100,000 population in 2013, 162.7 per 100,000 population in 2014, and 167.5 per 100,000 population in 2015), including the myocardial infarction mortality rate (23.6, 23.2, and 37.7 per 100,000 population, respectively), tends to rise, A certain role in this process belongs to MetS. This syndrome is one of the widely discussed problems in modern medicine. The International Diabetes Federation (IDF) estimates that about 25% of the world's population has MetS[(2)] although this estimate varies widely depending on the age, ethnicity, and gender of the population studied.[(3-7)] MetS is associated with increased CVD and all-cause mortality.[(8,9)] The prevalence of MetS increases with age, especially in an average age group (30%-40%).[(10-12)] According to studies conducted in the early 2000s, a significant increase in the prevalence of AH (44.6%) and overweight (42.4% in men and 51.7% in women) was noted among the indigenous population of Evenkia.[(13)] In Yakutia, besides Yakuts, the native inhabitants, there are several indigenous minorities. The prevalence of MetS among these ethnic groups has not previously been studied. However, carrying out such a study among indigenous minorities of the

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North is of great clinical importance, given the changes in the traditional way of life and nutritional habits, as well as the high prevalence of hypertension.

The aim of our research was to study the prevalence of MetS in the North of Yakutia.

Materials and Methods

The study was conducted under expeditionary conditions in the northern regions of Yakutia in the places where the indigenous peoples of the North live. A total of 686 people aged between 20 and 70 were examined in 4 districts: Anabarsky (Anabar), Nizhnekolymsky (Lower Kolyma), Verkhnekolymsky (Upper Kolyma), and Tomponsky (Tompo) (Table 1). In the compared groups, women prevailed. For the comparative analysis, we created five groups based on ethnic grounds living in the researched districts (Table 2).

Inclusion criteria: representatives of indigenous minorities of the North of Yakutia (the Dolgans, the Evens, the Evenks, the Chukchi, the Yukagir). Exclusion criteria: representatives of non-indigenous nationality and the Yakuts. The sample was formed according to the administrative lists of employees of the settlements. The response was 76%.

Table 1.

Gender characteristics of indigenous minorities of the researched districts of Yakutia

Variable	Anabar	Lower Kolyma	Upper Kolyma	Tompo
Total	274	182	89	141
Men	81(29.6%)	66(36.3%)	35(39.3%)	51(36.2%)
Women	193(70.4%)	116(63.7)	54(60.7%)	90(63.8%)
Average age, yrs	46.33±0.81	47.04±0.87	47.3±2.5	43.02±0.98

Table 2.

Gender characteristics of indigenous minorities of Yakutia based on ethnic grounds

Variable	Dolgans	Evens	Evenks	Chukchi	Yukaghirs
Total, n	85	141	67	40	77
Men, n (%)	26(30.6%)	51(36.2%)	13(19.4%)	20(50%)	34(44.2%)
Women, n (%)	59(69.4%)	90(63.8%)	54(80.6%)	20(50%)	43(55.8%)
Average age, yrs	44.93±1.56	43.02±0.98	48.37±1.64	39.73±1.93	46.49±1.54

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems. Written informed consent was obtained from each patient.

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

- Anthopometrical reference data: BMI was calculated using Quetelet's formula (in kg/cm^2). Measurement of WC was made at the uppermost lateral border of the ilium using a tape measure (in cm)
- Assessment of blood pressure by Korotkov's method.
- Assessment of FPG, OGTT, and blood levels of TG, HDL-C, LDL-C.

MetS was diagnosed according to the Russian national recommendations (the All-Russian Scientific Society of Cardiologists [VNOK, 2009])[14] based on the IDF consensus criteria (2006).[15] According to the that definition, the MetS is present when WC is increased (≥94 cm in males and ≥80cm in females (for Europids)) and at least two of the following factors are present: raised TG (≥1.7 mmol/l); reduced HDL-C (<1.0 mmol/l in males and <1.2 mmol/l in females); increased LDL-C (>3.0 mmol/l); systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg; raised FPG (>6.1 mmol/l) and or IGT (OGTT ≥7.8 mmol/l and ≤11.1 mmol/l). IGT 2Hr PG ≥7.8 mmol/l and ≤ 11 mmol/l .

Statistical analysis was performed using SPSS (version 17.0). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The frequencies of categorical variables were compared using the Chi-square test. A probability value of $P<0.05$ was considered statistically significant.

Results

The research was conducted within two research projects: "The contribution of MetS to the development of coronary artery atherosclerosis in Yakutia residents" and "Development of new technologies for the treatment and prognosis of the risk of arterial hypertension and stroke in RS(Y) (State Contract No. 1133)."

In all regions, there was a high prevalence of hypertension, with the highest frequency in the Anabarsky District (Fig.1). In all groups, there was a high frequency of AO (from 47.5% in the Chukchi to 79.1% in the Evenks) (Fig.2). We found statistically significant differences in the frequency of AO in women compared with men. However, it is necessary to notice that there was equally high frequency of AO in Yukaghirs men and women in comparison with other ethnoses.

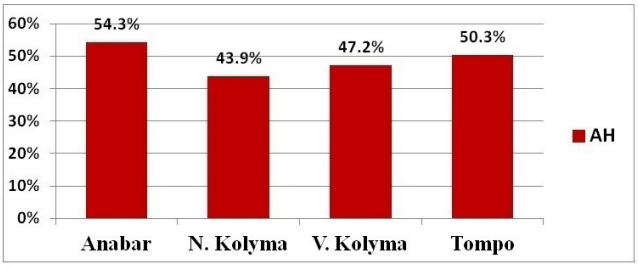

Fig. 1. *Prevalence of AH in the researched districts of Yakutia.*

We compared the frequency of MetS in the surveyed ethnic groups in accordance with the VNOK criteria (Fig.3). The greatest frequency of MetS was found in the Evenks (56.7%) and the lowest in the Chukchi (20%). Such a large difference in the MetS frequency was due to gender differences in these groups

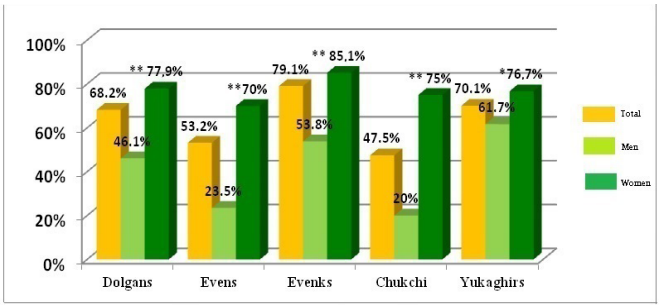

Fig. 2. *Prevalence of AO in indigenous minorities (**-P<0.001 and *-P<0.05 – between women and men).*

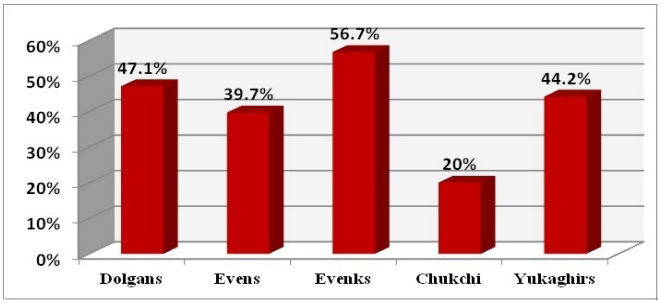

Fig. 3. *MetS frequency in the surveyed ethnic groups.*

Considering the traditional, historically developed, essential differences in the level of physical activity and other characteristics of lifestyle between men and women, the MetS frequency assessment in the compared groups was carried out separately for them (Fig.4). Women made a significant contribution to the frequency of MetS among the adult population. Women had higher MetS frequency than men, and differences between men and women are statistically significant, with the highest frequency in Evenks women (61.1%).

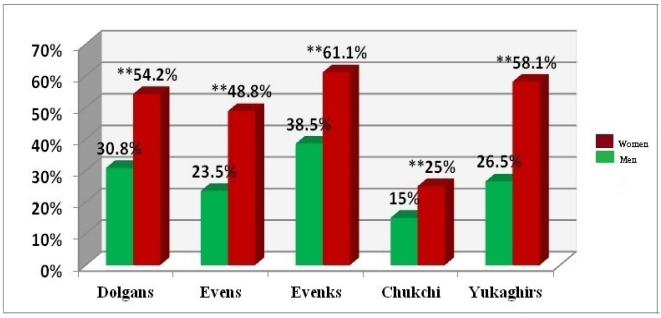

Fig. 4. *MetS frequency in the surveyed ethnic groups depending on gender (**- P<0.001 – between women and men).*

In conclusion, we found a high prevalence of arterial hypertension in the remote northern regions of the RS(Y), where the representatives of indigenous minorities of the North live. The high risk of cardiovascular complications points out the necessity of further in-depth study of all factors affecting the health of the population in the areas of compact residence of indigenous minorities of Yakutia. Our study shows a high frequency of metabolic syndrome in the examined ethnic groups, which is caused by a change in the traditional lifestyle, the nature of nutrition, and low physical activity. The highest frequency of metabolic syndrome was observed in women. In the public health arena, the desirable way to reduce MetS is by lifestyle intervention, especially weight reduction, increased physical activity, and an anti-atherogenic diet.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 235-239
http://dx.doi.org/10.21103/Article8(3)_OA14

ORIGINAL ARTICLE

Sports Medicine

INTERNATIONAL JOURNAL OF BIOMEDICINE

Complex Assessment of the Blood Oxidative Metabolism in Qualified Athletes

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Abstract

The purpose of this research was to study the structure of the shifts in the blood oxidative metabolism in professional athletes.

Materials and Methods: The study included 262 highly qualified athletes aged between 19 and 29 years. The control group consisted of healthy untrained volunteers of similar age. In blood plasma, we estimated the levels of 8-isoprostane, ox-LDL, alpha- and beta-carotene, alpha- and gamma-tocopherols, and tissue-specific antioxidants (lycopine, luteine and zeaxantine) and the activity of SOD and GP.

Results: Thus, in qualified athletes, characteristic changes in the state of oxidative metabolism, concerning the components of the pro- and antioxidant systems, were determined; however, the inhomogeneity of these metabolic transformations attracts attention. The revealed regularity allows confirming the previously stated hypothesis about the heterogeneity of shifts in oxidative metabolism in professional athletes, which suggests different approaches to their correction. **(International Journal of Biomedicine. 2018;8(3):235-239.)**

Key Words: oxidative stress • professional athletes • blood oxidative metabolism • antioxidants

Abbreviations

GPx, glutathione peroxidase; **GR**, glutathione reductase; **LPO**, lipid peroxidation; **OS**, oxidative stress; **ox-LDL**, oxidized low-density lipoprotein; **SOD**, superoxide dismutase.

Introduction

In the conditions of disadaptation, including professional sports, there are significant shifts in oxidative metabolism, typically characterized by an intensification of LPO against a background of decreasing antioxidant reserves in organs and tissues.[1-5] The pronounced manifestation of this trend, which is an independent pathogenetic mechanism known as OS, is considered as an independent syndrome.[3-8] Some researchers also give a 3-degree gradation of the severity of this syndrome,[9-12] implying a differentiated approach to the evaluation of the pathological state being studied and, consequently, its management.

In view of this circumstance, the possibility and necessity of diagnostics and pathogenetic correction of OS is assumed.[6,10,13-15] In relation to diagnostics, technologies and methods for estimating the shifts in oxidative metabolism have been proposed and are being developed. They are based on the determination of laboratory markers of varying degrees of specificity in biological substrates, the study of spontaneous biochemiluminescence of body fluids and tissues, and the use of modern instrumental techniques (instrumental techniques such as electron paramagnetic resonance and fluorescent probes. and others).[4,9,16,17] It should be noted that the determination of qualitative and quantitative criteria for the diagnosis of the state of pro- and antioxidant systems is necessary not only for the purpose of detecting OS,[2,6,13,16]

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but also for monitoring the adaptive capabilities of individual systems and the organism as a whole, including in sports medicine, adaptation, and environmental physiology.[13,18,19]

The second aspect of the problem, pathogenetic correction of OS, is related to the development of measures to correct the disturbances in oxidative metabolism.[10,14,15,20,21] This correction can be performed in two ways: by normalizing metabolism in general and/or by administering into the body natural and synthetic compounds that have antioxidant activity. In this case, the first path is nonspecific, since almost any pharmacological agents can be considered as compounds having indirect antioxidant activity and, therefore, as contributing to the optimization of one or more metabolic components.[6,15,20-22]

Taking into account the peculiarities of metabolic processes in professional athletes, who are forced to adapt to intensive regular physical training and to psychoemotional stress during competitive activity,[4,7,11,20,23] determining oxidative metabolism shifts and their severity in professional athletes seems very interesting. At the same time, in the special literature, there is not enough information about the nature of such disturbances, but there are single studies that assume the presence of OS in qualified athletes.[3,8,11,16,21,24]

The purpose of this research was to study the structure of the shifts in the blood oxidative metabolism in professional athletes.

Materials and Methods

The study included 262 highly qualified athletes—representatives of cyclic sports (ski races, rowing, cycling, athletics, and orienteering) with a sport title from Candidate for Master of Sport of Russia to Master of Sport of the International Class (Group 1) aged between 19 and 29 years. The control group (Group 2) consisted of healthy untrained volunteers of similar age (n=35).

The present study was approved by the local Ethics Committee of Federal Medical Biophysical Center named after A.I. Burnazyan (Record No.18 dated 10.12.2015). Written informed consent was obtained from each patient.

The serum level of 8-isoprostane was determined by ELISA using an 8-isoprostane ELISA kit ("USBiological", USA). Quantitative determination of ox-LDL was carried out by ELISA in a microplate format using the automatic immunoassay analyzer "Evolis" (Bio-Rad, Germany-USA) with Biomedica Gruppe reagents. The SOD activity was estimated by inhibiting the auto-oxidation of epinephrine in carbonate buffer at pH10.0 after the addition of blood hemolysate samples in proportions 1:50, according to the method of M. Sun and S. Zigman (1978). GPx activity was analyzed by measuring the oxidation of reduced glutathione in the presence of t-butylhydroperoxide (Moin 1986), and GR activity by its ability to catalyze NADPH-dependent reduction of oxidized glutathione (Karpischenko AI, 2002). Alpha- and beta-carotene and alpha- and gamma-tocopherol were determined according to the technique of Moisenok et al. (2009). The level of tissue-specific blood antioxidants (lycopene, lutein and zeaxanthin) was determined by chromatographic mass spectrometry, according to A.V. Grigoriev (2005) and N.L. Batsukova & E.R. Yaremko (2015).

Statistical analysis was performed using the Statistica 6.1 software package (StatSoft Inc, USA). The mean (M), standard error of the mean (SEM), and standard deviation (SD) were calculated. The Shapiro-Wilk test was used in testing for normality. Multiple comparisons were performed with a one-way ANOVA. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

In qualified athletes, the levels of most parameters of the studied metabolic component were significantly different from those in untrained individuals. Thus, in Group 1, the plasma level of 8-isoprostane (Figure 1) was 1.25 times higher than in Group 2 ($P<0.05$). Taking into account that the plasma concentration of 8-isoprostane is considered as an integral laboratory marker of OS,[25] the observed tendency indicates excessive stimulation of free radical oxidation processes induced by intensive physical training.

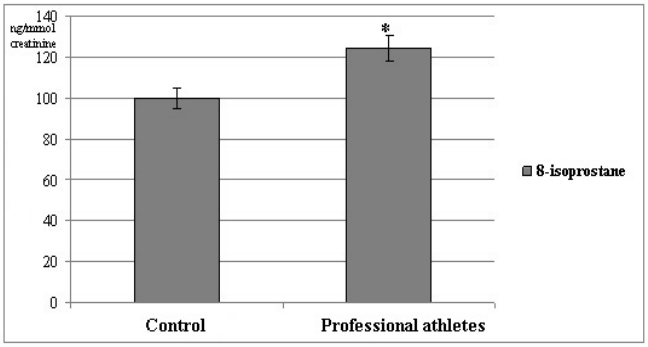

Fig. 1. *The plasma level of 8-isoprostane in professional athletes and healthy untrained volunteers (*- P<0.05).*

At the same time, the level of ox-LDL in Group 1 was 16% lower than in Group 2 ($P<0.05$, Figure 2), which is apparently related to the predominant effect of the studied factor, not on LPO, but on oxidative damage of other biomacromolecules, in particular, on the oxidative modification of proteins.

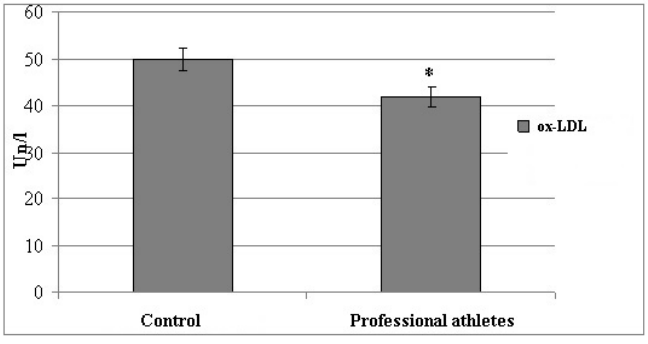

Fig. 2. *The plasma level of ox-LDL in professional athletes and healthy untrained volunteers (*- P<0.05).*

This is indirectly evidenced by the character of the shift in SOD

activity observed in highly trained athletes (Figure 3). Thus, in Group 1, a moderate inhibition of the catalytic properties of this enzyme was found in comparison with Group 2. Changes in the level of this parameter, on the one hand, indicate the active participation of the enzyme in the utilization of the free radicals formed (by removing the superoxide radical anion from the biological fluid), and, on the other hand, can reflect the partial oxidative modification of SOD as a large protein molecule.

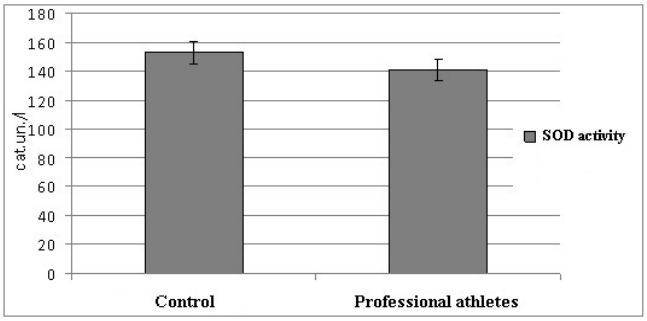

Fig. 3. *The catayitic activity of SOD in professional athletes and healthy untrained volunteers.*

The pronounced activation of free radical processes in qualified athletes is also evidenced by the dynamics of plasma concentrations of non-tissue-specific, non-enzymatic antioxidants. In particular, the level of alpha and gamma-tocopherol in Group 1 was significantly lower than in Group 2 (Figure 4). At the same time, this trend is most significant for gamma-tocopherol, the concentration of which in Group 1 was 1.68 times lower than in Group 2, whereas the level of alpha-tocopherol decreased only 1.23 times (P<0.05 in both cases).

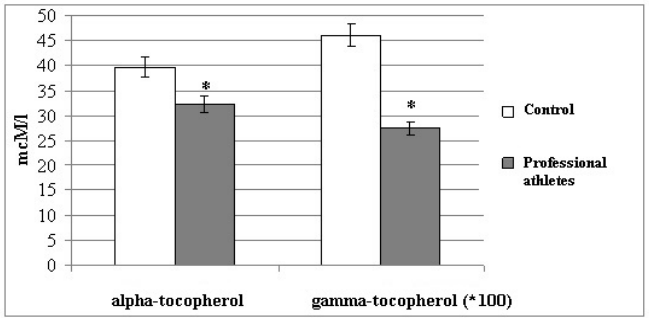

Fig. 4. *The plasma level of alpha-tocopherol and gamma-tocopherol in professional athletes and healthy untrained volunteers (*- P<0.05).*

It should be emphasized that not only does the absolute decrease in the levels of both tocopherols take place, but, taking into account the lipophilic nature of the latter, there is also a decrease in the "plasma vitamin E level/plasma cholesterol level" ratio, which decreased in Group 1 by 1.25 times compared to Group 2 (P<0.05).

Similar, but less pronounced, changes were recorded for another group of non-enzymatic antioxidants—carotenes (Figure 5). In professional athletes, the plasma level of alpha-carotene decreased more significantly than the plasma concentration of beta-carotene (by 1.3 and 1.1 times, respectively, P<0.05 in both cases). This further confirms the deficit of the antioxidant potential, which is formed under the influence of regular, intense physical activity and indicates the development of OS in these conditions.

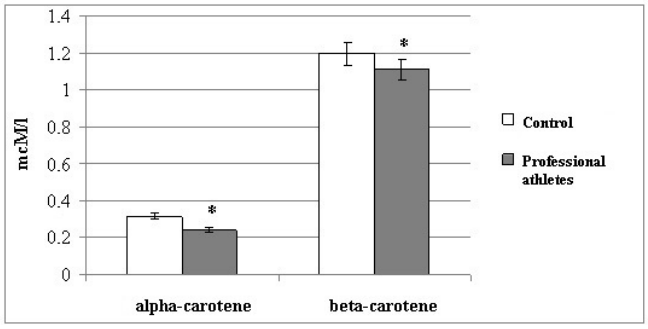

Fig. 5. *The plasma level of alpha-carotene and beta-carotene in professional athletes and healthy untrained volunteers (*- P<0.05).*

This tendency fully applies to tissue-specific antioxidants also (Figure 6). In particular, the plasma levels of zeaxanthin, lycopene and lutein were significantly reduced in Group 1 compared to Group 2 (up to 1.9 times, P<0.05).

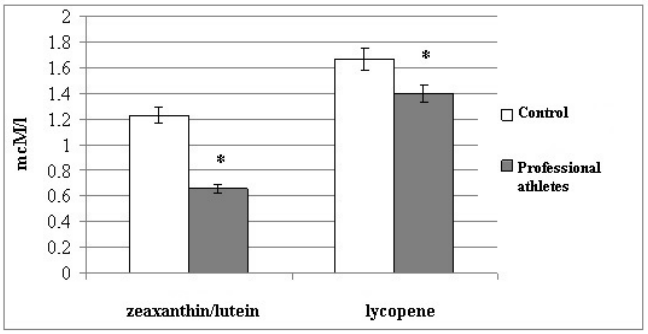

Fig. 6. *The plasma level of lycopene, zeaxanthin and lutein in professional athletes and healthy untrained volunteers (*- P<0.05).*

Thus, in qualified athletes, characteristic changes in the state of oxidative metabolism, concerning the components of the pro- and antioxidant systems, were determined. At the same time, the heterogeneity of the nature of the loads used, as well as the presence of individual features of free radical processes, allow one to assume the heterogeneity of their changes under conditions of regular intensive physical training. For a more detailed study of such trends, we used the method of assessing the state of oxidative metabolism, based on a joint examination of the values of parameters characterizing the activity and reserves of pro- and antioxidant systems (Figure 7).

Attention is drawn to the fact that in almost all cases of pairwise comparisons, structural diagrams allow us to distinguish 2 subgroups of athletes, which indicates the expediency of creating two variants of metabolic support oriented to the type of metabolism modification (Figure 7).

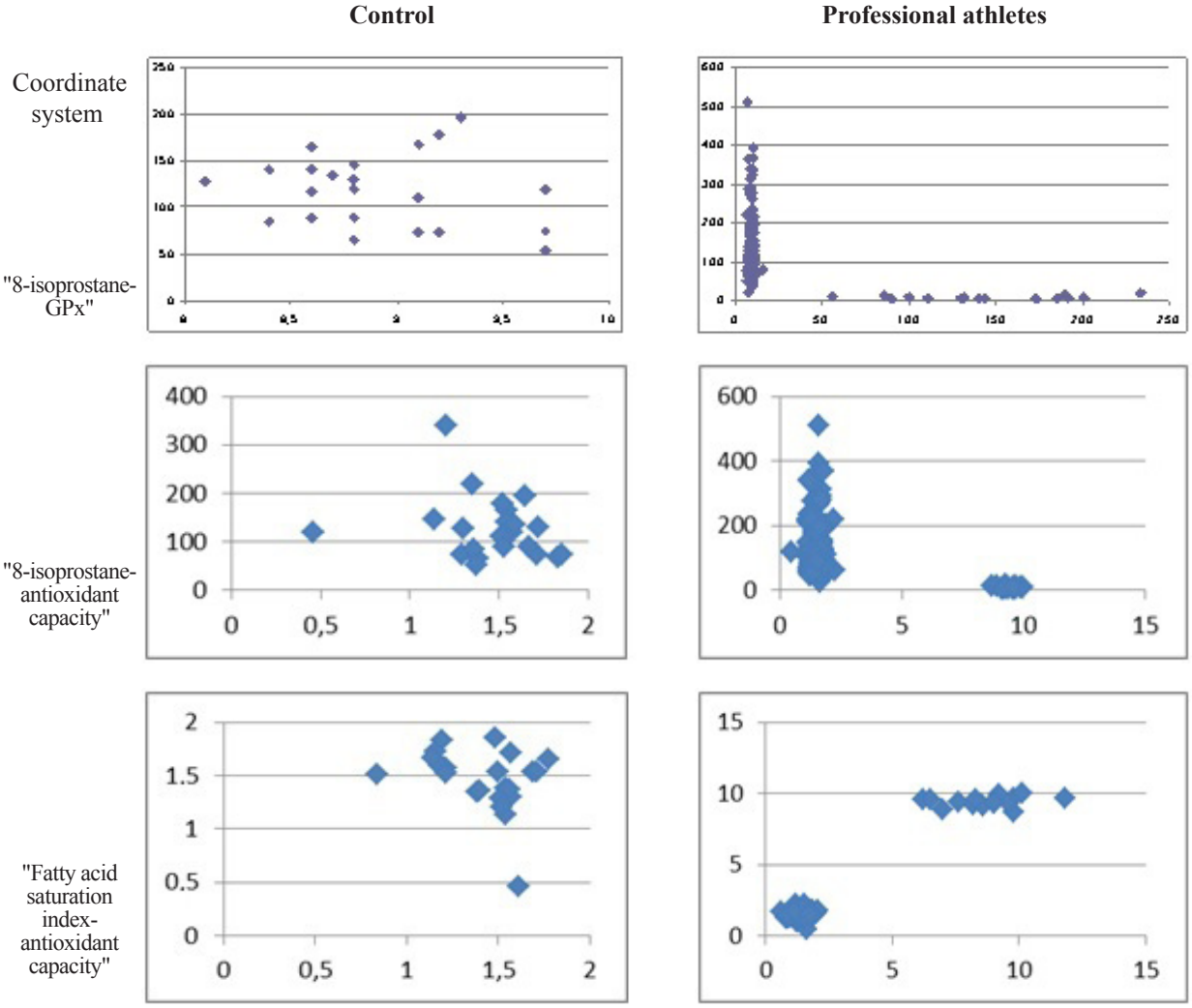

Fig. 7. *Two-dimensional comparative analysis of indicators of the state of pro- and antioxidant blood systems in professional athletes and healthy untrained volunteers.*

It is interesting that paired comparisons performed between the indices characterizing separately the state of pro- and antioxidant systems, as well as between 2 parameters of one of the listed components of oxidative metabolism, also demonstrate the dichotomous heterogeneity of Group 1. Thus, the representation of Group 1 in the coordinates "GP activity - SOD activity" allows us to identify 2 subgroups in this group (Figure 8).

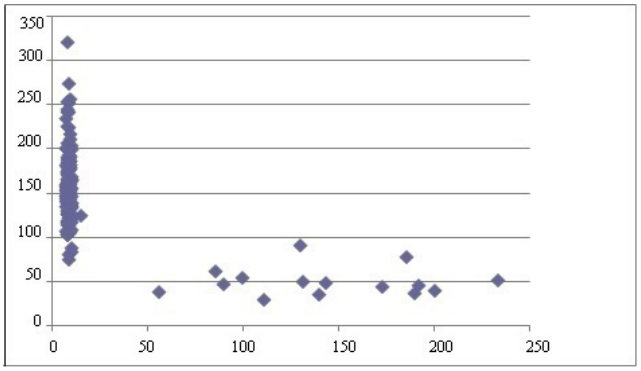

Fig. 8. *Distribution of the results of examination of professional athletes in the coordinate system "GPx activity-SOD activity."*

Thus, the conducted complex study made it possible to demonstrate the presence of shifts in the blood oxidative metabolism induced by occupations in professional sports. At the same time, the obtained data confirm the formation of OS in qualified athletes; however, the inhomogeneity of these metabolic transformations attracts attention. The revealed regularity allows confirming the previously stated hypothesis about the heterogeneity of shifts in oxidative metabolism in professional athletes, which suggests different approaches to their correction. Further research in this area can be aimed at developing the OS management, taking into account the type of reactions of the blood oxidative metabolism in conditions of regular and intense physical activity.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 240-243
http://dx.doi.org/10.21103/Article8(3)_OA15

ORIGINAL ARTICLE

Novel Techniques & Methods

INTERNATIONAL JOURNAL OF BIOMEDICINE

Comparison of Wrist Tapping Parameters in Healthy Adults with and Without Anxiety Using a Modified Original Technique

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Abstract

The aim of the present study was to assess the main characteristics of the tempo-rhythm in healthy adults.

***Materials and Methods*:** We examined 60 healthy adults without neurological and endocrinological pathology. Participants were divided into 2 groups. In Group 1 there were 33 adults without deviations on the scale of anxiety and depression were, and in Group 2, there were 27 persons with subclinically expressed anxiety according to the test results. The test was conducted using hospital anxiety and depression scale HADS. The study was conducted using a modified original technique "Method of exogenous rhythmic stimulation influence on an individual human rhythm."

Results: We found that anxiety statistically significant affects the quantitative and qualitative parameters of wrist tapping (individual rhythm and rhythm stability) in healthy adults.

Conclusion: The obtained data can be used in neurorehabilitation for adult patients with a wide range of neurological disorders, including epilepsy. **(International Journal of Biomedicine. 2018;8(3):240-243.)**

Key Words: rehabilitation • wrist tapping • neurology • epilepsy • anxiety

Introduction

Tapping is a psychomotor test that can be used to assess the psychophysiological brain functions, in particular, the time perception.[1,2] Tapping without any external influence, with the preferred test speed, is a "biological constant", which reflects the speed of nervous processes and endogenous rhythmic processes in the central nervous system.[3] Interest in the study of wrist tapping remains high due to its fundamental character. Specifically, according to V. A. Rudnev (1982), the cyclical nature of movements in wrist tapping is a natural statistical regularity that can be considered as a measurement standard to which different parameters can be compared. The study of these biologically appropriate movements makes it possible to establish a pattern of certain rates and rhythms that occurs in the pathology at different levels of the human nervous system.[4]

Methods of tempo-rhythm correction take a special place in modern neurology and neurorehabilitation.[5] The synergy of the wrist tapping was chosen as the object of our study. We studied three quantitative parameters: (1) movement from the tapping point – component "b," (2) movement to the tapping point – component "a," and (3) the time of the full cycle – "b+a").

The aim of the present study was to assess the main characteristics of the tempo-rhythm in healthy adults.

Materials and Methods

The present study was approved by the local Ethics Committee of Professor V.F. Voino-Yasenetsky Krasnoyarsk State Medical University (Record No. 77/2017 dated 26.06.2017). The study was performed at the Center for Neurology, Epileptology, Neurogenetics and Brain Research of the University Clinic; the Department of Nervous Diseases with a course of medical rehabilitation; the Department of Medical Genetics and Clinical Neurophysiology of the Institute of Postgraduate Education; and also at the Department of Personalized Psychiatry and Neurology of the V.M. Bekhterev National Research Medical Center for Psychiatry and Neurology (St. Petersburg), within the agreement on scientific cooperation with the center.

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A total of 60 healthy volunteers (28 men, 32 women) participated in the study. Participants were divided into 2 groups. In Group 1 there were 33 adults without deviations on the scale of anxiety and depression were, and in Group 2, there were 27 persons with subclinically expressed anxiety according to the test results. The test was conducted using hospital anxiety and depression scale HADS.[6] The study was conducted using a modified original technique "Method of exogenous rhythmic stimulation influence on an individual human rhythm" [RF patent №2606489 dated 10.01.2017]. Modification of the method included carrying out the study of the patients' individual rhythms without the use of exogenous rhythmic stimulation (Fig.1). The study was conducted in the morning with the exclusion of external sensory stimuli (loud sound, bright light) and other people, except the doctor and the volunteer, during the tapping procedure. Room temperature was maintained at 22-25 °C.

Fig. 1. *Method of exogenous rhythmic stimulation influence on an individual human rhythm [RF patent №2606489]*

A - *General view of the study*
B - *An example of the results of wrist tapping in healthy volunteers*

Inclusion criteria:
-Healthy adults
-Signed voluntary informed consent
-Male and female
-The age period: the youth (males 17-21; females 16-20 years); the first period of middle age (males 22-35 years; females 21-35 years); the second period of middle age (males 36-60 years; females 36-55 years)
-Russian-speaking Europeans

Exclusion criteria:
-Children and adolescents
-Refusal to participate in this study
-Participation in other studies
-Acute and chronic neurological, psychiatric and endocrinological disorders at the time of examination
-Alcohol intake (2 or more drinks during the last 2 weeks)
-Use of narcotic drugs at the time of the study and in history

Volunteers did not receive any payment for participating in this study. The researchers did not receive any payment for conducting the study.

Research procedure

The technique included finger tapping on the surface of the device (Xiaomi smartphone based on Android, China), followed by the registration of the time parameters of this process in the author's program based on the modified technique "Method of exogenous rhythmic stimulation influence on an individual human rhythm" [RF patent №2606489 dated 10.01.2017].

During the task implementation, the mechanogram where vertical strokes display the contact of the finger with the screen appears on the screen of the device. (Fig.1). We analyzed the following parameters: total number of taps per minute, average frequency of taps per second (Hz), the rhythm stability – the proportion of the frequency of taps (in percentage), the individual rhythm - the frequency with the highest percentage of occurrence in the sample; the delay time of the subsequent tap (in seconds), the intervals between taps, including the maximum interval (seconds), the minimum interval (seconds), and the average interval (seconds). Also, the registration of an individual "minute" was carried out with the use of a stopwatch. Volunteers were asked to count 60 seconds to themselves, after which they had to say "stop". Time on the stopwatch stopped, the result was recorded.

Participants

Group I consisted of 33 adults (13 men and 20 women) without deviations on the scale of anxiety and depression (0-7 points). In Group 1, the mean age was 33.69±12.06 years, the median age was 32 [22; 46.5] years. Group 2 consisted of 27 adults (15 men and 12 women) with subclinically expressed anxiety (8-10 points). In Group 2, the mean age was 32.4±11.46 years, the median age was 29 [22; 42] years.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 23. Continuous variables were presented as mean± SD and as median (interquartile range [IQR]). Means of 2 continuous normally distributed variables were compared by independent samples Student's t test. Mann-Whitney U test and Kruskal-Wallis test were used, respectively, to compare means of 2 and 3 or more groups of variables not normally distributed. A value of $P<0.05$ was considered significant.

Results and Discussion

The main characteristics of wrist tapping in Group 1 using a modified technique "Method of exogenous rhythmic stimulation influence on an individual human rhythm" are presented in Table 1. The main characteristics of wrist tapping in Group 2 are presented in Table 2.

In men of Group 1, the median of an individual "minute" was higher than in women of the same group (64 [58.5; 69] seconds against 55[49.25; 60.75] seconds, respectively), and the average value was 63.3±13.21 versus 56.05±9.6 seconds, respectively. However, gender differences were not statistically significant.

Table 1.

Tapping parameters in Group 1

Variable	Me [P_{25}; P_{75}]			M±SD			*P*-value
	All N	Male n1	Female n2	All N	Male n1	Female n2	
Volunteers age, yrs	32 [22;46.5]	39 [25.5;49]	22 [21;42.75]	33.69±12.06	37.53±12.05	31.2±11.68	0.6
Individual minute, sec	59 [50.5;66]	64 [58.5;69]	55 [49.25;60.75]	58.9±11,54	63.3±13,21	56.05±9,6	0.08
Individual rhythm, Hz	1.13 [0.99;1.21]	1.18 [0.91;1.23]	1.1 [1.02;1.2]	1.11±0.13	1.11±0.15	1.1±0.12	0.9
Rhythm stability, %	49 [40.7;52.69]	50.72 [40.7;51.71]	46.82 [40.1;56.03]	49.12±11.55	46.95±7.46	50.54±13.57	0.7
Delayed time of the tap, sec	0.1 [0.08;0.18]	0.09 [0.07;0.19]	0.11 [0.09;0.2]	0.13±0.09	0.11±0.04	0.15±0.11	0.5
Maximum interval between taps, sec	1.33 [1.09;1.62]	0.08 [0.07;0.16]	1.38 [1.17;1.8]	4.31±1.33	1.33±0.35	6.25±21.13	0.4
Average interval between taps, sec	0.87 [0.82;1]	0.85 [0.8;1.09]	0.9 [0.82;0.97]	0,91±0,11	0.91±0.13	1.1±0.12	0.9
Minimum interval between taps, sec	0.63 [0.51;0.7]	0.66 [0.63;0.7]	0.6 [0.48;0.73]	0.58±0.2	0.65±0.08	0.54±0.24	0.7
Total number of taps	68 [60;73]	71 [55;74.5]	66 [61.5;72.75]	66.8±8.08	66.84±9.34	64.66±12.44	0.9

Table 2.

Tapping parameters in Group 2

Variable	Me [P_{25}; P_{75}]			M ± SD			*P*-value
	All N	Male n1	Female n2	All N	Male n1	Female n2	
Volunteers age, yrs	29 [22;42]	29 [24;44]	28 [21.25;40.75]	32.4±11.46	33.53±12.33	31±10.62	0.24
Individual minute, sec	53 [44;59]	53 [43;58]	52 [46.5;62.5]	52.74±10.32	51.2±9.23	54.67±1.66	0.5
Individual rhythm, Hz	1.53 [1.45;1.98]	1.52 [1.45;1.81]	1.84 [1.38;2.63]	1.82±0.58	1.69±0.43	1.98±0.71	0.34
Rhythm stability, %	52.94 [48.8;54.65]	50.57 [47.75;54.65]	54.02 [50.92;56.51]	52.32±7.88	51.3±9.27	53.58±5.86	0.13
Delayed time of the tap, sec	0.1 [0.07;0.18]	0.1 [0.08;0.19]	0.08[0.07;0.18]	0.12±0.062	0.12±0.06	0.11±0.06	0.7
Maximum interval between taps, sec	0.8 [0.66;1.11]	0.75 [0.65;1.35]	0.82 [0.66;1.07]	0.92±0.38	0.94±0.39	0.89±0.38	0.6
Average interval between taps, sec	0.64 [0.49;0.69]	0.66 [0.55;0.69]	0.55 [0.38;0.71]	0.58±0.14	0.61±0.1	0.55±0.17	0.4
Minimum interval between taps, sec	0.41 [0.24;0.55]	0.44 [0.19;0.55]	0.39 [0.27;0.55]	0.38±0.17	0.36±0.2	0.41±0.15	0.9
Total number of taps	93 [87;121]	91 [55;74.5]	109 [83.5;158.5]	109±34.79	102±25.76	119.4±42.83	0.38

In Group 2, the median of an individual "minute" in men was 53 [43; 58] seconds versus 52 [46.5; 62.5] seconds in women; the average value was 51.2±9.23 seconds versus 54.67±11.66 seconds. As with Group 1, gender differences did not reach statistical significance in Group 2. Considering this fact, we neglected gender differences in both groups and compared the average values of individual minutes; we did not find a statistically significant difference between these parameters (Table 3). The median of individual rhythm in men and women of Group I was 1.13 Hz versus 1.53 Hz in Group 2.

Table 3.

Comparison of wrist tapping parameters in Groups 1 and 2

Variable	Me [P_{25}; P_{75}]		M±SD		*P*-value
	Group 1	Group 2	Group 1	Group 2	
Volunteers age, yrs	32 [22;46.5]	29 [22;42]	33.69±12.06	32.4±11.46	0.2
Individual minute, sec	59 [50.5;66]	53 [44;59]	58.9±11.54	52.74±10.32	0.06
Individual rhythm, Hz	1.13 [0.99;1.21]	1.53 [1.45;1.98]	1.11±0.13	1.82±0.58	0.0000
Rhythm stability, %	49 [40.7;52.69]	52.94 [48.8;54.65]	49.12±11.55	52.32±7.88	0.019
Delayed time of the tap, sec	0.1 [0.08;0.18]	0.1 [0.07;0.18]	0.13±0.09	0.12±0.06	0.07
Maximum interval between taps, sec	1.33 [1.09;1.62]	0.8 [0.66;1.11]	4.31±1.33	0.92±0.38	0.0001
Average interval between taps, sec	0.87 [0.82;1]	0.64 [0.49;0.69]	0.91±0.11	0.58±0.14	0.0000
Minimum interval between taps, sec	0.63 [0.51;0.7]	0.41 [0.24;0.55]	0.58±0.2	0.38±0.17	0.0000
Total number of taps	68 [60;73]	93 [87;121]	66.8±8.08	109±34.79	0.0000

We found a statistically significant difference between these parameters. A statistically significant difference was also found between the parameters of rhythm stability and the intervals between taps in Group 1 and Group 2. Thus, the most stable rhythm and the smallest interval between taps were detected in Group 2. We found no statistically significant differences in the delayed time of the tap between the study participants from Group 1 and Group 2. Thus, with the subsequent use of the reference corridors of the wrist tapping characteristics, we can ignore gender differences in the group of adult volunteers.[7] Also, it is necessary to take into account the effect of the presence of anxiety at the time of the study, since anxiety affects the quantitative and qualitative parameters of wrist tapping (individual rhythm and rhythm stability).

Conclusion

Studying how the anxiety level influences the parameters of wrist tapping in healthy adult volunteers, using a modified original technique "Method of exogenous rhythmic stimulation influence on an individual human rhythm," allowed us to determine the reference corridors for further application of this method in diagnostic testing and rehabilitation of patients suffering from various neurological disorders, including epilepsy.

Competing Interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 244-246
http://dx.doi.org/10.21103/Article8(3)_ShC1

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

Treatment of Acute Venous Thromboses and Pulmonary Embolism during Pregnancy

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Abstract

The aim of the study was to improve the results of the treatment of acute ascending varicothrombosis, deep vein thrombosis (DVT) and pulmonary embolism (PE) during pregnancy and the postpartum period. The study included 22 pregnant women aged between 20 and 41 years in different periods of pregnancy and in the early postpartum period, with acute thrombosis of deep and superficial veins of the lower extremities complicated by PE. All of the generally accepted methods for treatment of DVT, varicothrombophlebitis and PE were applied. In 16(72.8%) pregnant women with DVT of the lower limbs, in the absence of free-floating thrombus, conservative treatment was effective; an IVC filter was placed in only 1 patient. Combined phlebectomies of GSF with thrombosed tributaries were performed in 4(18.8%) patients with ascending varicothrombosis in the early postpartum period, resulting in early recovery and discharge from the hospital. Thrombolytic therapy in the patient with massive PE in the first trimester of pregnancy saved the life of the mother and child. **(International Journal of Biomedicine. 2018;8(3):244-246.)**

Key Words: pulmonary embolism • deep vein thrombosis • pregnancy • low molecular weight heparin

Abbreviations

DVT, deep vein thrombosis; **GSV**, great saphenous vein; **FFT**, free-floating thrombus; **IVC**, inferior vena cava; **LMWH**, low molecular weight heparin; **VTE**, venous thromboembolism; **PE**, pulmonary embolism; **USDS**, ultrasonic duplex scanning.

Introduction

In regions without specialized vascular hospitals, patients with phlebotrombosis are hospitalized in emergency general surgical hospitals, as patients with an acute condition that threatens their lives.[1-3] In general hospitals, the solution of tactics for diagnostic and treatment has a number of peculiarities: the standards of examination and treatment of patients with deep vein thrombosis (DVT) and its complications are not always observed. In addition, there are no unified algorithms for choosing the optimal tactics depending on the level and nature of thrombotic veins. Unfortunately, the most important tactical decisions are often based on a subjective view of the patient's condition.[4-6]

Clinical diagnosis of pulmonary thromboembolism in pregnancy remains difficult because of pregnancy associated physiological symptoms and signs, which can mimic those of enous thromboembolism.[7]

The aim of the study was to improve the results of the treatment of acute ascending varicothrombosis, deep vein thrombosis and pulmonary embolism during pregnancy and the postpartum period.

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Materials and Methods

In 2016-2017, 22 pregnant women aged between 20 and 41 years in different periods of pregnancy and in the early postpartum period, with acute thrombosis of deep and superficial veins of the lower extremities complicated by PE, were prospectively enrolled for surgical treatment at the Center for Emergency Medical Care. These women had been referred by the female consultations or departments of pathology at maternity hospitals. Superficial venous thromboses with ascending varicothrombosis with the threat of FFT were diagnosed in 4 women in the postpartum period. Thrombosis of the iliofemoral segment was diagnosed in 5 cases, of the femoropopliteal segment - in 6 cases, and of the popliteal segment - in 4. Acute ileofemoral thrombosis was complicated by massive PE in 1 patient in the first trimester of pregnancy and by non-massive PE in 1 patient in the third trimester of pregnancy.

The examination of patients at the pre-hospital stage included the collection of complaints and anamnesis, a physical examination, a laboratory blood test with the obligatory determination of clotting and bleeding time, ECG, amd chest X-ray. In patients with signs of PE, CT examination of the thoracic cavity was performed in vascular regimen, and X-ray imaging was performed by installing a catheter for targeted thrombolytic therapy. The main method of instrumental examination of all patients, both at a pre-hospital stage and during treatment, was USDS. In all cases, ultrasound diagnosis consisted of a series of successive stages: 1) Confirmation of the presence of DVT; 2) Determination of the localization and extension of DVT; 3) Determination of the nature of the thrombotic process - the presence of a FFT and its length; 4) Identification of the risk for PE development; 5) Determination of the massiveness of PE.

Patients with FFT underwent USDS monitoring daily for 5-7 days.[1,7,9,10] Anticoagulants of a low molecular weight were used to treat pregnant women in accordance with the national clinical recommendation of the Russian Society of Surgeons. (RSS,2015).[4] Sixteen patients received conservative treatment - LMWH (clexane, fraxiparine) in therapeutic doses, taking into account the weight of the patients, as well as DVT elastic compression stockings.[3-6] During treatment, all patients underwent monitoring via the coagulogram and ultrasonic venous duplex examination. After reducing leg edema, the threat for FFT, and active recanalization of thrombosed veins, the patients were transferred to the Perinatal Center of the Republican Hospital No.1 (National Center of Medicine) for further dynamic observation and treatment, as well as for maintaining pregnancy.

Active surgical tactics were used for ascending varicothrombosis of superficial veins of GSV in the early postpartum period. Combined phlebectomies were performed in 4(18.8%) patients. Patients were discharged 4-5 days after the operation. To prevent recurrent PE, at the beginning of the third trimester of pregnancy an inferior vena cava (IVC) filter was installed in a woman with non-massive PE, which was removed after the delivery.

Successful regional thrombolytic therapy was conducted for one patient with massive PE during the first trimester of pregnancy (development time of less than 24 hours). After thrombolytic therapy, a course of LMWH was administered according to body weight. An IVC filter was not installed. Subsequently, the patient was transferred to the Perinatal Center for further dynamic observation, treatment and maintenance of pregnancy**.** The patient continued to receive therapeutic anticoagulation during pregnancy. A Cesarean-born baby was full-term and healthy.

Results

In 16(72.8%) pregnant women with DVT of the lower limbs, in the absence of FFT, conservative treatment was effective; an IVC filter was placed in only 1 patient.

Combined phlebectomies of GSF with thrombosed tributaries were performed in 4(18.8%) patients with ascending varicothrombosis in the early postpartum period, resulting in early recovery and discharge from the hospital.

Thrombolytic therapy in the patient with massive PE in the first trimester of pregnancy saved the life of the mother and child. Pregnancy successfully ended with operative delivery and the birth of a full-term, healthy child.

The presence of FFT (from 2 cm to 4 cm in length) is an indication for the installation of a temporary IVC filter in pregnant women.

Conclusion

- All of the generally accepted methods for treatment of DVT, varicothrombophlebitis and PE can be used in pregnancy and the early postpartum period.[1,3-6]
- The analysis of scientific literature and expert evaluation of the outcomes of pregnancy and childbirth complicated by DVT, varicothrombophlebitis and PE, as well as our own experience in the treatment of pregnant women, prove the importance of creating special algorithms for managing such patients, taking into account the diagnostic and technical capabilities of the hospital.
- Termination of pregnancy as an initial stage in the fight against PE is an erroneous tactic. Pregnancy is not the cause of the disease, but only a factor that aggravates a woman's condition. Targeted thrombolytic therapy in pregnant women with massive PE is strongly recommended.
- Surgical treatment of acute ascending varicothrombosis in the early postpartum period avoids and minimizes the risk of thromboembolic complications.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 247-249
http://dx.doi.org/10.21103/Article8(3)_ShC2

SHORT COMMUNICATION

INTERNATIONAL JOURNAL OF BIOMEDICINE

The Influence of Tension on the Success of Aponeurotic Suture of the Anterior Abdominal Wall in Experiment

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Abstract

Background: The problem of predicting the failure of aponeurotic sutures today is of current interest in abdominal surgery, especially in herniology.

Methods and Results: The experimental study was carried out on 20 rabbits of the white giant breed (both sexes). Aponeurotic defects of various sizes were made to the animals' middle zone of the anterior abdominal wall in the area of the anterior rectus sheath. The defects were sutured with a different tension of the aponeurosis depending on the size of the defect. This tension was determined by traction over ligatures conducted through the borders of the reduced aponeurosis by a digital dynamometer where the edges contact. To increase the rigidity of the layers, titanium frames were installed in the retromuscular space. Thus, tension from 0.012 MPa to 1.2 MPa was created. The results were evaluated on the 30th day of the postoperative period Macroscopic assessment under a loupe and histological examination were used. It was found that aponeurotic sutures failed at a higher tension index (0.66±0.16 MPa vs. 0.26±0.16 MPa, $P<0.001$). At the same time, histological changes were characterized by signs of inflammation with a pronounced alterative component. We did not find that the direction of the incision had any effect on the tension value in cases of suture failure.

Conclusion: No failure of the suture in the early postoperative period was observed in cases of aponeurosis edge tension less than 0.4 MPa. Exceeding this value in 68.7% of cases led to the failure of aponeurotic sutures. **(International Journal of Biomedicine. 2018;8(3):247-249.)**

Key Words: tension • aponeurosis • abdominal wall • suture failure

Introduction

Tension of the aponeurosis of the anterior wall is a key concept in abdominal surgery, especially in herniology. Excessive strain on the aponeurosis during suturing is often accompanied by an increase of intra-abdominal pressure; such strain is also the cause of intraoperative and postoperative suture failure, which can lead to eventration and herniation. It is known that about 20% of laparotomies subsequently lead to the formation of postoperative hernia.[1] There is a large number of methods to reduce the tension on the aponeurosis (separation plastics, introducing botulinum toxin into the lateral abdominal muscles, applying of different incisions, acute stretching of the transverse abdominal muscle, etc.).[2-4] However, aponeurotic tissue is functionally related to tension; therefore, it is impossible to completely avoid the stretch of aponeurotic edges during an operation or in the postoperative period. To date, it is not known what value of tension is permissible. The only objective criterion, which is used by many authors, is intra-abdominal pressure.[5] At the same time, it often happens that intra-abdominal pressure does not depend on the tension on the aponeurosis.

In surgery, there are different ways to assess the tension in the wound.[6] However, there are no recommendations regarding the thresholds, exceeding which leads to a high degree of probability that aponeurotic suture will fail in the postoperative period. The determination of such thresholds would allow predicting the process of aponeurotic wound healing.

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The purpose of this study was to determine the thresholds for the tension of the edges of aponeurotic wounds, exceeding which leads to the failure of the sutures in the early postoperative period.

Materials and Methods

The study protocol was reviewed and approved by the Ethics Committee of Voronezh State Medical University named after N.N. Burdenko. 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.

The study was performed on 20 rabbits of the white giant breed (both sexes). The average weight of the animals was 4.7±0.41 kg (Me=4.8 kg). Under intravenous anesthesia with thiopental (25 mg/kg), an incision was made in the skin of the anterior surface of the abdomen, allowing dissection of dissection of the anterior rectus sheaths. Further, a defect of rectangular shape in the aponeurosis was created, symmetrical with respect to the axis of the *linea alba* of certain sizes (one side of the rectangle was 10 mm, and the second - from 5 mm to 35 mm, depending on the required tension). Closing the defect (the length of the joined edges of the defect was always equal to 10 mm) by bringing the edges of the aponeurosis together created some tension. This tension was determined by traction over ligatures conducted through the borders of the reduced aponeurosis by a digital dynamometer where the edges contact (measurement accuracy of ±0.05 Newton (N)). To calculate the aponeurosis strain in relative value (MPa), the obtained value was divided by the cross-sectional area of the aponeurosis edge. This area is equal to 10 mm (the size of the reduced edge of the wound) × the thickness of the aponeurosis. This thickness was determined by a micrometer. In the studied samples, the thickness was 0.2±0.04 mm (Me=0.2 mm). Taking into account the fact that during the experiment, tension index (TI) was from 0.02 N to 2.08 N (0.8±0.55 N), the aponeurosis tension in the region of one edge was from 0.012 MPa to 1.2 MPa (0.41±0.29 MPa, Me=0.36 MPa).

For modeling wound edge tension exceeding 0.72 N, an artificial increase in tissue rigidity was required. It was achieved by installing a rectangular metal frame made of titanium wire in the retro muscular space. This frame, when suturing the aponeurosis in the longitudinal direction with its outer edges resting against the lateral edges of the rectus sheath, created the necessary resistance when the medial edges approached. When suturing the aponeurosis in the transverse direction, the frame created such resistance, by the upper and lower edges resting on the *linea alba*. The need for such modeling arose because of the pronounced mobility of the layers of the abdominal wall of the rabbit. Since the frame itself was located in the retromuscular space, being almost isolated from the anterior rectus sheath, its influence on the healing of aponeurotic wounds can be ignored.

Thus, 120 aponeurosis defects were formed in laboratory animals, of which 60 were sutured in the longitudinal and 60 in the transverse direction. Closure of the defects was carried out by a continuous polypropylene (thickness 6/0) suture with "bytes" in 2 mm. That is, to suture one defect, it was required to overlap 5-6 stitches of the seam.

The results were evaluated on the 30th day of the postoperative period because until this time, pronounced infiltrative changes in the wound do not allow assessment of the success of sutures. After euthanasia, macroscopic assessment of the sutures was performed using small magnification (×10), as well as microscopy with H&E staining.

The statistical analysis was performed using the statistical software Microsoft Excel. The mean (M), median (Me) and standard deviation (SD) were calculated. The distribution was estimated by the Kolmogorov-Smirnov criterion with the Lilliefors correction. The Mann-Whitney U Test was used to compare the differences between the two independent groups. The level of statistical significance was 0.01.

Results

We did not find a lethal outcome, wound suppuration, or complete eventration with a divergence of cutaneous sutures. In macroscopic assessment under a magnifying glass, it was found that in the area of 87 aponeurotic defects (42 longitudinal and 45 transverse) there was a whitish scar, through which the suture material could be seen. The underlying muscle tissue was not visible. Such wounds are macroscopically evaluated as successful. The aponeurosis TI in such wounds ranged from 0.01 MPa to 0.45 MPa (0.26±0.16 MPa). Microscopically, we observed in the sections fragments of fibrous adipose tissue with manifestation of minor edema and hyperemia, and a group of transverse-striated muscle fibers of a typical histological structure with mild edema manifestation. Large fragments of fibrous tissue, sometimes with embedded muscle fibers of different thickness with weakly expressed diffuse-focal or focal lymph plasma-cell infiltration were observed. There were also fragments of foreign polymer material, surrounded on all sides by fibrous tissue.

During the inspection of 18 wounds, we found a complete divergence of edges of the aponeurosis. The bottom of the wound was either muscle or peritoneum. The aponeurosis TI in such wounds ranged from 0.49 MPa to 0.98 MPa (0.72±0.18 MPa). These sutures are determined to be failed. Of these, 9 were in the longitudinal and 9 in the transverse direction. At the same time, fragments of fibrous-fat tissue with signs of edema and vascular hyperemia were found in micro-preparations. Small groups, sometimes bundles of muscle fibers, were observed, in some cases with sites of diffuse, moderately expressed, interstitial inflammation. We also observed fibrous tissue with a few muscle fibers in it, with signs of severe atrophy, and around them pronounced diffuse focal lymph plasma-cell infiltration with moderate, in some cases, neutrophil infiltration.

In the area of 15 wounds, we observed partial divergence of edges of the aponeurosis. In these cases, the underlying muscle tissue was visible through the central part of the scar between the individual stitches. The aponeurosis TI in such wounds ranged from 0.4M Pa to 0.68 MPa (0.57±0.11 MPa). These sutures are determined to be partially failed. Of these,

9 were in the longitudinal and 6 in the transverse direction. Microscopically, the following changes were observed: fragments of fibro-adipose tissue with symptoms of edema and hyperemia; bundles of muscle fibers, sometimes separated by thin layers of fibrous tissue; large fragments of fibrous tissue with moderate diffuse-focal inflammatory infiltration, sometimes with an admixture of single neutrophils; and a few muscle fibers with signs of atrophy in areas with fibrosis.

In processing the results, it was found that the tension rate in sutures of aponeurosis defects with complete or partial failure have a statistically significant higher value compared to wounds with successful healing (0.66±0.16 MPa vs. 0.26±0.16 MPa; U=117.0, P<0.001).

Partially failed seams also had a statistically significant lower stress value compared to normal sutures (0.57±0.11 MPa vs. 0.72±0.18 MPa; U= 58.5, P<0.01). At the same time, we did not observe any influence of the direction of the suture on the tension index that led to a failure of wound healing (U=108, P=0.338).

The obtained data cannot serve as an absolute guide to surgery after recalculation for human aponeurosis. In the postoperative period, due to the verticalization and various reflex and motor acts, tissue tension can vary significantly.[7] However, exceeding the critical values during surgery gives grounds to predict the failure of aponeurotic suture with a high probability.

Conclusion

During the experimental study, it was found that an increase in the tension of aponeurosis increased the probability of suture failure. No failure of the suture in the early postoperative period was observed in cases of aponeurosis edge tension less than 0.4 MPa. Exceeding this value in 68.7% of cases led to the failure of aponeurotic suture.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 250-252
http://dx.doi.org/10.21103/Article8(3)_CR1

CASE REPORT

INTERNATIONAL JOURNAL OF BIOMEDICINE

Hemodialysis Induced Osmotic Demyelination Syndrome in a Eunatremic Patient

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Abstract

Osmotic demyelination syndrome (ODS) has been described in end-stage renal disease patients recently started on hemodialysis and is usually attributed to a rapid correction of hyponatremia. We describe a case of ODS developing after recent hemodialysis and unrelated to serum sodium changes. Our aim is to help provide more data about the pathophysiology and precipitating factors of this syndrome. ODS is seen in the setting of acute osmotic changes, which has been historically linked to sodium levels. We hope that our clinical case highlights the other possible contributing factors leading to this syndrome and will aid in proper avoidance and suitable management of this condition. **(International Journal of Biomedicine. 2018;8(3):250-252.)**

Key Words: osmotic demyelination syndrome • hemodialysis • chronic renal failure • hypertension

Introduction

ODS (formerly called central pontine myelinolysis or CPM) is a well-known clinicopathologic entity characterized by edema and demyelination in the pons and extra-pontine areas.[1] It is caused by an acute shift in serum osmolality, which is classically related to rapid correction of hyponatremia.[2] We present a case of ODS occurring after recent dialysis in a patient with end-stage renal disease and normal serum sodium level.

Case presentation

A 34-year-old male with a medical history of uncontrolled hypertension presented to the emergency department as a referral from his primary care doctor for hypertensive urgency. Upon presentation, he was noted to have an elevated blood pressure with a systolic of 185 mmHg and diastolic of 120 mmHg. He was asymptomatic and denied any headache, blurry vision, chest pain or shortness of breath. However, he confirmed decreased exercise tolerance for 3 weeks and dark urine for 2 days; he denied any dysuria or flank pain. His examination was unremarkable and there was no jugular venous distention, pulmonary crackles or lower extremity edema. His neurological exam was also intact with no focal deficits.

The initial basic metabolic panel showed an acute kidney injury with blood urea nitrogen (BUN) of 142 mg/dl and creatinine (Cr) of 16.6 mg/dl; sodium (Na) level was 133 mEq/L and potassium (K) was 4.3 mEq/L. Urine analysis was notable for +3 hematuria and +3 proteinuria. Patient was started on a nicardipine drip and admitted to the medical intensive care unit (MICU) for close monitoring. A few hours later, he was noted to be in respiratory distress with tachypnea, diaphoresis and accessory muscle use, and the decision was made to intubate the patient. A post-intubation chest X-ray showed acute pulmonary edema. Renal service was consulted and recommended starting hemodialysis. Patient was also started on oral antihypertensive medications and titrated off the nicardipine drip.

Apart from poor mental status off sedation and failure to tolerate the ventilation weaning trials, the remainder of his MICU course was unremarkable. Off sedation, patient was noted to open his eyes, but was unable to track movements or to follow simple commands; he had a right gaze preference, was able to withdraw to pain with a weak movement and had intact reflexes. In view of the unexplained deterioration in mental status, a brain CT (Fig. 1a, 1b) was obtained, which showed periventricular white matter changes necessitating a brain MRI for better visualization.

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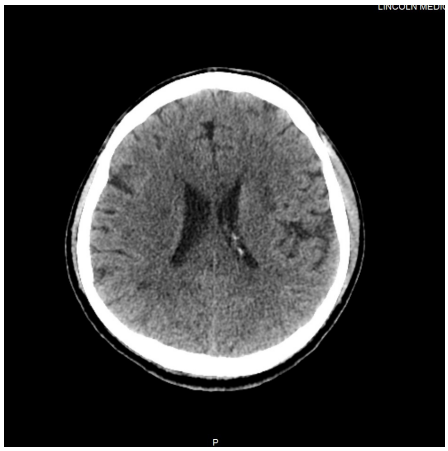

Fig. 1a

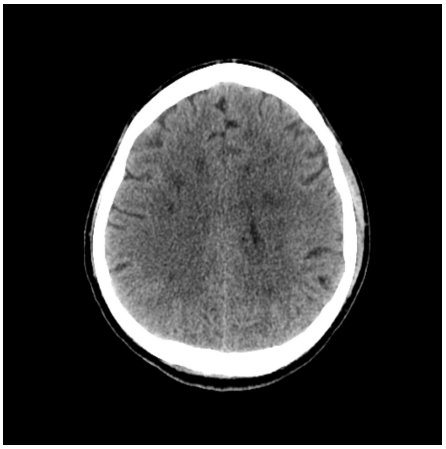

Fig. 1b

The brain MRI (Fig. 2a-d) was remarkable for numerous T2 hyper-intense lesions in the white matter, particularly seen within the corpus callosum and periventricular white matter. The majority of these lesions demonstrated diffusion restriction, suggesting active demyelination.

The trend of the patient's sodium and BUN levels before and after starting the dialysis is provided in Table 1. Lumbar puncture was done to exclude other etiologies, specifically multiple sclerosis and acute disseminating meningoencephalitis.

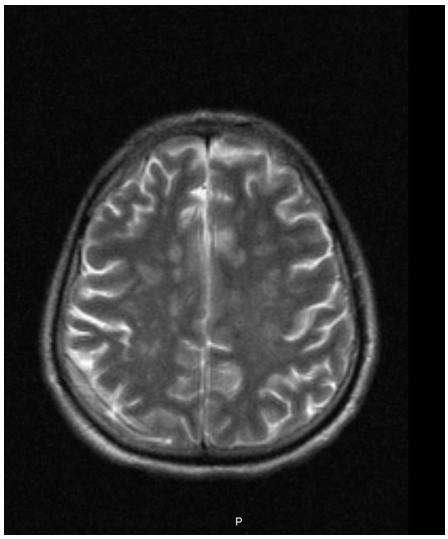

Fig. 2a

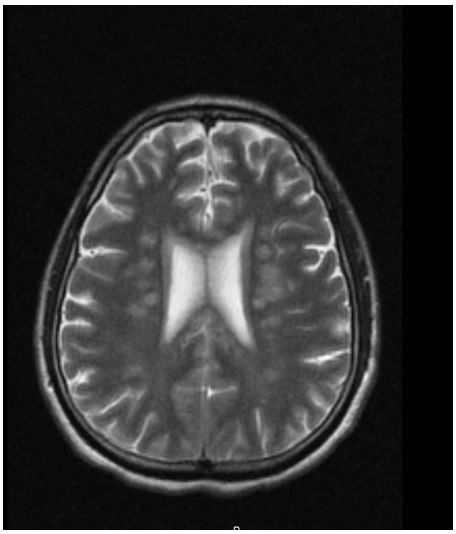

Fig. 2b

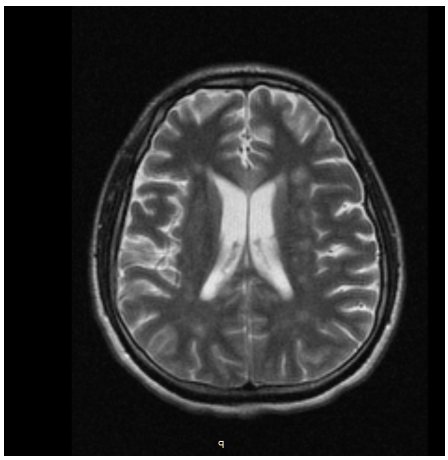

Fig. 2c

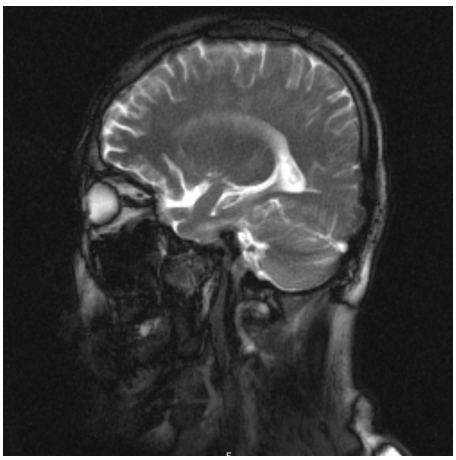

Fig. 2d

Table 1.

	Na (mEq/L)	BUN (mg/dl)
Before dialysis	133	142
Day 1 after dialysis	138	79
Day 2 after dialysis	138	51

Results were remarkable for normal CSF white cell count and protein level, as well as negative oligoclonal bands. The patient was started on high dose steroid therapy with methylprednisolone sodium succinate 1000 mg daily for 5 days; unfortunately, there was no improvement in the patient's mental status; hence, tracheostomy and percutaneous endoscopic gastrostomy were pursued.

Discussion

Central pontine myelinolysis was initially described by R.Adams in 1959 as a disease affecting alcoholics and malnourished patients.[1,3,4] The concept was extended in 1962 with the recognition that the disease can also affect extra-pontine sites (extra pontine myelinolysis or EPM).[4] Since then the name "osmotic demyelination syndrome" has become more popular.(1) Various mechanisms have been proposed for the development of ODS; the primary pathophysiology is reduced adaptive capacity of the glial cells to large shifts in serum osmolarity.[5] The condition is classically related to rapid correction of hyponatremia. However, it has also been reported in normonatremic patients, especially in patients with chronic renal failure, hypokalemia, and liver disease and in patients following liver transplantation.[6]

In patients with end-stage renal disease, ODS may develop as a result of the disease itself or because of osmotic changes during hemodialysis.[1] Y.Endo et al. reported a 14% incidence of ODS in patients with end-stage renal disease receiving hemodialysis.[7]

The proposed hypothesis of osmotic demyelination is osmotic injury to the endothelium from the rapid changes in serum osmolality, resulting in the release of myelinotoxic factors leading to disruption of the oligodendrocytes.[8] As mentioned before this has been historically related to changes in serum sodium, which is the most important solute contributing to serum osmolality. However, a few case reports described the syndrome in eunatremic patients, suggesting that other solutes also play an important role in the disease pathophysiology. Our case possibly developed ODS due to rapid changes in the serum BUN level as serum sodium has been stable through his hospital course and there were no exogenous solutes contributing to the effective serum osmolality.

Competing interests

The authors declare that they have no competing interests.

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International Journal of Biomedicine 8(3) (2018) 253-254
http://dx.doi.org/10.21103/Article8(3)_LE

LETTER TO THE EDITOR

INTERNATIONAL JOURNAL OF BIOMEDICINE

Late Start of Surfactant Therapy and Surfactant Drug Composition as Major Causes of Failure of Phase III Multi-Center Clinical Trials of Surfactant Therapy in Adults with ARDS

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To the Editor:

We have read, with great interest, the Letter to the Editor by Grotberg et al.,[1] which puts forward reduced alveolar delivery as the major cause of the unexpectedly disappointing results of phase III, multi-center, controlled clinical trials of surfactant therapy in adults with acute respiratory distress syndrome (ARDS). We do hope that this will fuel a long-anticipated discussion on the causes of the failure of surfactant therapy for ARDS, despite abundant evidence from animal models with ARDS and some clinical trials that surfactant therapy is efficacious. While we strongly support Grotberg and colleagues' suggestion to further explore Grotberg's hypothesis that higher volumetric doses are needed for better drug delivery,[1] we would like to stress that future research cannot be limited to this one direction; for apart from studies yielding positive results of therapy with natural surfactants at a volumetric dose ranging from 2.3 ml/kg to 5.4 ml/kg,[2-5] there are multi-center clinical trials that showed no mortality decrease in patients with ARDS despite a volumetric dose as large as 4.0 ml/kg for the drug Surfactant HL-10[6] and 6-8 ml/kg for the synthetic drug Surfaxin.[7] Moreover, Surfactant-BL from bovine lungs is administered at a dose of 6mg/kg and a volumetric dose of 0.5-1.0 ml/kg every 12 hours, with a mortality rate fluctuating from 14.9% to 20%.[4,8]

There is no doubt that the efficacy of surfactant therapy is influenced by drug composition[4,8,9] and the time when surfactant therapy is started.[4.8.10] Taeusch believes that complex natural multicomponent surfactants, which are most similar to the composition and properties of lung surfactants *in situ*, are more effective than simple surfactants.[9] Synthetic surfactant drugs, including Surfaxine, Venticute and Exosurf, are ineffective for ARDS. [7,11,12] Design by means of modern biotechnologies of a surfactant drug with properties similar to those of the lung surfactant *in situ* is deemed unfeasible.[14] This is in line with the fact that while surfactants used in most controlled multi-center clinical trials require a dose of 200-600mg/kg,[6,7,11] a highly native Surfactant- BL requires a dose of only 6mg/kg when administered twice a day.[4,8,10] The time when surfactant therapy is started is also a major factor in surfactant efficacy. In controlled multi-center clinical trials of both native and synthetic surfactants, the first dose is administered too late, within 48-72 hours after ARDS diagnosis.[6,7,11,13] Early administration within 24 hours after PaO_2/FiO_2 decreases to less than 200 mmHg has been shown to be effective in clinical trials of Surfactant-BL, while a later administration of this drug has proved ineffective.[4,10] Based on these data we believe that the major cause of the failure of phase III, multi-center, clinical trials of native surfactant drugs is late surfactant administration performed within 48-72 hours after patient intubation.[2,6]

Competing interests

The authors declare that they have no competing interests.

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