THE APPRAISAL OF THE ROLE OF BIOCHEMICAL INDICES IN DIAGNOSTIC PRESENTATION OF METABOLIC SYNDROME USING DISCRIMINANT ANALYSIS

UDC 612.015.38–074
Received 26.04.2012

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The aim of the investigation is to assess the possibilities of discriminant analysis to determine the significance of some biochemical indices in metabolic syndrome development.

Materials and Methods. There were studied the biochemical parameters in blood plasma of 136 patients with metabolic syndrome and 50 virtually healthy people using standardized test systems. The findings were statistically processed by software package of statistical analysis R 2.11.0 and BIOSTAT.

Results. According to discriminant analysis of biochemical indices there was developed a prognostic model enabling to refer an experimental subject either to a group of patients with metabolic syndrome or to healthy people.

According to the analysis of discriminant functions there were determined variables that make a major contribution to pathogenic picture of metabolic syndrome and contain significant diagnostic information. Among these are: free cholesterol, the intensity of free radical oxidation, and the main products of oxidative protein modification. Thus, it can be argued that the important criteria in the development and progression of metabolic syndrome are both indicators of lipid metabolism, and indicators of oxidative stress.

Key words: metabolic syndrome; discriminant analysis; oxidative stress.

The prevalence of such symptom complex as metabolic syndrome (MS), the severity of diabetic and vascular complications in younger people determine the urgency of MS study for its early detection [1, 2]. MS associated functional and biochemical changes at subclinical stage are reversible, i.e., in proper treatment it is possible to eliminate or at least reduce the intensity of MS manifestations [3].

The main MS manifestations that play a significant role in pathogenetic chain of events are generally thought to be insulin resistance and dyslipidemia [4]. However, emerging up-to-date data extend the idea of MS and require further clinical and fundamental studies.

The aim of the investigation was to assess the possibilities of discriminant analysis to determine the significance of some biochemical indices in metabolic syndrome development.

Materials and Methods. The analyzed (main) group included 136 patients with metabolic syndrome revealed by a combination of criteria. The control group consisted of 50 apparently healthy people comparable by age and gender with those of the main group. To study the components of carbohydrate, lipid metabolism and free radical oxidation in MS patients we examined blood plasma: measured glucose using glucose oxidase test; estimated lipid profile including total and free cholesterol (CH_{total} и CH_{free}), triglycerides (TG), nonesterified fatty acids (NEFA), phospholipids (PL) using standard test systems Diasys (Germany). Insulin content was determined by ELISA (enzyme-linked immunosorbent assay) using DRG test system (Germany). Free radical oxidation in blood plasma was assessed by induced chemoluminescence on biochemoluminometer БХЛ-06 (Russia) [5]. From chemoluminogram parameters parameters there were considered the values of free radical oxidation intensity Imax (mV), light sum S (mV), $tg\alpha_2$. The determination of lipid peroxidation product levels included the measurement of diene conjugates (DC), triene conjugates (TC), and Schiff's bases (SB). The degree of protein oxidative modification was determined by the level of carbonyl derivatives [6].

The obtained data were processed by software package of statistical analysis R 2.11.0 and BIOSTAT. The results were stated in the form of $M\pm\sigma$, where M — mean, and σ — mean square deviation. Validity coefficients are presented according to Student t-test (t). To assess the interaction between the studied parameters we carried out correlation analysis using Pearson coefficient. To develop prognostic

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models we used linear regression analysis, and to estimate the contribution of the studied indices in MS development — dispersion analysis.

Results and Discussion. One of the most important links in MS pathogenesis is insulin resistance, and for its assessment we used HOMA coefficient [3] considering the concentrations of glucose and insulin in blood plasma:

HOMA (mmol/L)=(glucose, mmol/L × insulin, micro units per mL) : 22.5, where 22.5 is design coefficient used to express the index in mmol/L.

The analysis of the obtained results in MS patients revealed statistically significant increase of the studied value in this pathology (See Table). It enabled to conclude that the disease of the examined patients had not passed into decompensation stage. Glucose content in 28.3% patients with MS exceeded the values obtained in the control group, and in the rest patients – varied in reference range. Mean values of this parameter were close to threshold (5.79±2.92 mmol/L) that is a signal of the necessity of blood glucose level monitoring. Even at subclinical stage there was impaired tissue resistance to insulin as evidenced by statistically significantly increased (nearly twice) values of HOMA-index. Thus, revealed hyperinsulinemia is associated with compensatory mechanisms of the body – overcoming of reduced tissue sensitivity to insulin due to the increased synthesis by β-cells of pancreas.

It is widely accepted that hypertriglyceridemia is one the main components of lipidosis in MS. However, in our research high TG values in blood plasma were revealed only in 44.3% of patients and statistically significantly exceeded TG level in control group (2.80±2.33 mmol/L). Thus, it may be concluded that the presence of hypertriglyceridemia is not a required component of MS. In its turn, TG content in the range of reference values is not the evidence of the absence of syndrome or the risk of its development.

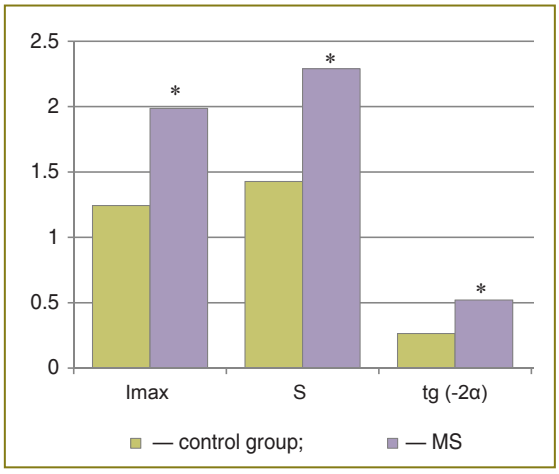
In patients with MS the level of total cholesterol was in reference range (4.99±2.57 mmol/L). It is significant that increased values of total cholesterol were found only in 41.5% of cases that, as in the case of TG, does not exclude MS risk in patients with normal CH values. For more complete diagnostic information we analyzed the level of free (nonesterified form) cholesterol. The increase of this value was stated in 78.3% of cases — 44.11±20.87% of total CH content that exceeded the values received in the control group by 73.8%. What stands out is that increased content of free CH was found in 46.2% of MS patients with normal total CH. Thus, it can be argued that determination of total

Table 1

Glucose and insulin content in blood plasma in metabolic syndrome (M±σ)

Index	Control group	Threshold values	MS
Insulin, micro units per mL	8.82±3.89	<11.00	20.23±14.92*
Glucose, mmol/L	4.83±0.85	<6.10	5.79±2.92*
HOMA-index, mmol/L	1.89±0.85	<2.70	5.08±4.43*

* — statistically significant differences with control values (p≤0.01).



Indices of chemoluminogram in metabolic syndrome. S index is given at a scale of 1 in 10; * — statistically significant differences with control values (p≤0.01)

CH does not reflect disorders of lipid-transport system, and a normal value of this index does not exclude cholesterol metabolism disturbance in this group of patients.

NEFA are not least important in MS pathogenesis. In our studies HEFA level in MS turned out to be increased in 100% of cases and almost twice exceeded normal values (p=0.001).

The increased amount of NEFA results in reduced insulin binding by hepatocytes, its degradation and insulin resistance development, inhibition of insulin suppressive effect on glucogenesis, as well as systemic hyperinsulinemia that contribute to the development of peripheral insulin resistance [7, 8].

Thus, obtained data enable to suggest the use of NEFA as universal diagnostic criterion of MS presence.

The appraisal of PL level in MS patients showed their normal content being in the range of threshold values. To increase the information value of this parameter we used design coefficient PL/ CH_{total}. It enables to reveal statistically significant decrease of this parameter by 1.6 times in 89.6% of MS patients. It is interesting to note that 26.4% of examined patients with normal CH and PL levels in blood plasma had pathological value of this coefficient. Thus, the calculation of ratio of PL to total CH enables to reveal patients fall under the risk group of vascular complications.

The assessment of chemoluminogram demonstrated statistically significant increase of Imax characterizing the intensity of free radical oxidation in plasma by 2.6 times. The indices S and tg (-2α) were also statistically significantly higher than normal values — by 60.4 and 200.0%, respectively, that indicated the decreased activity of antioxidant system. The obtained data enable to suggest the failure of adequate response of the body to free radical oxidation intensification, and as a result, the development of oxidative stress in MS (See Fig.)

Oxidative stress results in the level increase of both primary (DC and TC), and end (SB) products of peroxidation in plasma of MS patients. DC content was 0.20±0.07 relative units that exceeded the value of this parameter in apparently healthy people by 25% (0.16±0.03 relative units),

and TC content was 0.08±0.03 relative units, higher than in the control group by 82.9% (0.041±0.02 RU). The content of SB, the end products of lipid peroxidation, the most toxic and stable, demonstrated the increase of this parameter fivefold (15.97±12.83 RU) as to those of the control group (3.57±1.56 RU). In the course of the study there was observed statistically significant increase of oxidation-modified proteins (OMP) of both neutral and basic character. The most increase of carbonyl derivatives was observed at wavelength of 430 and 530 nm — by 45.1 and 58.3%, respectively. The recorded level increase of products of neutral character on an average exceeded normal values by 29.1%. Thus, the increase of OMP and peroxidation products confirms a significant contribution of free radical oxidation to pathological picture of MS and its complications.

We carried out discriminate function analysis of the findings to develop prognostic model that enables to refer an experimental subject to a group of patients or healthy people.

Based on a large bulk of statistical data, we derived the system of two equations: y_1 — the equation was derived as the result of analysis of values in healthy people, y_2 — in patients with MS.

$$y_1 = -25{,}3232 + 11{,}5571 \times 5571 \times NEFA + (-0{,}5066) \times TG + 0{,}2782 \times CH_{free} + 4{,}239 \times PL + 0{,}2378 \times insulin + 8{,}4436 \times Imax + 92{,}9218 \times TC + 0{,}0251 \times SB + 2{,}4019 \times OMP\ (430\ nm) + (-3{,}1973) \times OMP\ (530\ nm);$$

$$y_2 = -61.4927 + 21.4184 \times NEFA + 1.1613 \times TG + 0.5305 \times CH_{free} + 0.6497 \times PL + 0.4886 \times insulin + 14.9376 \times Imax + 169.4671 \times TG + 0.3167 \times SB + 4.0204 \times OMP\ (430\ nm) + (-5.4850) \times OMP\ (530\ nm)$$

Final prognosis is based on the fact what function takes the maximum value. If in the equation system $y_2 > y_1$, a patient is referred to "patients" class.

The calculation of correlation relationship of values in the group of apparently healthy people and in the group of MS patients revealed inverse correlation between the level of free CH and TC (r= –0.424; p=0.020). Moreover, there was found direct correlation relationship between insulin and SB (r=0.492; p=0.006). Inverse correlation between TG and the main products of protein oxidative modification (r=-0.408; p=0.025) in healthy people is due to the competition for substrate under the conditions of normal work of antioxidant system. The analysis of correlation in the group of MS patients revealed the differences that are consistent with pathological changes accompanying the development and progression of this symptom complex, namely, correlation relationship between TG and OMP products changed the correlation character — the correlation became direct (r=0.262; p=0.035).

Conclusion. The discriminate function analysis enabled to reveal variable values that make the most contribution to pathogenetic picture of metabolic syndrome and contain the most diagnostic information. Among these are free cholesterol, intensity of free radical oxidation, and the main products of protein oxidative modification. These data enable to conclude that the important criteria in development and progression of metabolic syndrome are both parameters characterizing lipid metabolism, and parameters characterizing oxidative stress.

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