Changes In Biochemical Characteristics In Correction Of Metabolic Misbalance With Low Doses Of Ozone

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Introduction

Metabolic syndrome (MS) is a complex disease caused by a combination of genetic factors with a person's life-style (nutrition characterized by a high content of carbohydrates, hypodynamia and hypokinesia, alcohol, smoking, mental and physical strain). The incidence of metabolic syndrome in different countries ranges from 10% to 30%. In Russia about 1,200,000 people die annually as a result of MS complications. MS incidence has been found to be increasing in female patients for the recent 10 years, compared with the one for male patients [1,2,3].

Criteria for metabolic syndrome were elaborated and unified for various ethnic groups, however, there are some discrepancies among MS criteria that have been suggested lately. WHO experts emphasize the role of misbalance in carbohydrate metabolism and insulin resistance. The expert group of the USA National Council in its Third Report on identification, evaluation and treatment of hypercholesterinemia in adult patients high-lights the role of lipid metabolism misbalance and fatty tissue condition. International Diabetes Federation worked out approved criteria for the disease [1,4,5].

Present-day MS criteria are subdivided into principal and additional ones, the principal being central obesity with waist circumference starting from 94cm for male and 80cm for female patients; additional criteria including serum triglyceride starting from 1.7 mmol/l or special treatment of hypertriglyceridaemia;Chol-LPHD should be less than 1,03 mmol/l for male and 1,03 mmol/l for female patients or special treatment of hypoalphalipoproteinaemia; systolic pressure being above 130mm m.c. and diastolic pressure - above 85mm m.c. or special treatment of arterial hypertension; serum fasting glucose starting from 5.5 mmol/l or Diabetes Mellitus of Type II, diagnosed earlier. MS diagnosis can be made in cases when the principal criterion is accompanied with any of the 2 additional criteria [6,5].

The aim of the work is to study biochemical characteristics in the blood of patients with metabolic syndrome under the influence of ozone in low doses and to compary the results in parallel groups.

Material and Methods

Evaluation of pathologic changes and MS complications was done with the use of specially elaborated number system, which allows to define the part of various nosologic forms in the combined clinical picture of each patient. A three-point scale was used to assess the manifestation degree of a disease being the part of metabolic syndrome (DM-II, hypertony, atherosclerosis, obesity, concomitant diseases). Numerical form of a diagnosis was a mathematical sum of a total score made by a doctor

Table 1 Numeral System to Evaluate the Degree of Pathologic Changes and Complications in Metabolic Syndrome

Pathology	*Manifestation Degree*
Obesity	0-3
Diabetes Mellitus, Type-II	0-3
Hypertension	0-3

Atherosclerosis	0-4
Concomitant Diseases	0-1
Numerical Form of a Diagnosis	Total

This system allows to make a clinical evaluation of examined patients in accordance with the accepted classifications taking into consideration the stage of the disease being the part of metabolic syndrome complex.
The examined group consisted of 111 patients, average age being 50 – 70, with different nosologic forms of metabolic syndrome manifestations.

Table 2. Correlation of Various Diagnostic Criteria of Metabolic Syndrome of Patients

Criterion	Visceral Obesity	Arterial Hypertension	Hyper-glyceridaemia	Hypo-alphalipoproteinaemia	Fasting glucose〉5.6mmol/l Or DM-II
Number of patients	111 (100%)	82 (65%)	59 (53%)	63 (57%)	71 (48%)

Metabolic misbalance was corrected with low doses of ozone in the clinic of Nizhny Novgorod Medical Center of Ozone Therapy.

The course of treatment consisted of 6-8 procedures depending on the severity of the disease, the procedures done every second day. Each procedure included intravenous infusions(200ml) of ozonated physiologic saline(0.9% NaCl) with ozone concentration of 300-400mcg/l. Barbotage of 0,9% NaCl was done simultaneously with intravenous infusions, ozone being generated by “Kvazar”-ozonator, holding European certificate of quality.

Preliminary study was done to investigate the kinetics of ozone decomposition in physiologic solution in different ozonation regimes by the method of ultraviolet spectrometry. Methods of analytical chemistry were used to study possible side products of ozone decomposition in water solutions. On having been ozonated, water solution revealed ozone level not exceeding 0.67mg/l and concentrations of hydrogen peroxide making 0,0004%. There was not found any significant increase in the content of chlorinated ions, NO_2^- and NO_3^-, ammonia and ammonium ions when ozone concentration in a sample was within 0.55mg/l.

Control group consisted of 29 healthy persons, within the same age range, that were included into the group regarding the following criteria: lack of chronic diseases in the stage of exacerbation, no acute respiratory viral diseases or alcohol intake within the period of 10 days either before or on the day of analysis.

Lipid profile(level of total cholesterin, triglycerides,Chol-LPHD, Chol-LPLD) was analyzed by standardized methods (Vital diagnostics, Russia). The content of non-etherified fatty acids(NEFA) was measured with Diasys test-system (Germany). The activity of the basic antioxidant enzymes(SOD, catalase, glutathione-peroxidase, glutathione-reductase) was estimated with the use of Rendox reagents (England) Potential binding capacity of albumin was studied by Albumin-Zond1 device with the use of Cintacom-sets (Russia) to define total and effective albumin concentration. The activity of detoxicating enzyme of glutathione-S-transferase was defined according to Karpischenko method(2002).

Results and Discussion

Initial increase of total cholesterin by 26.35% was revealed in 78% patients. It made up 6.57±0,20 mmol/l with the recommended level not exceeding 5.2mmol/l. TG level was found to be increased in i57%(47 patients) by 48% exceeding the recommended level and it was by 215% higher than the level in healthy persons(2,52±0,15 mmol/l)

The increased NEFA content was revealed in serum of 100% patients with the studied pathology and upon average made up 0,76±0,06 mmol/l.

Table 3 The Level of Biochemical Indices in Healthy Persons and Patients with Metabolic Syndrome

(M±m)

Index	*Recommended values*	*Healthy persons*	*MS patients*
Triglycerides mmol/l	less 1,7	0,80±0,12	2,52±0,15*
Total cholestrin mmol/l	less 5,2	4,61±0,20	6,57±0,20*
Chol-LPHD mmol/l male female	above 1,03 above 1,30	1,65±0,14	0,95±0,03*
Glucose mmol/l	less 5,6	4,78±0,20	10,25±0,94*
NEFA mmol/l male female	0,10-0, 60 0,10-0,45	0,40±0,04	0,76±0,06*

- - significant differences with indices for the normal group

Changes in the indices of lipid metabolism and glucose level after the course of ozone therapy are presented in Table 4.

Table 4 Changes in Lipid Metabolism and Glucose Level after Ozone Procedures

Index	*Group of patients*
Triglycerides (mmol/l)	Decrease by 35% (p<0,05)
Total cholesterin (mmol/l)	Tendency to decrease (5%)
Chol-LPHD (mmol/l)	No significant changes revealed
Fasting glucose (mmol/l)	Decrease by 9% (p<0,05)

The activity antioxidant enzymes of SOD and catalase in erythrocytes of MS patients was found to be significantly decreased by 77% and 34%, respectively while the activity of glutathione-peroxidase and of glutathione reductase appeared to be increased by 3 and 2 times, accordingly.

Under the influence of medical ozone used in low doses there was registered normalization of the indices of lipid metabolism(total cholesterin level, triglycerides, Chol-LPHD,Chol-LPLD), significant decrease of NEFA by 15 % in serum of MS patients.

The described changes seemed to be caused by activation of aerobic processes under the influence of ozone, ß-oxidation of excessive NEFA content.

Intravenous ozone therapy was found to facilitate the normalization of antioxidant enzymes balance, increasing SOD and catalase activity and decreasing glutathione-peroxidase and glutathione-reductase.

To evaluate the detoxicating function of the body it was necessary to study the activity and binding capacity of albumin. The levels of albumin total concentration in blood of healthy persons and MS patients were found to be significantly different. Effective concentration of albumin in MS patients upon average was lower by 18.5%, compared with healthy persons. According to the received data, albumin potential capacity _in healthy persons is equivalent to 96,10±1,08%. In MS patients APC was significantly lower(p<0,05) and made up 83,09±1,19. Informative criteria of albumin

detoxicating function point to the decrease in albumin effective concentration and potential binding capacity in blood plasma when metabolic syndrome develops. The study of informative parameters of albumin detoxicating capacity revealed the increase of albumin binding capacity in group 1, where it was 88,01±1,43, the normal one being 96,10±1,08. It should be noted that total concentration of albumin increased by 8% upon average ($p<0,05$) in patients who did not have any significant increase, of albumin potential capacity. It testified to the the fact, that ozone in low doses produces systemic effect on the liver protein synthesis function.

Evaluation of glutathione-S-transferase activity after the course of ozone therapy revealed the reduction of the activity in 76% of examined patients by 14%($p<0,05$), the increased activity of glutathione-S-transferase plasma fraction within the normal limits was found in 15% of patients and in 9% of patients it was increased by 37%. Glutathione-S-transferase is known to be the enzyme of the second phase of exogenic and endogenic toxins biotransformation. Neutralization of toxic substances is done by their reduction. Glutathione-S-transferase can attach the molecules of reduced glutathione to the substrate or to perform nucleophilic substitution of hydrophobic groups.

Conjugation of toxic LP products protein oxidative modification with glutathione results in their egestion. On studying glutathione-S-transferase activity in erythrocytes and in blood plasma there was found the increase of enzyme activity by 89%(up to14,42±0,76mol/l/min, with the norm being 7,63±1,29 mol/l/min. The enzyme erythrocytic fraction activity was found to be also increasing by 44% in MS patients, starting from 124,40±11,55 mol/l/min in healthy persons and up to 178,57±9,92 mol/l/min in erythrocytes of patients with metabolic syndrome

Conclusions

1. Initially pathologic characteristics of lipid peroxidation(total cholesterin level, the levels of triglycerides**,** Chol-LPHD, Chol-LPLD**,** NEFA)had a tendency to normalization after the course of ozone therapy.
2. Intravenouse ozone therapy helps to restore the balance of antioxidant enzymes and to be more precise, it results in increase of catalase and SOD activity and decrease of glutathione-peroxidase and glutathione-reductase
3. The administration of therapeutic low doses of ozone leads to reduction in the activity of erythrocyte and glutathione-S-transferase plasma fractions in 76% of examined patients($p<0,05$) and to increase in albumin binding capacity

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