CLINICAL RESEARCH

# Lipid-Lipoprotein and Oxidative Stress Markers in Patients with Metabolic Syndrome

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## Abstract

The lipid-lipoprotein and oxidative stress markers in the different levels of glucose-insulin homeostasis have been studied in patients with metabolic syndrome (MS).

The increase in the levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), as well as the decrease in High-Density Lipoprotein Cholesterol (HDL-C) was revealed to be one of the causes of hyperinsulinemia and insulin resistance (IR). Increased lipolysis activates the oxidative stress (OS). The leading role of TG as an indicator of high atherogenic potential of plasma in MS has been determined.

*Keywords: lipoproteins; oxidative stress; metabolic syndrome.*

## Introduction

MS is now considered an interrelated combination of the main cardiovascular risk factors - abdominal obesity (AO), arterial hypertension (AH), dyslipidemia and impaired glucose tolerance or type 2 diabetes mellitus[1,2]. Cardiovascular disease, particularly coronary *heart disease* (CHD) is the leading cause of death in patients with MS [1.3.4]. There is evidence to support that the pathophysiological basis and the unifying factor of most of the symptoms described for MS, arise from the resistance of the peripheral tissues to insulin [1.5].

The main and most important risk factor for atherosclerosis and CHD caused by them is atherogenic lipid metabolism [2,3,6]. In addition to IR, hyperglycemia is of great importance in the genesis of dyslipidemia in MS [1,4]. The combination of such complex CHD risk factors aggravates atherogenic lipid disorders in MS [2,3,7,8].

Most researchers believe that the commonest manifestations of dyslipidemia combined with IR are hypertriglyceridemia (HT) and reduced HDL-C. An increase in the TC and LDL-C is less typical for MS [3,6,8,9]. In addition to the lipid-lipoprotein markers in the pathogenesis of MS, the free fatty acids (FFA) also play a major role.

FFA (unesterified fatty acids) are formed by the hydrolysis of TG found in the adipose tissue [1,5,10]. In the myocardium, the FFA are rapidly metabolized by β-oxidation in the mitochondria and supply the heart with 65% to 70% of ATP. The remaining 20-25% of myocardial ATP results from glucolysis [10]. During ischemia, FFA consumption falls, while their level increases in the plasma, which leads to serious consequences [1,3,5,10]. However, IR is the main risk associated with the elevated FFA levels [10]. It has been shown that MS patients have an elevated plasma FFA level, which results in the IR of several tissues, viz., muscle, liver, adipose, and endothelial cells [1,10]. Any increase in the concentration of FFA also leads to OS and endothelial dysfunction, which results in ischemia and a further increase in the concentration of FFA in the blood, i.e. the development of a vicious circle in the disease process [1,5,10].

Thus, the putative mechanisms of the relationship of the lipid-lipoprotein biomarkers and glucose-insulin homeostasis determine the necessity of studying their roles in MS.

***The aim*** of the study was to determine the content of the lipid-lipoprotein biomarkers, FFAs, and the markers of OS in the patients with MS.

## Material and Methods

A total of 80 patients (32 men and 48 women) between 35 and 63 years of age (mean age 49.2±5.3 years) were included in our study. MS was diagnosed in 30 patients based on the criteria recommended by the experts of US *National*

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*Cholesterol Education Program* (NCEP, 2005). The criteria for MS considered a waist circumference greater than 94 cm in men and more than 80 cm in women, blood pressure of 130/85mmHg and above, and plasma glucose levels in the blood glucose, at 5.6 mmol/l or more. Control group included 30 healthy subjects without any objective manifestations of MS.

The blood glucose test was performed on a fasting basis (fasting blood glucose, FBG). The fasting serum insulin level was measured by immunoenzyme assay (Beckman Coulter ™). Insulin resistance status was calculated using the homeostatic model assessment-insulin resistance (HOMA-IR). The calculation formula employed is as follows:

HOMA-IR = (fasting insulin [µIU/mL]×fasting blood glucose [mmol/L])/22.5.

During fasting, an insulin level higher than 12.5 µIU/mL indicated a diagnosis of hyperinsulinemia, whereas those patients with a HOMA index above 2.27 were considered IR patients.

The total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were determined in the venous blood using «Roche Reflotron Plus» analyzer (Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated according to Fridvald's formula. Serum free fatty acid (FFA) concentrations were assayed using a NEFA FS kit (DiaSys, Holzheim, Germany) according to the manufacturer's protocol. The intensity of lipid peroxidation in the blood plasma was investigated by spectrophotometry for the level of end-product of lipid peroxidation – malondialdehyde (MDA).Antioxidant protection (AOP) was studied as the total antioxidant activity of red blood cells and its enzymatic chain - by the activity of catalase (CA).

The study was approved by the Andijan State Medical Institute Ethics Committee.

***Statistical analysis*** was performed using the statistical software «Statistica». *The mean (M)* and standard error of the mean (SEM) were deduced. Analysis of the distribution of values obtained was performed using the Kolmogorov-Smirnov test. *For data with normal distribution, s*ignificance was assessed using Student's *t*-test. Pearson's Correlation Coefficient (r) was used to determine the strength of the relationship between the two continuous variables. A value of P<0.05 was considered statistically significant.

# Results and Discussion

Investigation of the lipid-lipoprotein biomarkers identified dyslipidemia in varying degrees of severity in all the MS patients.

The increased plasma TG level in MS patients, as well as in the whole population, remains relevant, while the remaining unfavorable factors determine the development of vascular lesions [3,8]. We detected hypercholesterolemia in 50.2% of the patients. The average TC level in the group with MS patients was 6.5±0.31 mmol/l, which is 1.7-times higher than in the control (P<0.001) (Fig.1).

Increasing levels of LDL-C also revealed that it is the most aggressive fraction of cholesterol that determines the development of atherosclerotic vascular lesions [8,11].

The average LDL-C concentration in MS patients was 5.13±0.1 mmol/l, which is 2.4-fold higher than the control (P<0.001) (Fig.1). Both figures are considered "targets of hypercholesterolemia" for MS patients, as recommended by U.S. experts, where the cholesterol levels were significantly exceeded.
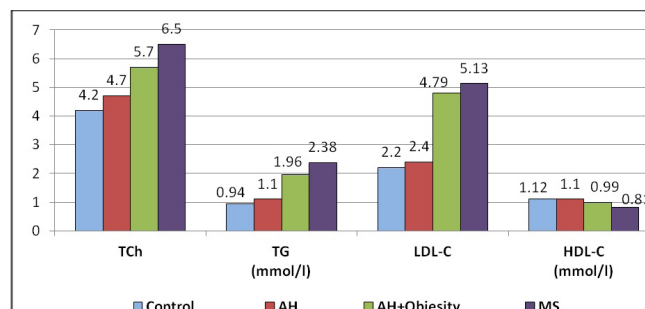


*Figure 1. Lipid-lipoprotein biomarkers in patients with MS*

The value of HDL-C as the most important factor in antiatherosclerotic protection is proved by numerous clinical and experimental data [3,11,12]. Reduced HDL-C, especially in MS patients, has significant prognostic value with regard to morbidity and mortality from coronary heart disease. In 70.2% of the MS patients examined, the HDL-C level was below 0.92 mmol/L (0.81 ± 0.01 mmol/l) (Fig. 1).

It is still an open question regarding the importance of triglycerides as an independent risk factor for atherosclerosis. Numerous studies have shown that the TG content can predict the risk of CHD, without considering other factors [1,2,3,8]. The plasma TG level more than any other indicator reflects the severity of the major clinical manifestations of MS. We found hypertriglyceridemia in 72.5% of the MS patients. The mean plasma TG concentration in MS patients was 2.8±0.07mmol/l, which was 2.5-fold higher than in the control (P<0.001) (Fig.1). Thus, the presence of hypertriglyceridemia in MS patients can be regarded as an indicator of high atherogenic potential of blood plasma.

To analyze the association between the main clinical manifestations of MS and the lipid-lipoprotein parameters in MS patients, Spearman's rank correlation was used.

As seen from Table 1, only the plasma TG concentration revealed a close relationship with the majority of clinical and biochemical parameters of the patients with MS, examined. There were statistically significant correlations between the TG content and important clinical characteristics of MS: diastolic blood pressure, fasting glucose levels and waist circumference (WC).

*Table 1.*

***The correlations between TG levels and clinical parameters in patients with MS***

| | Indicators | r | p |
|---|---|---|---|
| **TG** | Diastolic blood pressure | 0.681 | 0.027 |
| | WC | 0.435 | 0.043 |
| | Fasting glucose | 0.421 | 0.046 |
| | TC | 0.695 | <0.001 |
| | HDL-C | -0.437 | 0.029 |
| | LDL / HDL-C | 0.549 | 0.008 |

It was interesting to observe when a strong positive correlation was detected between the TG content and diastolic blood pressure levels. The probable point of view taken is that the development of hypertension and hypertriglyceridemia is determined by the presence of IR and the structural features of the skeletal muscles [11]. From this point of view, the relationships found between TG and WC, TG and DBP, as well as the positive relationship between DBP and BMI (r=0.560; p =0.004) could be interpreted as an indirect confirmation of the common pathophysiological roots of AH, AO and HT.

We have found a direct correlation between the TG content and the other atherogenic factors (TC, HDL-C, and the LDL-C/HDL-C ratio) as well as a negative correlation between TG and HDL-C. In the analysis of the plasma lipid profile, based on the TG level in MS patients, we found that patients with HT had significantly higher levels of TC and LDL-C.

A positive correlation relationship between the TG content and WC is legitimate. The WC and WC/HC (hip circumference) ratio are the most sensitive indicators of the severity of AO and atherosclerotic changes. Enhanced lipolysis, which is typical for AO and the atherosclerotic process, is accompanied by an increase in the FFA concentration in the portal vein blood that leads to the increased secretion of TG-rich very-LDL-C [3,8,12].

Based on the results of the regression analysis, it can be argued that, when the WC/HC ratio >1.0, the plasma HDL-C concentration is significantly decreased (0.91±0.02 and 0.80±0.01 mmol/L; p=0.031), showing a trend toward an increased plasma TG level (1.99±0.09 and 2.39±0.11 mmol/L; p=0.071), which is detected. The changes detected were independent of patient age, duration of MS, the degree of control of carbohydrate metabolism and BMI. The results obtained suggest a close relationship between AO and hypertriglyceridemia in MS patients.

The positive correlations between the plasma TG content and fasting glucose indicate an increase in the atherogenic properties of the plasma with deterioration in the control of the carbohydrate metabolism, which is, in turn, associated with a reduction in the lipoprotein lipase activity.

The violation of blood transport and FFA uptake by the cells precedes IR formation. In MS patients, we found a significant increase in the plasma FFA level, by up to 0.87±0.19 mmol/l (2.4-fold) (P<0.001) (Fig. 2).
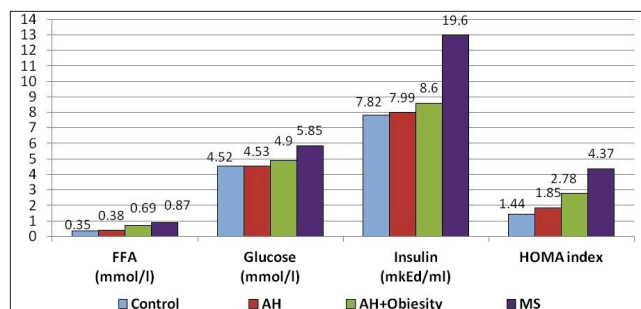
**Figure 2. Indicators of FFA and glucose homeostasis in patients with MS**

Consequently, the functioning of the insulin receptors, the secondary system of signal transmission and glucose uptake

by the cells are broken. Hyperglycemia and increased FFA level are accompanied by hyperinsulinemia. The insulin level in the blood increases to 19.6±1.72 μIU/mL (Fig. 2). The violation of the lipid-transport pathway causes the changes in the carbohydrate homeostasis and MS development. The adaptation of cells to this type of transport activates the FFA lipolysis and enhances the insulin secretion, which results in hyperinsulinemia. The violation of the receptor-mediated FFA transport, causing a change in the structure of the cell membranes, is the basis of IR formation and hyperinsulinemia [1,2,4,6]. Elevated FFA levels cause the overproduction of the mitochondrial reactive oxygen species which leads to LDL-C oxidation and induces an inflammatory process [4,10].

Excess hypertriglyceridemia and FFA in the blood activate radical formation and lipid peroxidation, which contribute to the peroxide modification of the LDL-C. The oxidized LDL-C then damages the endothelium, increases its permeability, and causes the endothelial dysfunction by developing vasoconstriction [13]. Arteriole narrowing leads to tissue ischemia, energy shortages and further activation of the lipid peroxidation [1,9].

The study of the lipid peroxidation activity in the MS patients showed that the plasma level of MDA, which is the product of peroxidation, was increased by 2.2-fold (P<0.001) (Fig. 3). In a study of the anti-radical potential, the reduced activity of catalase to 37.6% caught our attention (P<0.001) (Fig. 3). In the study of the anti-radical potential of the plasma, catalase activity reduced by 37.6% was found, which was the main enzyme involved in anti-radical protection. Therefore, in MS patients, the activation of lipid peroxidation is associated with a reduction in the antioxidant potential.
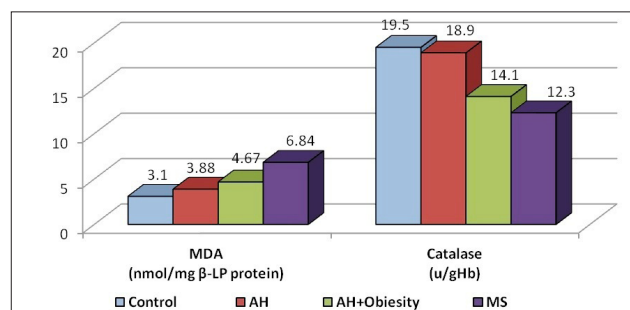
**Figure 3. Markers of oxidative stress in MS patients**

## Conclusion

MS is associated with a significant impairment of the lipid-lipoprotein plasma biomarkers: increased levels of the plasma TG, FFAs, and LDL-C and a decrease in HDL-C level. The plasma TG level, more than any other indicator, reflects the severity of the major clinical manifestations of MS: AO, AH and decompensation of the carbohydrate metabolism. Hypertriglyceridemia in MS patients is most often associated with lipid metabolism disorders such as high TC, LDL-C, and FFAs levels. The presence of HT in the MS patients can be considered as an indicator of the high atherogenic potential of the blood plasma. The dysfunction of the lipid biomarkers

activates the OS with an increase in the lipid peroxidation and a decrease in the antioxidant defenses.

Therefore, we can conclude that the lipid-lipoprotein biomarkers and OS markers are component parts of MS.

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