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Markers of Endothelial Damage, Inflammation, Oxidative and Cellular Stress in Patients with Coronary Artery Disease and Type 2 Diabetes

Julia A. Kotova, PhD*; Anna A. Zuikova, PhD, ScD; Aleksander N. Pashkov, PhD, ScD; Natalia V. Strahova, PhD; Olga N. Krasnorutskaya, PhD, ScD; Veronika I. Shevzova, PhD; Elena Yu. Esina, PhD, ScD

Voronezh State Medical University named after N.N. Burdenko
Voronezh, the Russian Federation

Abstract

Results of this study present the features of the severity of coronary atherosclerosis, evaluated by the Gensini score, depending on the presence of T2D (type 2 diabetes). The presence of T2D aggravates the course of coronary heart disease due to the more pronounced processes of inflammation, endothelial dysfunction, and oxidative and cellular stress. (**International Journal of Biomedicine. 2020;10(2):104-107.**)

Key Words: coronary heart disease • type 2 diabetes • oxidative stress • endothelial dysfunction • inflammation

Abbreviations

ADPH, aldehyde derivative of DNPH; **BMI,** body mass index; **CVDs**, cardiovascular diseases; **CHD**, coronary heart disease; **CAG**, coronary angiography; **DNPH**, 2.4-dinitrophenylhydrazine; **ED**, endothelial dysfunction; **FPG**, fasting plasma glucose; **GS**, Gensini score; **hs**CRP, high-sensitivity C-reactive protein; **HDL-C**, high-density lipoprotein cholesterol; **Hsp70**, heat shock protein 70; **KDPH**, ketone derivative of DNPH; **OS**, oxidative stress; **OMP**, oxidative modification of proteins; **SOD**, superoxide dismutase; **T2D,** type 2 diabetes; **tHcy**, total homocysteine; **WC**, waist circumference.

Introduction

Usually caused by atherosclerosis, CHD constitutes a high level of mortality among CVDs—a leading cause of morbidity and mortality globally.[1-4] Atherosclerotic plaque formation is a complex process that involves several mechanisms, including lipid accumulation, ED, OS, vascular proliferation, matrix degradation, chronic inflammation, and thrombosis.[5-7] Oxidation of low-density lipoprotein cholesterol is one of the key factors for the development of atherosclerosis. Many factors are involved in the progression of atherosclerosis in patients with T2D; however, the most important factors are insulin resistance and hyperglycemia. [8-10] T2D is characterized by accelerated atherosclerosis with widely distributed vascular lesions. People with diabetes are more likely to have carotid plaque with calcification and lipid-rich necrotic cores than people without diabetes.[11] People with diabetes have a high incidence of two or more vessel diseases, compared with subjects without diabetes.[12]

Hyperglycemia is an important factor in cardiovascular damage, working through different mechanisms, such as activation of protein kinase C, polyol and hexosamine pathways, and production of advanced glycation end-products. All of these pathways, in association with hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, promote accumulation of reactive oxygen species and activation of OS.[13,14] The impact of chronic hyperglycaemia might induce damage on vascular homeostasis, mainly attributable to the endothelium function. Numerous observational studies have found increased levels of the mediators of inflammation, such as C-reactive protein (CRP), interleukin-6 (IL-6), plasminogen activator inhibitor 1 (PAI-1) Many studies have demonstrated an

***Corresponding author:** Julia Kotova, PhD. Voronezh State Medical University named after N.N. Burdenko. Voronezh, Russia. E-mail: kotova_u@inbox.ru*

association between insulin resistance and accelerated CVD in patients with T2D.[15-18] The strong association between insulin resistance and adverse cardiovascular outcomes in non-diabetic individuals and individuals with T2DM has been summarized in several meta-analyses.[19-21]

The aim of the present study was to evaluate the changes in the markers of endothelial damage and oxidative and cellular stress in CHD patients with T2D.

Materials and Methods

We examined 336 patients (178/53% women and 158/47% men aged between 47 and 75 years, mean age of 61.8±8.1 years) with CHD verified by standardized validated criteria and clinical-functional methods. T2D was detected in 70 out of 300 CHD patients.

All patients underwent the following examinations: assessment of traditional risk factors, 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. The levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and FPG were determined in the blood plasma. All patients underwent OGTT.

CAG was performed using General Electric Innova 3100 (GE Healthcare, USA). The transfemoral approach (the Seldinger technique) was used.

The severity of coronary atherosclerosis was evaluated by the GS.[22] The severity of the disease is expressed as the sum of the scores for individual lesions and the functional importance index of the area of each lesion in the coronary tree. The sample was continuous.. According to the calculated GS, patients were divided into three groups: GS0 – 162 patients with normal coronary arteries, GS1 (1-15 points) – 80 patients with hemodynamically insignificant coronary atherosclerosis, and GS2 (>15 points) – 94 patients with hemodynamically significant coronary atherosclerosis. As markers of OS, oxidized modified proteins and the SOD activity were determined. The determination of OMP in the blood serum was carried out using the methods by Dubinina et al.[23] The assay is based on the spectrophotometric detection of the reaction between 2.4-dinitrophenylhydrazine (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 356 nm and 370 nm, 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 tHcy was determined by EIA using «Axis-Shield» test kit.

Hsp70 and their chaperone activity were considered as markers of cell stress. Extracellular Hsp70 was measured by ELISA (Elisa Kit for Hsp70, Cloud-Clone Corp.) in blood samples. Hsp70 chaperone activity was measured by monitoring the DTT-induced aggregation of insulin using recombinant Hsp70 (HSPA1A) (Cloud-Clone corp.).[24]

The level of high-sensitivity C-reactive protein (hsCRP) was determined using the High Sensitive Elisa Kit for CRP (Cloud-Clone Corp., USA).

Statistical analysis was performed using statistical software package SPSS version 20.0 (SPSS Inc, Chicago, IL). Median values are presented with interquartile (IQ) ranges (IQR; 25th to 75th percentiles). The Mann-Whitney test was used to compare median values. The frequencies of categorical variables were compared using Pearson χ^2. The Spearman correlation coefficient (r_s) was used to assess the relationship between variables. A probability value of $P<0.05$ was considered statistically significant.

Results

All patients were further divided into two groups, depending on the presence of T2D. Group 1 included 70 CHD patients (45.7% women and 54.3% men) with T2D; Group 2 included 266 CHD patients (54.9% women and 45.1% men) without T2D. The characteristics of the compared groups are presented in Table 1. GS was significantly higher in Group 1 (P=0.005) (Table 1). In Group 2, GS0 was determined in 51.8% of patients, GS1 in 25.6%, and GS2 in 22.6%. In Group 1, we saw the opposite trend: GS0 was determined in 23.3 of patients, GS1 in 17.1%, and GS2 in 48.6%.

Table 1.
The characteristics of the compared groups

Variable	Group 1	Group 2	*P*-value
Age, yrs	60.1±6.9	58.8±5.8	0.236
BMI, kg/m^2	27.6 [25.7;31.4]	26.7 [25.7; 27.9]	0.118
WC, cm	92 [82;110]	90 [80;98]	0.071
SBP, mmHg	160 [140;180]	140 [140;160]	0.116
DBP, mmHg	90 [90;100]	90 [80;98]	0.005
GS, score	11 [0;29]	0 [0;12]	0.005

According to the parameters of the lipid spectrum, no significant differences between the two groups were found, with the exception of HDL-C, the level of which was lower in Group 1 (P=0.032). The glucose level in Group 1 was 1.5 times higher than in Group 2. The levels of tHcy and hsCRP were significantly higher in Group 1 than in Group 2. At the same time, the SOD activity was lower in Group I than in Group 2 (P=0.002). Markers of cell stress showed a tendency to increase in Group 1, compared with Group 2, indicating a more pronounced mitochondrial dysfunction and endoplasmic reticulum dysfunction in CHD patients with diabetes (Table 2). When evaluating OMP, a significant difference was established between these two groups in the blood levels of ADPHn (P=0.009), KDPHn (P=0.004), and KDPHb (P=0.045) (Fig.1). As is known, an increase in ADPHn indicates the activation of free radical processes, and an increase in KDPHn/b indicates a depletion of the adaptive performance.

Table 2.

Markers of endothelial damage, inflammation, oxidative and cellular stress in the compared groups

Variable	Group 1	Group 2	*P*-value
TC, mmol/L	5.9 [4.5;6.9]	5.5 [4.55;6.2]	0.311
LDL-C, mmol/L	2.7 [2.3;3.7]	2.6 [2.1; 3.1]	0.384
HDL-C, mmol/L	1 [0.9;1.1]	1.1 [0.9;1.1]	0.032
TG, mmol/L	1.3 [1;1.4]	1.2 [1.1;1.5]	0.757
FPG, mmol/L	7.9 [6.4;9.8]	5.4 [4.9;5.8]	5.96E-13
tHcy, µmol/L	10.9 [9.8;12]	10 [9.5;11.34]	0.020
hsCRP, mg/L	0.15 [0.01;0.76]	0.02 [0.005;0.21]	0.022
SOD, %	35.21 [32.1;37.6]	38.3 [35.21;39.26]	0.002
Hsp70, ng/ml	1.43 [1.02;1.9]	2.12 [1.52;2.88]	0.0002
Hsp70 chaperone activity, %	65.9 [52.7;68.5]	71.2 [61.5; 76.1]	0.001

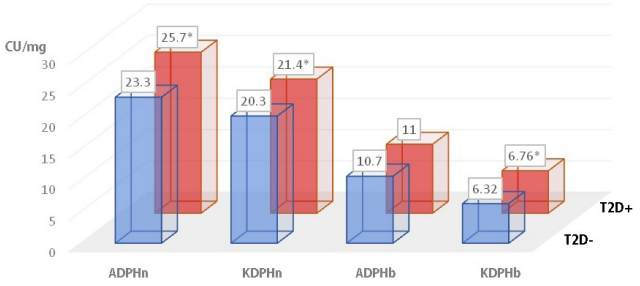

Fig.1. *Parameters of OMP in the compared groups.*

The lower chaperone activity was found in Group 1. As is known, chaperones play a huge protective role during cellular stress and pathologic conditions. Important redox modifications of chaperone proteins have been described in OS conditions. The activation of Hsp70 may play a role in protecting the cells against OS and inflammatory damage.[25] HSP70 is susceptible to S-glutathionylation under oxidative stress conditions. It has been suggested that S-glutathionylation of HSP70 may potentiate its chaperone activity.[26] In the setting of inducibly elevated Hsp70, cardiomyocyte protection was identified.[27] Additionally, other studies have found a lower incidence of post-operative atrial fibrillation in patients with high levels of HSP70 (also known as HSP70-1a, HSP70-1b), in contrast to those with low HSP70 (or a HSP70 polymorphism with decreased function) who have an increased risk of post-operative atrial fibrillation.[28,29] Hsp70 participates in cardioprotection induced by exercise preconditioning, early and late protection, where Hsp70 repairs unfolded proteins or may stabilize the function of the endoplasmic reticulum.[30]

Correlation analysis revealed the relationships between the presence of T2D and GS (r_s=0,217, *P*=0,005), the blood levels of ADPHn (r_s=0.201, *P*=0.009), KDPHn (r_s=0.221, *P*=0.004), KDPHb (r_s=0.155, *P*=0.044), SOD activity(r_s=-0.237, P=0.002), Hsp70 (r_s=-0.284, *P*=0.000), and chaperone activity (r_s=-0.249, *P*=0.0001).

Thus, our study showed that the course of coronary atherosclerosis in CHD patients with T2D is more severe than in patients without T2D. The presence of T2D aggravates the course of CHD due to the more pronounced processes of inflammation, endothelial dysfunction, and oxidative and cellular stress.

Competing Interests

The authors declare that they have no competing interests.

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