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THE ENDOTHELIAL DYSFUNCTION FACTORS IN DIABETES MELLITUS 2 TYPE

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Abstract

Aim: to investigate the association of EDF factors: endothelin 1 (ET1), endothelial NO-synthase (eNOS), nitric oxide (NO), tumor necrosis factor (TNFα), and diene conjugates (DC) with severity of type 2 diabetes.

Material and methods. Data were used for 152 hospital patients with type 2 diabetes at the age from 34 to 80 years (53.9±8.4 years). Women were 95 (62.5%), men – 57 (37.5%). According to the degree of severity of patients was divided into three groups: 1st (37.5% of patients) – the average stage in the compensation stage (HbA1s 7-9%), 2nd (41.4%) – the average stage in the stage of decompensation (HbA1s more than 9%), 3rd (21,1%) – a severe degree in the stage of decompensation. The control group included 95 practically healthy individuals. The plasma levels of the blood were determined by the enzyme-linked method: ET1 (Biomedica Immunoassays, Austria), eNOS (BCM Diagnostics, USA) і TNFα (Bender Medsystems, Austria). The level of blood NO and DC were determined biochemically (spectrophotometer Specord, Germany). Statistica 10 (StatSoft, Inc., USA) was used to statistically process the data obtained.

Results. Levels of EDF factors depended on the severity of DM 2 type. Thus, the level of ETI in patients exceeded control in 3.7-4.7 times ($p<0.001$) with the maximum values in

the 2nd and 3rd groups; also increased blood levels of NO (1.4-1.5 times; p<0.001). The highest increase was observed in TNFα levels (4.2-6.5 times; p<0.001) and DC (2.3-2.7 times; p<0.001). The blood content of eNOS in the patients' groups was lower when compared with control (1.3-1.9 times; p<0.001).

Conclusion. Factors of EDF are closely linked with clinical and laboratory indicators of severity of DM 2 type, which highlights them in the pathogenesis of the disease.

Key words: diabetes mellitus 2 type, severity, endothelin 1, nitric oxide, eNOS, TNFα, diene conjugates.

Relevance. Currently, diabetes mellitus (DM) and diabetic vascular complications have a major impact on the medical and demographic indicators associated with early disability and premature mortality of the working-age population [1-3]. The clarification of the role of the vascular endothelium has evolved over the last two decades and has come to the understanding that it is a dynamic regulatory system and plays a key role in both physiological and pathological processes [4, 5]. Endothelial cells perform a barrier role and actively regulate vascular tone, circulation and platelet function [4, 6, 7]. Endothelial dysfunction (EDF) is formed by vascular inflammation, atherosclerosis, hypertension, cardiomyopathy, retinopathy, neuropathy [8, 9]. Hyperlipidemia, hyperglycemia, and other metabolic factors leading to the development of vascular complications and EDF [10, 11].

In the case of diabetes, a whole cascade of pathological reactions unfolds in the endothelium of the vessels that afflict glucose toxicity, excessive action of stimulating hypertensive and inflammatory factors, thrombotic activators, and the intensification of oxidative stress [1, 6, 12]. According to J.F. Bermejo-Martin (2018) to the development of EDF leads several factors: 1) increased oxidative stress and systemic inflammation; 2) degradation and proliferation of glycocalyx; 3) violation of endothelial intercellular contacts and the blood-tissue barrier; 4) increase of leukocyte adhesion and extravasation; 5) induction of procoagulants and antiphibrinolytic systems [13]. On the other hand, according to X. Pi et alt. (2018), the damaged endothelium itself is included in the pathogenesis of diabetes and causes the development of further violations [4]. The main factors of EDF are endothelial hormone endothelin (ET1), nitric oxide (NO), endothelial NO synthase (eNOS), tumor necrosis factor (TNFα), and oxidative stress marker - diene conjugates (DC) that accumulate in blood and cause EDF [5, 14, 15].

Aim: to investigate the relationship of factors EDF (ET1, eNOS, NO, TNFα, DK) with severity of DM 2 type.

MATERIAL AND METHODS

The study involved data from 152 hospital patients with type 2 diabetes. The age of patients ranged from 34 to 80 years, on average 53,9 ± 8,4 years. Women were 95 (62.5%), men - 57 (37.5%). For the duration of the disease, the patients were distributed as follows: up to 1 year - 5 (3.3%), from 1 to 3 years - 40 (26.3%), from 4 to 5 years - 23 (15.1%), from 6 up to 7 years - 12 (7.9%), from 8 to 10 years - 31 (20.4%), from 11 to 15 years - 26 (17.1%) and more than 15 years - 15 (9.9%) of patients. In relatives of patients - type 2 diabetes was found in 35 (23.0%) cases. According to clinical practice guidelines [1, 2] the results of clinical and laboratory examinations determined the presence of retinopathy, nephropathy on levels of albuminuria and glomerular filtration rate (GFR), sensory polyneuropathy, angiopathy of lower extremities and hypertension.

According to the clinical classification [1, 2], 1 grade of severity was not detected in any patient, 2 grade - in 120 (78,9%) and 3 grade - in 32 (21,1%) patients. Patients with grade 2 were divided into 2 groups: first - 57 (37.5%) patients, and the second - 63 (41.4%) patients. For their distribution was selected a certain criteria that can represent degree of compensation of DM2T: level of glycated hemoglobin (HbA1c). In the first group were included patients with compensated state of hyperglycemia or with satisfactory level (level of HbA1C around 7-9%). In the second group were included patients in the state of decompensation (HbA1C more than 9%). Patients with 3 grade of severity were included in 3rd group. The control group included 95 practically individuals of similar age and gender distribution, who had no violations of carbohydrate metabolism and clinical manifestation of the corresponding symptoms, similar to the micro- and macrovascular complications of diabetes.

In blood plasma by the ELISA method were determined the levels of EDF indicators: ET1 (Biomedica Immunoassays, Austria), eNOS (BCM Diagnostics, USA) and TNFα (Bender Medsystems, Austria). The color intensity of product of the enzymatic reaction quantitatively measured on the photometer PR2100 SANOFI DIAGNOSTIC PASTEUR (France). The level of NO in blood was determined by its final metabolite nitrite in the Gris reaction by the spectrophotometer (spectrophotometer Specord, Germany) at a wavelength of 546 nm. The level of DC of unsaturated fatty acids was determined by the method of Z. Placer in the modification of VB. Gavrilov (1983).

For statistical analysis of the data was used Statistica 10 software (StatSoft, Inc., USA). After Kolmogorov-Smirnov, Anderson-Darling and χ-squared tests, a distinction was found between the normal distribution of variation series ($p < 0.05$). In this case, for the descriptive statistics of quantitative data, we used the median (Me) and the first and third

quartiles (Q1; Q3) of variation series. Paired independent samples was compared using the Mann-Whitney (U) and Crackel-Wallis (H) criteria. In order to compare categorical variables, the coupling tables and the nonparametric criterion of the x-square ($\chi 2$) of Pearson in the Yates modification were used. In all cases of statistical evaluation, the significance of the differences was taken into account at a value of $p < 0.05$.

RESULTS AND DISCUSSION

The summarized data of EDF indicators depending on the severity of the disease are presented in Table 1.

Table 1. Differences between EDF indicators (Me; Q1; Q3) in patients groups

Indicators	Groups				p (H)
	Control (n=95)	First (n=57)	Second (n=63)	Third (n=32)	
ET1, fmol/ml	0,55 (0,40; 0,75)	2,01 (1,87; 2,29)	2,44 (2,10; 2,80)	2,61 (2,09; 3,05)	<0,001
NO, μmol/l	4,35 (4,08; 4,61)	6,00 (5,69; 6,34)	6,69 (6,29; 7,10)	6,06 (5,99; 6,71)	<0,001
eNOS, pg/ml	364 (333; 390)	288 (232; 358)	231 (185; 254)	193 (166; 239)	<0,001
TNFα, pg/ml	22,3 (16,2; 29,0)	93,3 (70,1; 104,7)	113,0 (90,5; 124,0)	145,0 (102; 230)	<0,001
DC, U/ml	2,06 (1,67; 2,24)	4,70 (4,39; 5,17)	5,62 (5,48; 5,87)	4,77 (4,05; 5,24)	<0,001

Notes: p (H) is the statistical significance of the differences between the groups according to the criterion H (Kruskal-Wallis ANOVA by Ranks test)

The level of ET1 in the blood, there was a clear tendency in increasing of it in groups: from 0,55 fmol/ml in control to 2,61 fmol/ml in group number 3. Statistical analysis by U (Mann-Whitney U Test) showed that all differences were statistically significant ($p < 0.001$), except for differences in the levels of ET1 in groups 2 and 3 ($p = 0.206$). Thus, it was found that the level of ET1 in the groups of patients with type 2 diabetes statistically significantly exceeded the control (3.7-4.7 times). With the development of the disease, in general, there was a tendency towards higher values of ET1, but for medium and severe degrees of DM2 the certain difference was not found.

The level of NO in blood (see. Table. 1) increased in groups of patients compared with

controls (1,4-1,5 times), which, in general, had a high statistical significance (p <0,001). It reached the highest level in the second group - 1,5 times more (p = 2,3e-26). At the same time, no significant difference was found between the 1st and 3rd groups (p = 0.581). So we could assume that the level of NO in the presence of type 2 diabetes increases, which may not depend on the severity of the disease.

As shown in Table 1, the blood level of eNOS was lower in the groups of patients compared with control (1.3-1.9 times; p <0.001), with the lowest in the third group. The presence of a statistically significant difference between all groups confirmed the relationship between the development of type 2 diabetes and the level of eNOS in blood. Obviously, with increasing severity of diabetes was growing inhibition of eNOS expression and the progressive reduction of its level in the blood, which coincided with the data [16].

The level in the blood of a major pro-inflammatory interleukins - TNFa repeatedly increased, particularly in the 3rd group (6.5 times). Also there was very high statistical significance of differences between groups. The level of TNFα exceeded control in the 1st and 2nd groups, respectively, in 4.2 and 5.1 times. Preferably, the high values of TNFα grouped into Group 3 showed that the cytokine cascade activation depended on the severity of type 2 diabetes, and consistent with the literature [5, 7, 14].

The last indicator which was chosen to describe EDF was the level of diene conjugates in blood. The role of this indicator as a key parameter for the intensification of the lipid peroxidation in the case of type 2 diabetes was also evident in our previous publications [15]. Moreover, DC, as malonic dialdehyde, progressively accumulated in the blood during the first five years, after which it remained at a stable high level. A similar trend is observed in this study (see Table 1): in all groups, the level of DC was significantly increased (2.3-2.7 times; p <0.001). When comparing the groups, there was no statistically significant difference between the 1st and 3rd groups (p = 0.385), while the level of DC was significantly higher in group 2 (p <0.001).

Thus, there were established certain patterns of EDF development with increasing severity of DM 2 type. Levels of ET1, TNFα, NO, and DC in the blood - repeatedly increased significantly, which, in general, corresponded to the severity of the pathological process, especially for TNFα. Blood levels of eNOS - decreased according to the severity of the disease process.

CONCLUSION

1. Levels of EDF factors depended on the severity of DM 2 type. Thus, the level of ET1 in patients exceeded control in 3,7-4,7 times (p <0,001) with the maximum values in the

2-nd and 3-rd groups; Also, blood levels of NO increased (1.4-1.5 times, p <0.001).

2. The highest increases were observed in TNFα levels (4.2-6.5 times; p <0.001) and DC (2.3-2.7 times; p <0.001).

3. Blood levels of eNOS in groups of patients was lower when compared with the control (in 1,3-1,9 times; p <0,001).

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