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COMPREHENSIVE ASSESSMENT OF AGE-RELATED, GENETIC AND BIOCHEMICAL FACTORS IN THE DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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The purpose of the study was to assess the role of hereditary factors, antioxidant status, and nitric oxide metabolism in the development of endothelial dysfunction in patients with diabetes mellitus. The study included thirty-three patients. Descriptive evaluation of clinical and biochemical parameters, odds ratio analysis, correlation, and cluster analysis were performed. The results showed that hereditary predisposition increases the risk of endothelial dysfunction by ten times. Strong positive associations were found between hereditary factors, antioxidant activity, and markers of oxidative stress. Cluster analysis identified two subgroups of patients: the first characterized by a high level of oxidative stress with compensatory activation of the antioxidant system, and the second with a more effective antioxidant defense and lower levels of inflammatory markers. The findings emphasize the important role of hereditary factors in the development of endothelial dysfunction in diabetes mellitus and suggest the possibility of distinguishing different phenotypes of the disease course.

Key words: diabetes mellitus, endothelial dysfunction, heredity, antioxidant system, oxidative stress, nitric oxide.

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КОМПЛЕКСНА ОЦІНКА ВІКОВИХ, ГЕНЕТИЧНИХ І БІОХІМІЧНИХ ФАКТОРІВ РОЗВИТКУ ЕНДОТЕЛІАЛЬНОЇ ДИСФУНКЦІЇ У ПАЦІЄНТІВ ІЗ ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ

Метою дослідження було оцінити роль спадкових факторів, антиоксидантного статусу та метаболізму оксиду азоту у формуванні ендотеліальної дисфункції у пацієнтів з цукровим діабетом. У дослідження було включено 33 пацієнти. Проведено описову оцінку клініко-біохімічних показників, аналіз відношення шансів, кореляційний та кластерний аналіз. Результати показали, що спадковість у десять разів підвищує ризик розвитку ендотеліальної дисфункції. Виявлено тісні позитивні зв'язки між спадковістю та показниками антиоксидантного статусу і маркерами оксидативного стресу. Кластеризація дозволила виділити дві підгрупи пацієнтів: першу з високим рівнем оксидативного стресу і компенсаторною активацією антиоксидантної системи та другу з більш ефективним антиоксидантним захистом і нижчими показниками маркерів запалення. Отримані результати підкреслюють важливу роль спадкових факторів у розвитку ендотеліальної дисфункції при цукровому діабеті та вказують на можливість виділення різних фенотипів перебігу захворювання.

Ключові слова: цукровий діабет, ендотеліальна дисфункція, спадковість, антиоксидантна система, оксидативний стрес, оксид азоту.

The study is a fragment of the research project "Development of means to correct pathological changes in the organs of the digestive system in the context of civilization diseases", state registration No. 0124U001922.

Diabetes mellitus (DM) is a major global public health issue, associated with population aging, socio-economic development, unhealthy dietary habits and a sedentary lifestyle [8]. Recent estimates report that in the near future more than 10% of the world's adult population (aged 20–79 years) will be living with diabetes [8].

Chronic hyperglycemia, the oxidative stress (OS) it induces and the activation of inflammatory processes play a key role in the development and progression of type 2 diabetes mellitus (T2DM) and its complications. T2DM is among the leading factors contributing to cardiovascular pathologies, which significantly worsen disease prognosis and reduce patients' life expectancy. The central pathogenetic mechanism of these complications is endothelial dysfunction (ED) – a disturbance of the regulatory function of the endothelium, accompanied by reduced bioavailability of nitric oxide (NO) and the predominance of vasoconstrictive, prothrombotic and pro-inflammatory responses [1, 2]. ED is one of the most critical links in the pathogenesis of atherosclerosis – the morphological basis of coronary heart disease – and represents a major pathogenetic mechanism in obesity and preeclampsia [2, 4, 12].

The persistently elevated blood glucose level, characteristic of diabetes, promotes increased production of reactive oxygen species (ROS) through mechanisms such as glucose auto-oxidation, activation of the polyol pathway and the formation of advanced glycation end-products (AGEs). In addition, mitochondrial dysfunction, which is common in diabetes, leads to further release of ROS [5].

Mitochondrial dysfunction can lead to increased production of free radicals and cellular damage, including damage to endothelial cells, thereby causing endothelial dysfunction [10].

The pathogenesis of endothelial dysfunction in T2DM is multifactorial. Its development is driven by chronic hyperglycemia, insulin resistance, dyslipidemia, activation of oxidative stress and inflammatory processes [7]. Excessive production of ROS disrupts the balance between the antioxidant system and pro-

oxidant influences, reducing the synthesis of biologically active NO [9]. In this process, antioxidant defense enzymes, namely, superoxide dismutase (SOD) and catalase, play a key role by neutralizing ROS, as well as malondialdehyde (MDA), which is a marker of lipid peroxidation. NO synthesis is regulated by constitutive (cNOS) and inducible (iNOS) isoforms of NO synthase, whose activity changes influence vascular tone and the course of inflammatory reactions [8].

In addition to metabolic and biochemical disorders, hereditary factors make a significant contribution to susceptibility to endothelial dysfunction. Genetically determined features of metabolism, the activity of antioxidant system enzymes, the regulation of NO synthase and the inflammatory response may account for individual differences in the development of endothelial dysfunction, even under identical clinical conditions [1, 5, 9].

The study of the combined impact of oxidative stress, NO synthase activity and genetic predisposition on endothelial status in T2DM patients is crucial for developing personalized strategies for the prevention and treatment of vascular complications.

The purpose of the study was to analyze the impact of age-related, hereditary and biochemical factors on the development of endothelial dysfunction in T2DM patients.

Materials and methods. The study was approved by the Ethics and Bioethics Committee of Poltava State Medical University (Minutes No. 239, July 22, 2025).

The study was conducted at the Municipal Enterprise "2nd City Clinical Hospital of the Poltava City Council" (Poltava) from September to December 2023. A total of 33 patients of both sexes, with a confirmed diagnosis of T2DM were involved in the study. The diagnosis was established in accordance with the "Unified Clinical Protocol for Providing Primary and Specialized Medical Care to Adults with Type 2 Diabetes Mellitus" (Order of the Ministry of Health of Ukraine No. 1300, July 24, 2024).

Inclusion criteria for the study were a confirmed diagnosis of T2DM and written informed consent to participate. Exclusion criteria included patients who had experienced an acute coronary syndrome or stroke, as well as patients with uncontrolled arterial hypertension; heart failure; arrhythmias; renal or hepatic insufficiency; acute illnesses; pregnancy or lactation.

In patient blood samples, the activities of inducible (iNOS) and constitutive (cNOS) forms of NO synthase, arginase, superoxide dismutase (SOD), and catalase, as well as the concentration of free malondialdehyde (MDA) were measured spectrophotometrically on Ulab 101 spectrophotometer by standard methods. All reagents used for biochemical studies were analytical grade purchased from certified vendors.

Statistical analysis of the obtained results was performed using the software packages GraphPad Prism (version 8) and Microsoft Excel 2010. Normality of distribution was assessed using the D'Agostino-Pearson test. For normally distributed data, the results were presented as mean ± standard error of the mean (M±m). For non-normally distributed data, results were presented as median (Me) and interquartile range [25th; 75th percentiles].

Correlations between parameters were assessed using Pearson's correlation coefficient (r), Spearman's rank correlation (R), and Kendall's rank correlation (τ).

Hanley's method was used to calculate areas under the curve (AUC) and to construct ROC curves. Cluster analysis was performed using hierarchical cluster analysis, with Euclidean distances calculated and dendrograms constructed.

Regression modeling was used to explain the dependence of endothelial dysfunction on several parameters, namely, SOD, catalase, MDA, cNOS, iNOS, Arg. The quality of the regression model was assessed using Fisher's criterion.

Differences were considered statistically significant at $p<0.05$.

Results of the study and their discussion. The age of patients included in the study ranged from 40.0 to 78.0 years. The mean age of the examined patients was 57.12±1.48 years. The duration of T2DM ranged from 2 to 19.0 years, with a mean of 8.88±0.85 years. In patient groups, HbA1c levels ranged from 5.26 to 12.4%. The mean glycated hemoglobin (HbA1c) level was 8.65±0.26%. BMI in the groups was set at 24.4-41.9 kg/m². The mean body mass index (BMI) was 31.82±0.88 kg/m². The values of the studied parameters: SOD, catalase, MDA, cNOS, iNOS, arginase in the study group are presented in Table 1.

Table 1

Characteristics of indicators in T2DM patients (n=33)

	M	m	Me	CI [Q1-Q3]
SOD	7.361	0.3045	-	-
Catalase	-	-	1.558	1.295-1.938
MDA	28.76	0.7813	-	-
cNOS	-	-	0.039	0.02-0.0557
iNOS	-	-	2.083	0.444-2.362
Arginase	1.226	0.07861	-	-

A strong direct relationship was established between heredity and key antioxidant parameters: catalase (τ=0.90; p<0.00001) and MDA (τ=0.85; p<0.00001), a very strong relationship with SOD (τ=0.94; p<0.00001), a moderate direct relationship between heredity and cNOS activity (τ =0.61; p<0.00001), and a notable inverse relationship between diabetes duration and catalase (r=–0.34; p=0.0504)

Based on the studied parameters, the group of T2DM patients was conditionally divided into two subgroups: patients with endothelial dysfunction and patients without endothelial dysfunction.

A high diagnostic efficacy of the biochemical markers: arginase (AUC=0.92 units, p<0.0001) and iNOS (AUC=1.0 units, p<0.0001) was established as predictors of ED (Fig. 1).

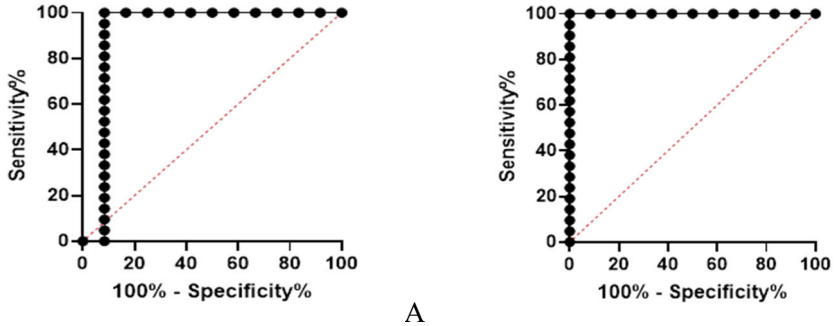

Fig. 1. ROC curves of the diagnostic role of biochemical markers as predictors of ED: A – ROC curve of the diagnostic role of arginase as a predictor of endothelial dysfunction; B – ROC curve of the diagnostic role of iNOS as a predictor of endothelial dysfunction.

For a more detailed study of T2DM patients, cluster analysis of the examined clinical and biochemical parameters was performed. Figure 2 shows the dendrogram of clustering of T2DM patients based on the studied clinical and biochemical parameters.

The dendrogram shows a division into seven subclusters, grouped into two main clusters. It demonstrates the similarity between subclusters 1.1.1.1 and 1.1.1.2, which included patients with and without ED. A similar trend was observed for subclusters 1.2.1 and 1.2.2.

Figure 3 shows a detailed dendrogram of clustering of T2DM patients based on refined clinical and biochemical parameters: catalase and iNOS.

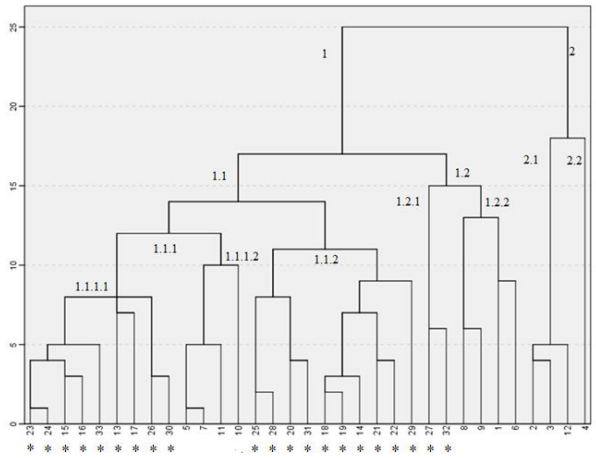

Fig. 2. Dendrogram of clustering of T2DM patients based on the studied clinical and biochemical parameters: On the Y-axis: patients 1–12 had no ED, patients 13–33 had ED;* patients with endothelial dysfunction.

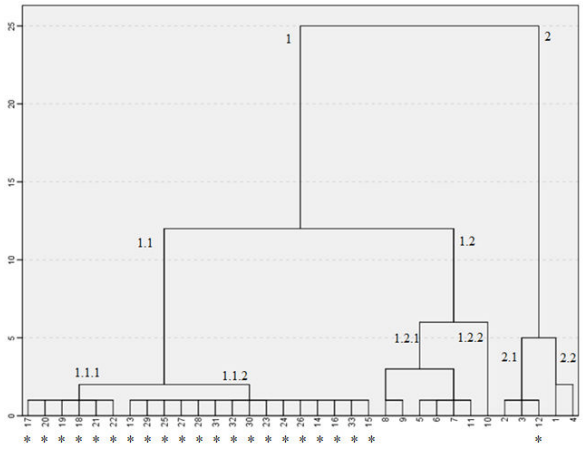

Fig. 3. Dendrogram of clustering of T2DM patients based on the studied clinical and biochemical parameters: catalase and iNOS: On the Y-axis: patients 1–12 had no ED, patients 13–33 had ED; * patients with endothelial dysfunction.

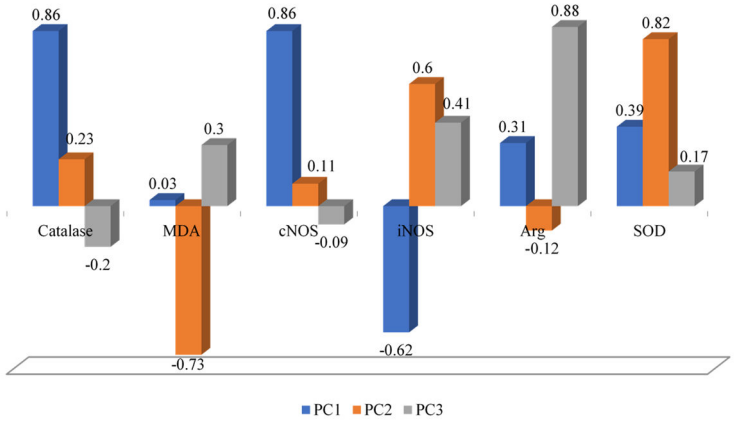

Fig. 4. Factor loadings for the principal components PC1, PC2, PC3.

The detailed dendrogram shows Cluster 2, which included patients without ED, except for one patient with ED, as well as Subcluster 1.2, which included patients without ED who were related to the patients in Subcluster 1.1 with ED.

Principal component analysis revealed that 80.83% of the total variance in the study group is explained by three components. The first principal component (PC1) is primarily associated with catalase, cNOS and iNOS; the second (PC2)

with SOD, MDA and iNOS; and the third (PC3) with arginase and iNOS. For the catalase indicator, the percentage of variation in the total variance reached 34.91; for MDA – 27.51; for cNOS – 18.41; for iNOS – 10.64; for arginase – 6.06; and for SOD – 2.47.

Figure 4 shows the factor loading for the three identified principal components based on biochemical indicators in the study group.

Taking the occurrence of ED as the dependent variable (Y), and SOD, catalase, MDA, cNOS, iNOS, and Arg as the independent variables (vector X), a linear regression model was calculated: $y=a_1\times x_1+a_2\times x_2+\ldots +a_6\times x_6$, where: $x_1, x_2, \ldots, x_6$ are the variables, $a_1, a_2, \ldots, a_6$ are the coefficients for the variables (Table 2).

Table 2

Parameters of the regression model of biochemical predictors of endothelial dysfunction

Variables	Coefficients for the variables	CI [Q1-Q3]	p
x_1 – 0.066	a_1 – 0.1674	-0.1625-0.02971	0.1674
x_2 – 0.2428	a_2 – 0.142	0.0137 - 0.472	0.0387
x_3 – 0.11	a_3 – 0.0387	-0.013 - 0.035	0.3547
x_4 – -2.53	a_4 – 0.3547	-8.377 - 3.318	0.3820
x_5 – 0.4738	a_5 – 0.382	0.321 - 0.627	<0.0001
x_6 – 0.1302	a_6 – 0.4593	-0.226 - 0.487	0.4593

According to the given parameters, the reliability of the model (R^2) was 0.84; the reliability by Fisher's criterion (F) was 22.45 ($p<0.0001$).

The regression model equation obtained was:

$y=0.167\times0.066+0.142\times0.243+0.039\times0.11-0.355\times2.53+0.382\times0.474+0.459\times0.13$

Coefficients x_1, x_3, x_4, x_6 do not demonstrate sufficient reliability.

The main pathogenetic mechanism of endothelial dysfunction (ED) development is the uncoupling of eNOS from its substrate (L-arginine) or under conditions of tetrahydrobiopterin (BH4) deficiency. The uncoupling of eNOS from its substrate occurs in several stages:

1. L-arginine deficiency, caused by the "deprivation" of eNOS due to high arginase activity, leads to a shift in the eNOS product from nitric oxide to the superoxide anion radical. At this stage, the process is reversible, since the levels of L-arginine in the blood and tissues are sufficiently high to restore eNOS activity if arginase activity decreases.

2. Excessive formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS are generated directly by "uncoupled" eNOS, whereas peroxynitrite ($ONOO^-$) forms through the interaction of the superoxide anion radical produced by eNOS with nitric oxide (NO), which is generated by iNOS activated in response to oxidative stress induced by ROS. This process can be halted at this stage through activation of the body's antioxidant system (genes encoding SOD, catalase and enzymes of the glutathione cycle).

3. Inactivation of BH4 by peroxynitrite leads to persistent uncoupling of eNOS from its substrate. At this stage, without external intervention, the body is unable to restore normal eNOS activity [5].

Heredity also plays an important role in the development of ED. In the studied patient group, age and diabetes duration did not show a statistically significant correlation with the development of ED, indicating a possible influence of genetic mechanisms in the pathogenesis of this disorder. However, a direct correlation was observed between family history of DM and increased levels of oxidative stress markers (MDA) and antioxidant activity (SOD, catalase). This confirms a genetically determined vulnerability of the endothelium, which, combined with hyperglycemia and insulin resistance, accelerates the development of vascular complications. Our findings regarding the importance of hereditary changes in cNOS activity are consistent with data from research teams at Poltava State Medical University and Shupyk National Healthcare University of Ukraine, which identified certain mechanisms by which maternal metabolic syndrome affects the development of corresponding disorders in newborns, including impaired nitric oxide synthesis [6].

The predominance of iNOS and shifts in the NO metabolism system observed in this study are consistent with previously reported data on the role of chronic hyperglycemia and insulin resistance in reducing NO bioavailability, altering the balance between iNOS and cNOS, and promoting the progression of atherothrombosis [12].

A high diagnostic efficacy of the biochemical markers: arginase (AUC=0.92, $p<0.0001$) and iNOS (AUC=1.0, $p<0.0001$) as predictors of ED was established, confirming previously reported findings [11]. The high diagnostic significance of arginase and iNOS activity is likely related to their direct involvement in the initiation and progression of eNOS uncoupling from its substrate, which is the primary mechanism underlying ED development.

The cluster analysis revealed the presence of seven subclusters grouped into two major clusters. The identification of a subcluster (n=4) of patients without ED, yet closely associated with clusters of patients with ED, also indicates clinical variability in complications. The two major clusters identified in

the study group differed in biochemical parameter variability: one was characterized by high oxidative stress and low antioxidant activity, while the other exhibited a more compensated state. This indicates that endothelial heterogeneity is not only a theoretical concept but also a clinically significant phenomenon, underscoring the rationale for personalized therapeutic approaches.

Principal component analysis showed that over 80% of the variance in the study group is explained by the variation in catalase, MDA and cNOS, once again highlighting the importance of cNOS as a pathogenic factor in DM complications [10].

The regression equation showed a dominant influence of MDA and iNOS on the development of endothelial dysfunction, while other biochemical parameters in this model may be statistically negligible. However, the high R^2 value indicates the adequacy of the obtained model and the collectively high explanatory power of the variables, even if their individual explanatory ability is lower. Therefore, the main therapeutic goals in the prevention and treatment of ED in patients with diabetes should be: first, enhancing antioxidant defense activity, and second, reducing iNOS activity.

Conclusions

1. Hereditary factors significantly increase the risk of developing endothelial dysfunction in DM patients: a strong positive correlation was established between heredity and the levels of SOD (τ=0.94; $p<0.00001$), catalase (τ=0.90; $p<0.00001$) and MDA (τ=0.85; $p<0.00001$).

2. Age and duration of diabetes do not show a significant impact on the ED development, indicating a possible influence of genetic mechanisms in the pathogenesis of this disorder.

3. A family history of DM should be considered a significant predictor not only for the development of the disease but also for an increased risk of ED, which underscores the need for early screening and more aggressive prevention in this patient category.

4. The combined values of MDA, cNOS, iNOS, Arg, SOD, catalase have a high explanatory power for the development of ED in DM patients with diabetes, even if their individual explanatory power is lower.

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