Dynamics of Endothelial Desquamation in Patients with Diabetic Kidney Disease

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Abstract: The objective of our study - to determine the effect of glucose-lowering therapy on blood glucose indices, vessels’ structure and markers of functional conditions of kidneys in patients with diabetes mellitus.

Methods: 11 type-1 and 39 type-2 diabetic patients were studied with assessment of vascular endothelium state and renal function parameters for a period of 3 months. Endotheliocytemia level was estimated using Hladovec method.

Results and discussion. 3-month course of insulin glargine in combination with short-acting insulin in type-1 diabetic patients and the administration of oral hypoglycemic therapy in combination with insulin glargine in type 2 diabetic patients has been associated with clinically significant improvement in glycemic control. The number of circulating endothelial cells was established to be 738 ± 67.8 cells/ml, which is 29.1% lower after 3-months of treatment (p < 0.001). The decrease in the level of endothelial desquamation was observed in all groups of patients with diabetes, regardless of the initial functional state of the kidneys.

Conclusion: Improvement of glycemic control and complex therapy of existing complications of diabetes leads to a significant reduction in endothelial damage in patients with diabetes, and, therefore, contributes to the protection of blood vessels (p < 0.001). The analysis of the functional state of the kidneys for a period of 3 months indicates that it is possible to prevent or slow down the progression of diabetic kidney disease using the prescribed treatment scheme.

Key words: diabetes mellitus; endothelial dysfunction; diabetic kidney disease, circulating endothelial cells, vascular endothelium, vascular complications

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1. Introduction

This work is a fragment of the research and development work ‘Investigation of endothelial dysfunction in diabetes mellitus and the possibility and effectiveness of the use of a new method for treating patients with diabetes mellitus’. No of state registration 0118U001852.

The global prevalence of diabetes mellitus among the adult population reached a worldwide epidemic. A steady increase in the incidence of diabetes mellitus (DM) in all age and ethnic groups has been observed. About 5% of the world's population already suffers from this disease, and the number of patients, despite the active efforts of modern medicine, is increasing annually by 5-7% [1].

DM is accompanied by the lesion of almost all organs and systems. It should be noted that the main mechanism of the pathogenesis of all vascular diabetic complications is endothelial damage with endothelial dysfunction (ED). One of the most serious vascular complications of diabetes is diabetic kidney disease (DKD), which is one of the main causes of chronic kidney disease (CKD) with an outcome in chronic renal failure[2],[3].

DKD is an integral component of the cardiorenal continuum, which determines the relationship between the pathological processes of the cardiovascular system and the kidneys. The development of DKD is associated with structural and functional changes caused by metabolic and hemodynamic factors that can lead to irreversible life-threatening outcomes[3].

Different pathogenesis links are closely related due to hyperglycemia, so we cannot highlight any specific mechanism for the development of ED. Under condition of hyperglycemia, the following pathological processes are developed: the polyol pathway, glucose metabolism, glycocalyxation of proteins with increased formation of glycation end products, hyperactivation of protein kinase C and renin-angiotensin-aldosterone...
system, the oxidative stress, mitochondrial and endoplasmic reticulum stress, low-grade inflammation, desquamation of endotheliocytes, etc. [4]-[6].

The increased endothelium desquamation intensity is regarded as the first stage of endothelial dysfunction [3],[7]. Therefore, the determination of the plasma level of circulating endothelial cells (CECs), a recognized morphological marker of structural damage of the endothelium, makes it possible to reveal the presence and degree of endothelial damage both in the early stages of ED and in the development and progression of vascular complications, including DKD[8].

**Aim of the study**

To determine the possibility of reduction of endothelial damage due to normalization of glycemia in patients with type 1 (T1DM) and type 2 diabetes (T2DM) with normal and depressed glomerular filtration rate (GFR) within 3 months of treatment.

**Data analysis.** Statistical analysis and the visualization of the obtained data carried out using Plotus software [9]. Data were presented as mean (M) and the standard error of mean (SEM) in comparison of group means. Levene’s test was used to assess the homogeneity of variances; the analysis of normal distribution was performed using the D’Agostino-Pearson test. The relationships and the correlations between the studied parameters were determined using Pearson’s rank correlation coefficient (r). The difference between the groups was determined using one-way analysis of variances (ANOVA). The difference was considered statistically significant at p < 0.05.

**II. Methodology**

50 patients with T1DM and T2DM, whom the eligibility criteria and agreed to enroll into the study, were involved. Patients were hospitalized in the Odessa Regional Clinical Medical Center from April to December 2018.

The study included an assessment of vascular endothelium state and renal function parameters in examined patients. Patients with T1DM received combination of insulin glargine, a long-acting human insulin analogue, and short-acting insulin as hypoglycemic therapy. For type 2 Patients with type 2 DM received metformin, glimepiride ± voglibose in combination with insulin glargine. The correction of a concomitant pathology and progression rates of micro- and macrovascular diabetic complications were carried out with the agreed decision of the physician and the patient and in accordance with the instructions of the drugs’ application.

Diabetes has been diagnosed within at least a year since the initial diagnosis. DKD was exposed on the basis of either of the following criteria: abnormalities of kidney structure or function presented for > 3 months [9]:

- Urine sediment abnormalities, albuminuria or albumin-to-creatinine ratio (ACR) ≥ 30 mg/g
- Electrolyte and other abnormalities due to tubular disorders
- Abnormalities detected by histology
- Structural abnormalities detected by imaging
- History of kidney transplantation
- Decreased GFR < 60 ml/min/1.73 m²

The National Kidney Foundation guidelines recommend using the CKD-EPI Creatinine Equation (2009) to estimate GFR as the best overall index of kidney function [9]. Endothelial desquamation intensity was determined using Hladovec J. method with the estimation of both the total number of CECs and the determination of CECs at different stages of decay in blood plasma [11]. Depending on the morphological changes of CECs, 3 stages of decay are conditionally distinguished: the initial (S1), the stage of the expressed changes (S2), and the final stage (S3) [10].

**III. Results and Discussion**

Among diabetic patients, 25 men (50%) and 25 women (50%) were included into the study. The average age of the patients was 56.32 ± 12.4 years. Duration of DM was an average of 10.3 ± 7.3 years. T1DM patients (n = 11) suffered from diabetes on average of 6.81 ± 5.11 years; T2DM patients (n = 39) – 11.28 ± 7.6 years, respectively.

The initial level of glycosylated hemoglobin (HbA₁c) was 8.42 ± 1.2%. The initial level of albuminuria was an average of 0.06 ± 0.1 g/l, serum creatinine concentration was 91.46 ± 4.3 μmol/l, with a high value group of T2DM patients. Concentration of creatinine exceeded the upper limit of the reference value by 28% (n = 14). The mean value of GFR was 73.17 ± 18.7 ml/min/1.73 m².

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The concentration of CECs varied from 1200 to 4600 cells/ml. The mean level of CECs in patients with DM was 2530 and the indicators after hospital treatment and on the final visit, have shown that prescribed treatment of patients with T1DM and T2DM has been associated with clinically significant improvements in glycemic control.

The highest values of the linear correlation coefficients were observed in the following pairs: urea and creatinine concentrations \( (r = 0.6; \ p < 0.001) \), GFR and urea concentration \( (r = -0.57; \ p < 0.001) \), GFR and serum creatinine concentration \( (r = -0.76; \ p < 0.001) \).

After the hospital treatment, the average fasting plasma glucose decreased from 13.72 ± 0.5 to 6.53 ± 0.3 mmol/l \( (p < 0.001) \). The percentage of patients with plasma glucose up to ≤ 7.0 mmol/l increased to 84%.

The level of fructosamine in the period of hospital treatment decreased by 0.75 mmol/l, with a statistically significant difference of 16.7% \( (p < 0.001) \).

At the end of the study, the mean level of HbA1c decreased from 8.42 ± 0.2% to 7.36 ± 0.3%, with a difference of -1.05 ± 0.2% \( (p < 0.001) \).

3-month course of insulin arginine in combination with short-acting insulin in patients with T1DM and head administration of oral hypoglycemic therapy in combination with insulin arginine in patients with T2DM has been associated with clinically significant improvements in glycemic control.

### Table 1: The functional state of the kidneys during the study period

<table>
<thead>
<tr>
<th>Indicators</th>
<th>main group</th>
<th>T1DM ( n = 11 )</th>
<th>T2DM ( n = 39 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial serum creatinine concentration, ( \mu \text{mol} /\text{l} )</td>
<td>91.46 ± 4.3</td>
<td>81.81 ± 4.9</td>
<td>94.17 ± 3.8</td>
</tr>
<tr>
<td>Initial GFR, ml/min/1.73 m²</td>
<td>73.17 ± 7.1</td>
<td>89.85 ± 6.6*</td>
<td>68.46 ± 2.7*</td>
</tr>
<tr>
<td>Initial level of albumin in urine, g/l</td>
<td>0.06 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Serum creatinine concentration after hospital treatment, ( \mu \text{mol} /\text{l} )</td>
<td>86.16 ± 4.4</td>
<td>77.72 ± 5.1</td>
<td>88.53 ± 2.7</td>
</tr>
<tr>
<td>GFR after hospital treatment, ml/min/1.73 m²</td>
<td>77.52 ± 8.1</td>
<td>95.02 ± 5.3*</td>
<td>72.58 ± 2.4*</td>
</tr>
<tr>
<td>Final serum creatinine concentration, ( \mu \text{mol} /\text{l} )</td>
<td>85.78 ± 4.5</td>
<td>81.09 ± 3</td>
<td>87.1 ± 3.7</td>
</tr>
<tr>
<td>Final GFR, ml/min/1.73 m²</td>
<td>78.58 ± 7.3</td>
<td>89.89 ± 3.9</td>
<td>75.39 ± 3.4</td>
</tr>
<tr>
<td>Final level of albumin in urine, g/l</td>
<td>0.04 ± 0.007</td>
<td>0.02 ± 0.009</td>
<td>0.04 ± 0.009</td>
</tr>
<tr>
<td>Final creatinine concentration in urine, ( \mu \text{mol} /\text{l} )</td>
<td>7.53 ± 0.7</td>
<td>8.27 ± 2</td>
<td>7.32 ± 0.8</td>
</tr>
<tr>
<td>ACR, mg/mmol</td>
<td>10.9 ± 2.69</td>
<td>9.54 ± 7.7</td>
<td>11.28 ± 2.7</td>
</tr>
</tbody>
</table>

Notes: *\( p \leq 0.05 \) difference between indicators of T1DM and T2DM groups; data are presented as M ± SEM.

### Figure 1: Comparative characteristics of GFR levels among patients with diabetes during the study period.

Notes: data are presented as medians and interquartile ranges.

The analysis of baseline indicators of kidneys’ functional state before treatment, and the indicators after hospital treatment and on the final visit, has shown that prescribed treatment of patients with T1DM and T2DM could prevent slow down the progression of kidney disease in diabetic patients.

The expression of endothelial glycemiawas revealed in the blood of all patients.

The concentration of CECs varied from 1200 to 4600 cells/ml. The mean level of CECs in patients with DM was 2530.
The number of CECs was established to be 738 ± 67.8 cells/ml, which is 29.1% lower after 3 months of treatment (p < 0.001). The reason for the decreased endothelium desquamation intensity is probably due to normalization of glycemic control and complex therapy of existing complications of DM.

The level of endothelial desquamation was analyzed according to the degree of endothelial desquamation (statistically significant decrease in the level of endothelial desquamation was observed in all groups of patients with diabetes, regardless of the functional state of the kidneys, which is shown in Figure 2).

Table 2: Levels of endothelial desquamation during the study in dependence of GFR

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Stages of study</th>
<th>GFR ≥ 90 ml/min·1.73 m²</th>
<th>GFR 60-89 ml/min·1.73 m²</th>
<th>GFR 59-45 ml/min·1.73 m²</th>
<th>GFR 35-44 ml/min·1.73 m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>General level of CECs, cells/ml</td>
<td>Baseline stage</td>
<td>2828.57 ± 2000</td>
<td>2366.66 ± 346.9</td>
<td>2711.11 ± 394.49</td>
<td>2825 ± 523.8</td>
</tr>
<tr>
<td></td>
<td>Stage after hospital treatment</td>
<td>1900 ± 450</td>
<td>1733.33 ± 199.8</td>
<td>1844.44 ± 119</td>
<td>1925 ± 321.4</td>
</tr>
<tr>
<td></td>
<td>Output stage</td>
<td>1771.42 ± 180</td>
<td>1635.48 ± 76.2</td>
<td>1844.44 ± 133.5</td>
<td>1866.66 ± 150</td>
</tr>
<tr>
<td>S1, cells/ml</td>
<td>Baseline stage</td>
<td>400 ± 100</td>
<td>310 ± 89.7</td>
<td>322.22 ± 125.8</td>
<td>350 ± 88.1</td>
</tr>
<tr>
<td></td>
<td>Stage after hospital treatment</td>
<td>214.28 ± 50</td>
<td>243.3 ± 47.1</td>
<td>266.66 ± 100</td>
<td>200 ± 100</td>
</tr>
<tr>
<td></td>
<td>Output stage</td>
<td>214.28 ± 44.7</td>
<td>183.87 ± 21.1</td>
<td>155.55 ± 36.8</td>
<td>300 ± 8150</td>
</tr>
<tr>
<td>S2, cells/ml</td>
<td>Baseline stage</td>
<td>2014.28 ± 200</td>
<td>1700 ± 246.3</td>
<td>1866.66 ± 150</td>
<td>1873 ± 392.9</td>
</tr>
<tr>
<td></td>
<td>Stage after hospital treatment</td>
<td>1442.85 ± 500</td>
<td>1220 ± 158.1</td>
<td>1322.22 ± 85.3</td>
<td>1200 ± 145.2</td>
</tr>
<tr>
<td></td>
<td>Output stage</td>
<td>1285.71 ± 168.5</td>
<td>1135.48 ± 72.7</td>
<td>1300 ± 110</td>
<td>1133.3 ± 250</td>
</tr>
<tr>
<td>S3, cells/ml</td>
<td>Baseline stage</td>
<td>428.57 ± 100</td>
<td>356.66 ± 40.8</td>
<td>522.22 ± 179.6</td>
<td>600 ± 66.6</td>
</tr>
<tr>
<td></td>
<td>Stage after hospital treatment</td>
<td>242.8 ± 100</td>
<td>273.33 ± 37.2</td>
<td>255.55 ± 75</td>
<td>525 ± 176.3</td>
</tr>
<tr>
<td></td>
<td>Output stage</td>
<td>271.42 ± 50.9</td>
<td>270.42 ± 26.2</td>
<td>388.88 ± 57.14</td>
<td>433.33 ± 100</td>
</tr>
</tbody>
</table>

Notes: data are presented as M ± SEM

The decrease in the level of endothelial desquamation was observed in all groups of patients with diabetes, regardless of the functional state of the kidneys, which is shown in Figure 2.

Figure 2: Dynamics of the level of endothelial desquamation in diabetic patients with different initial GFR.

Laboratory data characterizing endothelial dysfunction did not differ significantly between groups of patients with different GFR. Despite different initial nephropathic changes in these patients, statistically significant decrease in the level of endothelial desquamation was observed in all groups of patients with diabetes (p < 0.001).

In conclusion, the key role in the vascular wall lesions pathogenesis in patients with DM belongs to hyperglycemia and to the hypoinsulinemia. The correction of this disturbance leads to a decrease in the structural and functional state of the kidneys and decrease of the degree of endothelial desquamation.

IV. Conclusion
1. Improvement of glycemic control and complex therapy of existing complications of diabetes lead to a significant reduction in endothelial damage in patients with DM, and, therefore, contribute to the protection of blood vessels (p < 0.001).

2. A persistent decrease in endothelial desquamation due to treatment is observed in all diabetic patients, regardless of the initial GFR.

3. The analysis of the functional state of the kidneys for a period of 3 months indicates that it is possible to prevent or slow down the progression of diabetic kidney disease using the prescribed treatment scheme.

4. Determination of the degree of endothelial damage is an informative research tool, allowing by analyzing both the general level of CECs and the level of endothelial cells at different stages of decay, to predict the formation and progression of vascular complications of diabetes, timely detect and correct prescribed therapy and prevent further development of cardiovascular complications.

5. Based on our findings, it is proposed to determine the concentration of CECs as an independent marker of ED in patients with T1DM and T2DM mindynamics.

**PROSPECTS FOR FURTHER RESEARCH**

The study results indicate the necessity of effective measures for endothelial dysfunction correction development in patients with DM

**Acknowledgements**

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**Abbreviations:** ACR: albumin-to-creatinine ratio, CECs: circulating endothelial cells, DKD: diabetic kidney disease, DM: diabetes mellitus, ED: endothelial dysfunction, GFR: glomerular filtration rate, M: mean, S1: the initial stage of endothelial cell decay, S2: the stage of increased expression of endothelial cell decay S3: final stage of endothelial cell decay, SD: standard deviations, SEM: standard error of mean, T1DM: Type-1 diabetes mellitus, T2DM: Type-2 diabetes mellitus

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[9]. https://www.plotus.xyz/Datavisualisationandstatisticalanalysssoftware


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