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CIRCULATING ENDOTHELIAL CELLS: A NOVEL MARKER OF EFFECTIVENESS OF TREATMENT OF PATIENTS WITH DIABETES MELLITUS

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Abstract

Diabetes mellitus (DM) causes damage to small and large vessels and causes angiopathy. The issue of reversibility of vascular diabetic complications, which are based on endothelial damage with endothelial dysfunction (ED), is still a controversial topic. Against this backdrop, we sought to determine the endothelial dysfunction in patients with type 2 DM (T2DM) and to create a model to estimate the possibility of reduction of endothelial damage due to using our treatment strategy in patients with T2DM. Normalization of carbohydrate metabolism and correction of diabetic macro- and microangiopathies were carried out within 10 days of 39 T2DM patients. Preliminary therapy of patients with T2DM was changed to insulin glargine biosimilar and a combination of oral hypoglycemic drugs: metformin, glimepiride and voglibose. This therapy leads to a normalization of postprandial and fasting glycemia, fructosamine after 10 days and 3 months of treatment with an HbA_{1c} assessment ($p < 0.001$). Endothelial desquamation intensity was determined using Hladovec J. method with the estimation of both the total number of circulating endothelial cells (CECs). The mean level of baseline level of CECs ($CECs_b$) in patients was 2469.23 ± 687.92 cells/ml. There was a statistically significant decrease in the level of endothelial desquamation both after 10-days inpatient treatment ($CECs_{tr}$) and 3 months later ($p \leq 0.001$). The prognostic model was developed that can predict the level of $CECs_{tr}$ based on our treatment strategy for T2DM depending on the initial level of endotheliocythemia. The determination coefficient of this model was 60%.

All human studies were conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study". Informed consent was obtained from all participants.

Key words: *type 2 diabetes mellitus; endothelial dysfunction; circulating endothelial cells, correction of diabetic vascular complications, treatment of diabetes mellitus*

Introduction

Diabetes mellitus (DM) is an important medical and social and economic problems of our time, leading to early disability and premature mortality due to the development of vascular complications, and the cost of treating increases as complications develop [1].

DM has a negative effect on small and large vessels and causes angiopathy. The development of micro- and macroangiopathy in diabetes begins at the level of the endothelium, which is the first to be affected by impaired glucose metabolism [2].

The effect of diabetes on the vascular bed, based on molecular mechanisms, combines oxidative stress, damage to the endothelial wall, chronic low-level inflammation, mitochondrial and endoplasmic reticulum dysfunction, insulin resistance, hypoinsulinemia, dyslipidemia, arterial hypertension, etc. [3, 4]. Based on the number of circulating endothelial cells (CECs), it is possible to assess the activity of endothelial desquamation, and, consequently, the degree of endothelial damage, which is the morphological basis of endothelial dysfunction (ED). It also becomes possible to assess the response of the endothelium to fluctuations in the level of glycemia, especially in the dynamics of the treatment process [5, 6].

Correction of ED may become a new therapeutic target for treatment and prevention of diabetic vascular ccomplications in DM.

There is a wide variety of therapeutic strategies available for the management of type 2 diabetes mellitus (T2DM), so choosing the right treatment is important. A prerequisite for the successful therapy of DM is the effect on all known links of the pathogenesis of this disease [7].

As diabetes progresses, insulin becomes a necessary option of pathogenetic therapy in a significant part of patients with T2DM, with the discovery of which at the beginning of the 20th century the fate of a patient with diabetes changes. There is growing evidence that early initiation of insulin therapy is more effective in achieving adequate glycemic control. Many international guidelines contain recommendations on the need to prescribe insulin when, against the background of lifestyle modification and treatment with a combination of 2-3 oral antidiabetic drugs, the target glycemic levels are not achieved and not maintained [7, 8, 13].

Aim of the study is to determine the endothelial dysfunction in patients with T2DM and to create a model to estimate the possibility of reduction of endothelial damage due to using our treatment strategy in patients with T2DM.

Data analysis: Statistical analysis of the obtained data carried out using R 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 correlation coefficient (*r*). The difference between the groups was determined using one-way analysis of variances (ANOVA). The difference was considered statistically significant at $p < 0.05$.

Simple linear regression analysis was performed to create the prognostic model of CECs reduction in T2DM patients. The Dummy variable coding technique was used to encode quality variables. To assess the prognostic significance of the model, parameters such as coefficient of determination (R^2), statistical significance of regression coefficients, 95% confidence intervals and variance inflation factor were estimated.

Methods

Open, controlled study in clinical practice with a follow-up period of 12 weeks was conducted by the State Enterprise "Ukrainian Research Institute of Transport Medicine, Ministry of Health of Ukraine" on the basis of MNE 'Odessa Regional Clinical Medical Center' ORC in the period from April to December 2018.

39 patients with T2DM, who met the eligibility criteria and agreed to enroll into the study, were involved. Normalization of carbohydrate metabolism and correction of diabetic macro- and microangiopathies were carried out within 10 days of inpatient treatment. Except for the initial visit, the 10-day inpatient treatment and the visit after 3 months of treatment, systematic monitoring of patients was not performed.

On the background of previous insulin therapy or oral hypoglycemic therapy in combination with

basal insulin, examined patients had poor glycemic control or the target level of HbA_{1c} was not achieved due to the progressive nature of diabetes.

Preliminary therapy of patients with T2DM was changed to insulin glargine biosimilar and a combination of oral hypoglycemic drugs by agreed decision of the doctor and the patient and in accordance with the instructions for use of drugs.

A combination of metformin (biguanides), glimepiride (sulfonylureas), and voglibose (α-glucosidase inhibitor) was prescribed to patients with T2DM as oral hypoglycemic drugs. Insulin glargine was administered at a dose equivalent to 50% of the total daily dose of medium-acting insulin, which was part of previously used therapy. in insulin naive patients, dose titration began with 4 IU, with further adjustments once every 3 days. This titration algorithm is known as treat-to-target.

Selection of combined therapy was conducted considering comorbidity and the rate of progression of micro- and macrovascular complications of T2DM. On the table 1 infusion therapy is presented, which was prescribed to patients during inpatient treatment.

Endothelial desquamation intensity was determined using Hladovec J. method with the estimation of both the total number of CECs [10, 12].

Results

Among diabetic patients, 20 men (51,3%) and 19 women (48,7%) were included into the study. The average age of the patients was 60.69 ± 9,38 years. Duration of T2DM was on an average of 11.28 ± 7.61 years. Body mass index (BMI) was 31,71 ± 5.55 kg/m^2. Most patients were overweight and normal BMI was observed in 2 patients only.

Under the conditions of reaching the target levels of fasting glucose and postprandial glycemia, the daily dose of insulin glargine was 19.33 ± 3 IU. This was lower by 11.38 ± 7 IU than the previous dose of human insulin with a decrease of 36.2% (p <0.001).

In the tabl. 2 baseline and hospital levels of fasting plasma glucose, postprandial glycemia, and fructosamine were demonstrated. The study showed that at the end of inpatient treatment, the average fasting plasma glucose decreased from 13.85 ± 0.5 to 6.57 ± 0.2 mmol/l (p < 0.001). The average level of postprandial glycemia decreased from 14.73 ± 1 to 6.79 ± 0.3 mmol/l (p < 0.001). The average level of fructosamine during inpatient treatment decreased by 0.81 mmol/l, with a statistically significant difference of 18.04 % (p < 0.001).

The expressiveness of endotheliocytemia was revealed in the blood of diabetes patients at the different stages of study period. The concentration of baseline level of CECs ($CECs_b$) varied from 1200 to 4600 cells/ml. The mean level of $CECs_b$ in patients with T2DM was 2469.23 ± 687.92 cells/ml.

There was a statistically significant decrease in the level of endothelial desquamation both after 10-days inpatient treatment ($CECs_{tr}$) and 3 months later, as shown in Fig. 1 ($p \leq 0.001$). No significant differences were found between the output level of CECs and the level of endotheliocytemia after inpatient treatment.

Simple linear regression analysis was performed to assess the prognosis of the level of endothelial desquamation in patients with T2DM. Levels of $CECs_b$ and $CECs_{tr}$ were used to construct a simple linear regression model in patients with T2DM.

The Scatter chart (Fig. 2) shows the relationship between the $CECs_b$ and the number of $CECs_{tr}$ in T2DM patients, as well as 95% confidence intervals, r = 0.77, p < 0.001.

R^2 was 0.6, which indicates that the $CECs_b$ can explain the level of CECs after inpatient treatment by 60%.

Tab. 3 shows the main results of ANOVA: degrees of freedom, sum of squares, mean squares, Fisher's F-test and significance levels. Since ANOVA shows statistical significance, we can conclude that this model describes the data well.

Tabl. 4 shows the linear regression coefficients, standard errors of the regression coefficients, Student's t-tests, the statistical significance of the regression coefficients, as well as 95% confidence intervals.

The equation of the model we constructed has the following form:

$$\hat{Y}_i = b_o + b_1 X_i$$

Based on table 4, our equation takes the next form:

$$\hat{Y}_i = 453.8839935 + 0.538925485 * CECs_b$$

Using this model, we can predict how the level of endotheliocythemia will change as a result of the proposed inpatient treatment strategy. Suppose

than an initial level of endotheliocythemia in diabetes patient was 3000 cells/ml. Therefore, we expect that the number of $CECs_{tr}$ in blood plasma will decrease to 2070.660449 cells/ml.

Consequently, as a result of application of the linear regression method, the prognostic model was developed that can predict the level of $CECs_{tr}$ based on our treatment strategy for T2DM depending on the initial level of endotheliocythemia. The determination coefficient of this model was 60%.

Conclusions

1. Therapy of patients with T2DM using insulin glargine biosimilar and a combination of oral hypoglycemic drugs composed of metformin, glimepiride and voglibose leads to a normalization of postprandial and fasting glycemia, fructosamine after 10 days and 3 months of treatment with an HbA_{1c} assessment ($p < 0.001$).
2. Improvement of glycemic control and complex therapy of existing complications of diabetes accompanied by a significant decrease in endothelial damage in patients with T2DM, although it exceeds the indicators in healthy individuals ($p < 0.001$).

The prognostic model that can predict the level of $CECs_{tr}$ based on our treatment strategy for T2DM depending on the initial level of endotheliocythemia using linear regression method was developed.

Acknowledgments

The authors declare that there are no conflicts of interest.

Relationship of the publication with the planned research works. The work presented is a fragment of the research project "Diabetic nephropathy pathogenesis and substantiation of chronic kidney disease diagnostics, № state registration 0120U102210.

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Table 1. Type of infusion therapy depending on the complications that have developed on the background of diabetes

Infusion therapy	Dosing Regimen	T2DM
L-ornithine-L-aspartate	5 g IV* infusion № 5-10	n = 38 (97,4 %)
Alpha-lipoepic acid	600 mg IV infusion № 10	n = 39 (100 %)
Meldonium	1 g IV injection № 10	n = 39 (100 %)
Emoxipin	40 mg intramuscularly № 10	n = 8 (20,5 %)
B vitamins**	Vit. B1 100 mg, vit. B6 100 mg, vit. B12 1 mg, intramuscularly № 5	n = 35 (89,7 %)
Enoxaparin sodium	2000-4000 IU anti-Xa activity subcutaneously № 5	n = 33 (84,6 %)
Citicoline	4 g IV infusion № 5	n = 18 (46,1 %)
Nicergoline	4 mg intramuscularly № 5	n = 7 (17,9 %)
Phosphatidylcholine	500 mg IV injection № 10	n = 38 (97,4 %)

Note: *IV – intravenous; **B vitamins: thiamine hydrochloride (Vitamin B_1), pyridoxine hydrochloride (Vitamin B_6), cyanocobalamin (Vitamin B_{12}).

Table 2. Dynamics of fasting plasma glucose levels, postprandial glycemia, fructosamine in patients with diabetes

Indicators	**Initial level**	**After inpatient treatment**
Fasting plasma glucose, mmol/l	13,85 ± 0,5	6,57 ± 0,2
Postprandial glycemia, mmol/l	14,73 ± 1	6,79 ± 0,3
fructosamine, mmol/l	4,49 ± 0,2	3,68 ± 0,2

Note: Data are presented as M ± SEM

Figure 1. Dynamics of CECs levels during the study period

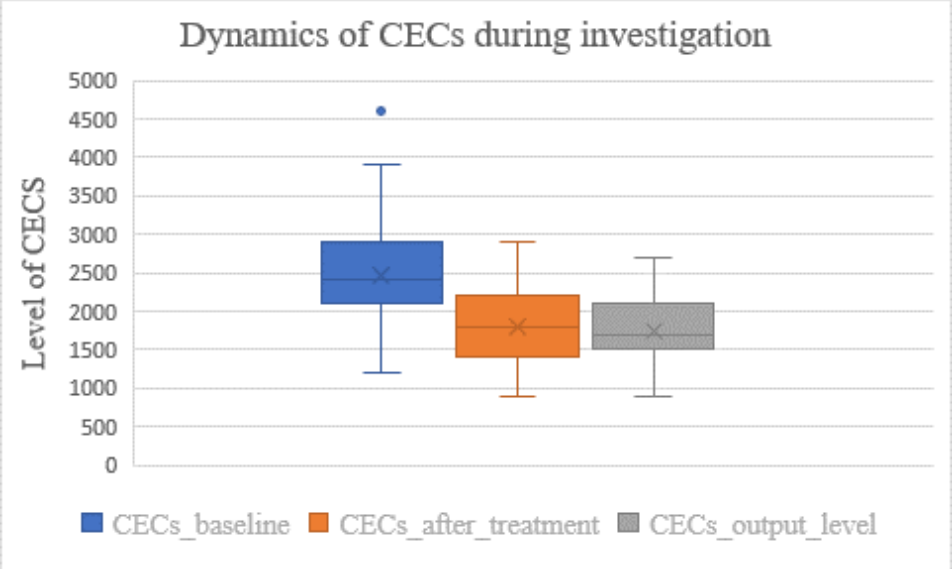

Figure 2. Scatter chart that displays the correlation between the $CECs_b$ and $CECs_{tr}$ in patients with T2DM

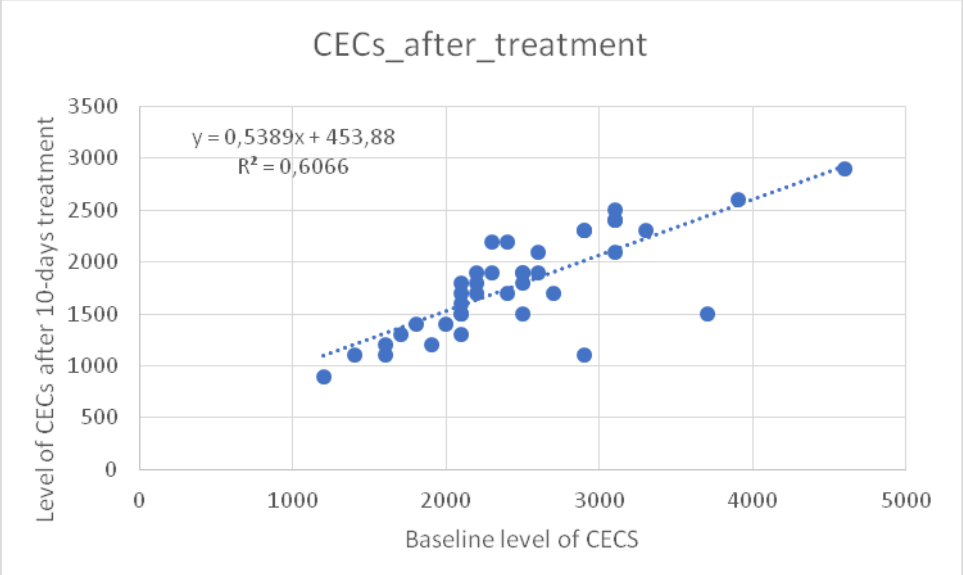

Table 3. Model quality assessment using ANOVA

	df	*SS*	*MS*	*F*	*p-value*	*sig*
Regression	1	5223017	5223017	57.04421	5.27E-09	yes
Residual	37	3387752	91560.87			
Total	38	8610769				

Note: *df* – degrees of freedom, *SS* – sum of squares, *MS* – Mean Square, *F* – Fisher's F-test, *p-value* – statistical significance

Table 4. Linear Model Parameters

	coeff	*std err*	*t stat*	*p-value*	*lower*	*upper*
Constant	453.884	182.7324	2.483873	0.01765	83.63307	824.1349
$CECs_b$	0.538925	0.071355	7.552761	5.27E-09	0.394347	0.683504

Note: *coeff* – linear regression coefficient, *std err* – standard error of the regression coefficient, *t stat* – Student's t-tests, p-value – statistical significance of the regression coefficients, lower and upper – lower and upper boundaries of the 95% confidence interval.