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THE NUMBER OF CIRCULATING ENDOTHELIOCYTES IN THE BLOOD PLASMA OF THE PATIENTS WITH DIABETES MELLITUS INCREASES

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Abstract

The research task included the detection of endothelial dysfunction (ED) in patients with diabetes mellitus (DM). The study involved 60 people, including 53 patients with type 1 and type 2 DM, with a severe course (state of decompensation), in which ED was assessed for the number of circulating endothelial cells (CECs). CECs are desquamated mature cells that have detached from the intimal monolayer in response to endothelial injury.
In patients with diabetes, the amount of CECs increases in 3-5 times and is in the range from 1800 to 11,200 $^{cells}/_{ml}$. The average amount of CECs in patients with diabetes was 3358.5 ± 366.3 $^{cells}/_{ml}$. Endothelium is involved in the pathological process at DM. This is evidenced by a significant increase in CECs in the blood plasma. ED appears at different stages of DM and correlates with the duration and severity of diabetes.

Keywords: diabetes mellitus, endothelial dysfunction, circulating endothelial cells, glycosylated hemoglobin, vascular endothelium.

Introduction

The prevalence of diabetes mellitus (DM) has acquired the character of a noninfectious pandemic of the XXIst century. The first WHO Global report on diabetes demonstrates that the number of adults living with diabetes has almost quadrupled since 1980 to 422 million adults [1].

The disease inevitably progresses and leads to complications, among which the main place is occupied by micro- and macroangiopathies [2]. According to the current hypothesis of vascular complications, the primary damage begins at the level of the vascular endothelium and, thus, endothelial dysfunction (ED) is considered an early predictor of damage to the vascular system [3].

During the last 2 decades, it has been shown that the vascular endothelium is an active paracrine, endocrine and autocrine organ that is indispensable for the regulation of vascular tone and the maintenance of vascular homeostasis [4].

Essentially, the maintenance of vascular homeostasis by endothelium is accomplished through the release of vasoprotective factors (eg, NO/endothelium-derived relaxing factor, prostacyclin, bradykinin, and endothelium-derived hyperpolarizing factor) and harmful substances (eg, endothelin, Reactive oxygen species (ROS), endothelium-derived COX-dependent vasoconstricting factor, and angiotensin II). Damage to endothelium will disrupt the balance between vasoprotective factors and harmful substances, initiating a number of events/processes that promote or exacerbate atherosclerosis via increased endothelial permeabilization, platelet aggregation, leukocyte adhesion, and cytokine production [3].

ED is involved in lesion formation by the promotion of both the early and late mechanisms of atherosclerosis including up-regulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, increased cell permeability, enhanced low-density lipoprotein oxidation, platelet activation, cytokine elaboration, and vascular smooth muscle cell proliferation and migration [5]. The presence of endothelial damage in diabetes can be established even before macroscopically significant damage to the vessel [6].

About 30 years ago circulating endothelial cells (CECs) were first observed in peripheral blood. Identification of CECs began in the 1970s [7]. A key step in their investigation occurred in 1992 when monoclonal antibodies to surface CECs antigens were discovered [8], leading to novel markers of CECs [9]. Since then CECs have been established as a sensitive and specific indicator of vascular injury and damage [10]. In this study we were exploring the role of ED in pathogenesis of DM by measuring the concentration of CECs in blood plasma of DM patients.

Materials and Methods

The pathophysiology of ED at DM is a complex interplay between several interconnected hyperglycaemia-induced pathological occurrences which damage endothelial cells. The impact of diabetes in vascular beds focusing on molecular mechanisms involving ED, the oxidative stress, mitochondrial dysfunction, insulin resistance, fatty acids, dyslipidemia [11].

Prolonged exposure to hyperglycemia is now recognized as a major factor in the pathogenesis of diabetic complications, including atherosclerosis, mechanistically involving enhanced enzymatic and nonenzymatic protein/lipid glycosylation, protein kinase C activation, inflammation, and ROS production [11, 12].

60 people, including 53 type 1 and type 2 DM, with a severe course (state of decompensation) participated in the present study. We have used the method of estimating ED by the number of circulating desquamation endothelial cells (CECs) [13].

Among the patients with diabetes there were 32 men (60,4%) and 21 women (39,6%), aged 19-80 y.o. The average age of the patients with diabetes was 55,6±3,9. The control group consisted of people without endocrine pathology, aged from 32 to 80, the average age was 62,57±15.

It was found that the level of CECs in the control group ranged from 200 to 800 $^{cells}/_{ml}$, with the average value of 500±200. There is a correlation between the obtained results. The level of CECs increases with age, which confirms the hypothesis of a causal relationship of aging with functional and structural damage to the vascular endothelium [14, 15].

Results

In patients with diabetes, the amount of CECs increases in 3-5 times and is in the range from 1800 to 11 200 $^{cells}/_{ml}$. The average amount of CECs in patients with diabetes is 3358±366,4, which significantly exceeds the number of cells in healthy individuals ($p<0,05$).

Depending on the age, patients with diabetes were divided into 3 groups: the 1st group included patients under the age of 50 y.o., in the second group - from 50 to 60, in 3 - older than 60 y.o. The average level of glycosylated hemoglobin (HbA1c) was 8,3±0,3%. Fig. 1 shows the dependence of the amount of CECs on the level of Hb1Ac.

In the group of patients with HbA1c less than 7%, the average amount of CECs was 2855,5±510 $^{cells}/_{ml}$. At HbA1c levels between 7% and 8%, the average level of the CECs was 3175±745,5 $^{cells}/_{ml}$, at HbA1c levels between 8% and 9% - 3414,3±289 $^{cells}/_{ml}$, at HbA1c greater than 9%, the amount of CECs was the highest and in average was 3863,6±755 $^{cells}/_{ml}$.

In Fig. 2, the level of endotheliocythemia is shown to increase with the age of the patients. In patients younger than 50 years, the average level of CECs was 2852,9±420 $^{cells}/_{ml}$, at the age of 50 - 60 years – 3170,5±429 $^{cells}/_{ml}$, in patients older than 60 years the highest level of endotheliocythemia was found and it was 3978,9±472,7 $^{cells}/_{ml}$.

Depending on the duration of the DM, all patients were divided into 3 groups. As it can be seen from Table 1, the 1st group consisted of patients with duration of DM up to 10 years, the second group - from 10 to 15 years, in the third group - more than 15 years.

In the 1st group the number of CECs was 3100±713,8 $^{cells}/_{ml}$, in the 2nd – 3166±526 $^{cells}/_{ml}$, in the 3rd - 3700±660,8 $^{cells}/_{ml}$. Proceeding from this, a direct correlation between the duration of diabetes and the amount of CECs was found, which increased according to the duration of diabetes.

Accordingly, involvement of the vascular endothelium in the pathogenesis of DM is confirmed by a significant increase in the level of endotheliocythemia. The use of this method allows to detect ED before clinically considerable vascular impairment and reflects the severity of the course and duration of DM. Consequently, the number of desquamated endotheliocytes in blood plasma increases in patients with diabetes, depending on the age of the patients, and also on the severity and duration of the disease.

Conclusions

In patients with Diabetes mellitus the level of circulating endothelial cells in blood plasma was shown to increase.

It was found that the number of CECs is due to the severity and duration of DM and is associated with age of patients.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Table 1: The number of CECs depending on the duration of the DM

Index	Group of examined patients			
	Control group	Patients with DM of various duration		
		up to 10 years	*from 10 to 15 years*	*more than 15 years*
The level of endotheliocythemia in 1 ml blood plasma	500±200	3100±713,8	3166±526	3859±660,8

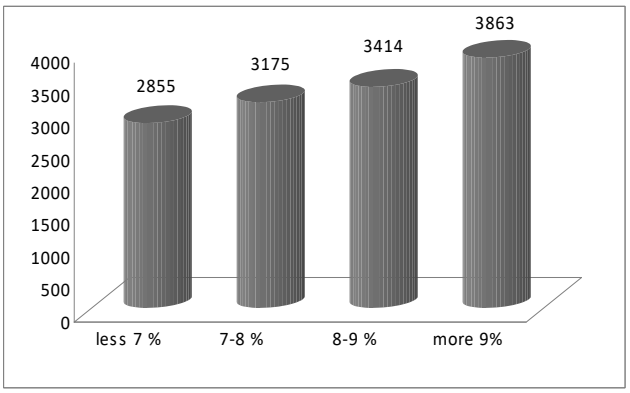

Figure 1: The number of CECs depending on the level of HbA1c

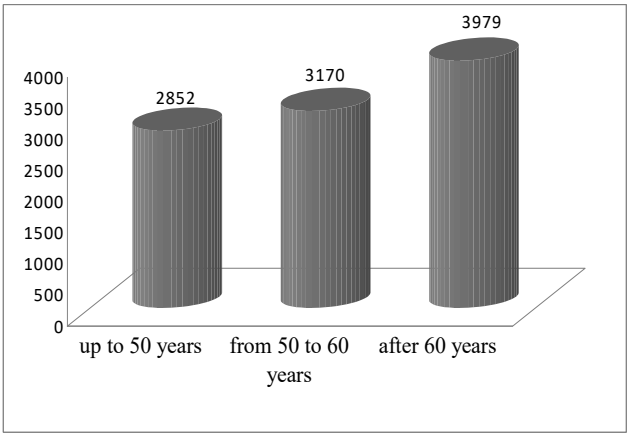

Figure 2: The number of CECs depending on the age of patients with DM