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THE DESQUAMATION OF THE ENDOTHELIUM DUE TO NORMALIZATION OF GLYCEMIA DECREASES IN PATIENTS WITH DIABETES MELLITUS

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Abstract

The objective of the research was to assess the role of hyperglycemia in the development of endothelial dysfunction (ED) in patients with diabetes mellitus (DM). The study involved 18 patients with DM type 1 (6) and type 2 (12) in the state of decompensation, in which ED was assessed by the number of desquamated endothelial cells.

The significant decrease of the circulating endothelial cells (by 22,5±3,4%, p<0,01; r=+0,76) as a result of treatment of diabetic patients clarify, that hyperglycemia is apparently one of the main factors that damage the endothelium and reduction of hyperglycemia leads to a decrease in desquamation of the endothelium. The results of this study show that it is possible that endothelial dysfunction is a reversible process regardless of the stage.

Keywords: *diabetes mellitus, endothelial dysfunction, circulating endothelial cells, glycemia, vascular endothelium*

Introduction

The pathophysiology of endothelial dysfunction (ED) at diabetes mellitus (DM) is a complex interplay between several interconnected hyperglycemia – induced pathological occurrences which damage endothelial cells (EC). The impact of diabetes in vascular beds focusing on molecular mechanisms involving ED, the oxidative stress, mitochondrial dysfunction, insulin resistance, dyslipidemia. Prolonged exposure to hyperglycemia is now recognized as a major factor in the pathogenesis of diabetic complications, including atherosclerosis, associated first of all with enhanced enzymatic and nonenzymatic protein and lipid glycosylation, inflammation, and reactive oxygen species (ROS) production. The hyperglycemic state causes EC damage by promoting advanced glycation end products, by activating protein kinase C, and through polyol pathway activation [1-3].

However, the biochemical and cellular links between elevated blood glucose levels and endothelial dysfunction remain incompletely understood. This work will focus on the conception of ED at DM.

Functional restructuring of the endothelium under the influence of pathological factors passes several sequential stages [4]:

Stage I – type 1endothelial activation. Type I EC activation occurs immediately following stimulation. The vascular endothelium can be activated by cytokines, adipocytokines, endotoxins, thrombin, complement components, neutrophils and EC autoantibodies [5], inflammatory markers 9 (C-reactive, protein, CD40 ligand, and interleukin-18) [6] and mechanical stimuli during balloon angiography and stenting [7].

In this stage, the synthetic activity of the EC increases, and previously synthesized proteins such as endothelial adhesion and antithrombotic molecules (P-selectin, thrombin, heparin, von Willebrand factor (vWF), antithrombin III and thrombomodulin) are liberated on the surface of the activated endothelium, thereby does not require *de novo* protein synthesis or gene transcription. In addition, a number of protective genes (NF-κB inhibitor-α – specific inhibitor of nuclear factor-κB and anti-apoptotic genes A20 and BcL-2, which downregulate the expression of the transcription factor NF-κB) are constitutively expressed within the EC.

Stage II – type 2endothelial activation is a delayed response that promotes de novo the secretion of pro-inflammatory cytokines, elicitation of T-cell–mediated immune responses, the elaboration of chemokines, *de novo* induction of procoagulant molecules. At this stage, the balanced secretion of factors regulating vascular tone, hemostasis system, intercellular interaction processes, the natural endothelial barrier function is disturbed, the permeability for various plasma components is increased as a result of de novosynthesis of such factors as E-selectin, vWF, chemokines (IL-8), platelet-activating factor (PAF), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), tissue factor, monocyte chemoattractant protein-1 (MCP-1), etc. [8].

As a result of endothelial activation, occurring changes lead to a morphological rearrangement of EC. The ultrastructural alterations of the type II EC activation are characterized by protrusion of ECs into the lumen of blood vessels, hypertrophy of ECs (plump cuboidal appearance), increased biosynthetic organelles (Golgi complex, rough endoplasmic reticulum, and ribosomes), and increased permeability (pinocytotic vesicles), but without loss of endothelial integrity. Monocytes and lymphocytes appear in the vicinity of the activated ECs, and harboring Fc receptors (FcR), may be exposed to the basement membranes, extravasated through the interaction of selectins and integrins, and further activated by binding of FcR to deposited immune complex [9].

However, the chronic EC activation process (over months), if uncontrolled, can progress to EC apoptosis [10]. The interaction of activated ECs with activated neutrophils, mast cells, and macrophages may amplify vascular inflammatory responses to produce a cascade of other reactions. The close association of EC activation with that of other cells suggests that some biomarkers of EC activation are not exclusively produced by activated ECs but are also produced by other activated cells [8].

Stage III – apoptosis of EC as a result of uncontrolled endothelial activation, characterized

by fragmentation of the endothelium, denaturation of the vessel wall and the endothelial separation from the underlying basement membrane, thereby resulting in circulation endothelial cells (CECs) as well as the release of endothelial cell microparticles (EMPs) and endothelial cell caleolin-1 (Cav-1) from the plasmalemmal membranes.

Stage IV – endothelial necrosis as a result of chronic exhaustion, mitochondria undergo a progressive edema that leads to death of EC. Moreover, endothelial necrosis indicates that the cellular injury is severe and persistent. Necrosis of the EC is accompanied by a violation of the processes of the endothelial regeneration [8].

Circulating ECs (CECs) are ECs detached from the basement membrane at sites of vascular injury [11]. This phenotypic evidence of an endothelial derivation supports the use of CECs as a sentinel for EC activation and injury and/or vascular dysfunction [12].

Whether it's the beginning of ED or ED is reversible has long been a subject of researchers' attention. In the literature there are contradictory data on the positive effect of therapy on ED, the conflicting effects of antihypertensive drugs on ED from selected clinical trials.

In support of the concept of reversibility or irreversibility of ED, different methods of endothelial evaluation have been used. In the number of researches it was shown, that clinically effective antihypertensive therapy does not restore impaired endothelium (does not restore impaired endothelium-dependent vascular relaxation) [13, 14]. Another clinical trials show the possibility of restoration of ED by antihypertensive drugs [15].

In addition, it was found out that many drugs (cerivastatin, atorvastatin, simvastatin, pentoxifylline, vitamin E, and vitamin C) have no effect on ED [16]. In contrast to the above-mentioned opinions, it has been suggested that that ED might be reversible with certain interventional strategies [17].

Methods

Patients with well established history of DM, who were treated at the ME «Odessa Regional Clinical Medical Center» (Odessa, Ukraine), were recruited for the study. The duration of inpatient treatment was 11 days.

The study was conducted during the period from December 2017 to February 2018, consent were obtained. Exclusion criteria: patients with poorly controlled DM (HbA_{1C} > 11%), or with chronic heart disease, e.g., dilated cardiomyopathy, recent acute coronary syndrome or coronary revascularization, as well as patients with cerebrovascular stroke. No patient had a history of peripheral vascular disease, coagulopathy or any disease predisposing them to vasculitis or Raynaud's phenomenon.

All participants involved in this study were subjected to: detailed history taking with stress on duration of DM, hypoglycemic drug intake either oral or insulin, full clinical examination including; body mass index (BMI) determination, cardiovascular, respiratory, abdominal and neurological systems examination. Clinical and laboratory evaluations were done to assess the glycemic control, including fructosamine and glycosylated hemoglobin (HbA_{1C}). Lipid profile was evaluated by total cholesterol, triglycerides, low density lipoprotein, high density lipoprotein. CECs were determined by the method of J.Hladovek [18, 19].

Treatment was carried out with the following criteria: antihypertensive therapy to target levels**.** In order to normalize glycemia, a hypoglycemic therapy was prescribed in accordance with the recommendations of the European Association for the Study of Diabetes (EASD)/American Diabetes Association (ADA). To patients with DM type 1was prescribed all-analog basal-bolus regimen, patients with DM type 2 were receiving oral antidiabetic drugs (Metformin, Glimepiridum ± Voglibose) ± human basal insulin with the use of low-carbohydrate diets and lifestyle modification. Infusion therapy was prescribed in view of existing complications of diabetes, such as micro- and macroangiopathies and polyneuropathies.

18 patients (13 male and 5 female) with DM type 1 (6) and type 2 (12) were examined (Fig.1). Patients' age ranged between 16 and 75 years, with the mean age of 49,2±4,3 years. 61,1% of patients did not smoke, 38,8% smoked.

The duration of diabetes was 10,3±1,47 years. BMI was 29,1±1,5 kg/m^2; 66,6% of patients were overweight (BMI 25 to <30 kg/m^2), 75% of them were obese (BMI ≥ 30 kg/m^2).

In all patients with DM dyslipidemia was observed. Total cholesterol level was in average of 6,7±0,5 mmol/L, mean level of low-density lipoproteins was 4,3±0,3 mmol/L, mean level of high-density lipoproteins was 1,5±0,08 mmol/L, mean level of triglycerides was 2,0±0,5 mmol/L.

The mean systolic blood pressure level was 130±5,2 mm Hg, with 38,8% of patients having a systolic blood pressure level of >140 mmHg. The mean diastolic blood pressure level was 76,1±3,4 mmHg, with 16,6% of patients having a diastolic blood pressure level of >90 mm Hg.

Statistical analysis was performed using STATISTICA for Excel program. In describing and comparing quantitative variables, the arithmetic mean and their standard errors and the standard deviation were calculated. Relations between variables were assessed by means of Pearson's correlation coefficient.

Results

All of the patients with diabetes were in decompensated stage. The baseline level of HbA_{1C} was 8,1±0,2%, level of fasting plasma glucose (FPG) was 12,25±0,71 mmol/L, level of 2-h postprandial plasma glucose (2h-PPPG) was 13,56±0,77 mmol/L. The baseline level of fructosamine in the patients was in the range 3,5–8,4 mmol/L with an average value of 5,2±0,35 mmol/L. Correlations between HbA_{1C} and fructosamine were not found (r=+0,1). Prescribed treatment led to normalization in glucose level: level of FPG was 5,8±0,24 mmol/L, level of 2h-PPPG was 6,47±0,27mmol/L. To control the effectiveness of hypoglycemic therapy, after 10 days the level of fructosamine was repeatedly determined, amounted to 3,7±0,2 mmol/L. For 10 days, fructosamine decreased by 26,1±3,8%. The tendency to normalization of glycemia was significant (r=0,52, p=0,001) (Table 1).

The level of the CECs was studied on two occasions: before the medical treatment when the patients were at the stage of decompensation and after the medical treatment that reduce glycemia and blood pressure to normal limits in each patient. The level of the CECs in patients before the treatment was ranged from 1300 to 3900 cells/ml, an average of 2395,4±165,4 cells/ml. The significant decrease in the CECs as a result of treatment were admitted (Fig. 2). After treatment, the level of the CECs was in the range of 3100 to 1000 cells/ml, with an average value of 1816,6±128,6 cells/ml (this decline was by 22,5±3,4% (p<0,01; r=+0,76)).

These results clarify that hyperglycemia is apparently one of the main factors that damage the endothelium, and reduction of hyperglycemia leads to a decrease in desquamation of the endothelium.

Discussion

It is believed that the 1st and the 2nd stages of ED are reversible, and the 3d and 4th stages are irreversible and the targeted treatment cannot stop irreversible ED and cannot restore normal function of EC [4]. At the same time, our studies demonstrate that complex treatment of patients with diabetes both 1 and 2 types reduces endothelial damage, which, according to our data, directly correlates with a decrease in glycemia. Nevertheless, the role of other possible mechanisms of endothelial damage, especially arterial hypertension, can not be completely excluded (Fig. 3). The factors listed on the scheme, such as oxidative stress, increase of polyol pathway and hexosamine pathway, increase of protein glycosylation and of the number of advanced glycation end products (AGEs), increase of protein kinase C (PKC) activation, increased blood pressure as the result of renin-andiotensin-aldosterone system (RAAS) hyperactivation, dyslipidemia, hypervolemia, mitochondrial dysfunction, increasing synthesis of fibronectin, dicturbances in EC regeneration and gene expression can ultimately increase the desquamation of the endothelium. However, hyperglycemia and glycosylation of proteins have the primary importance. The positive influence of complex diabetes therapy on endothe

Conclusions

1. The results of this study show that it is possible that endothelial dysfunction is a reversible process regardless of the stage.

2. The process of desquamation of the endothelium depends on hyperglycemia in many respects and decreases with the normalization of glycemia.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Figure 1. Risk factors of CVD

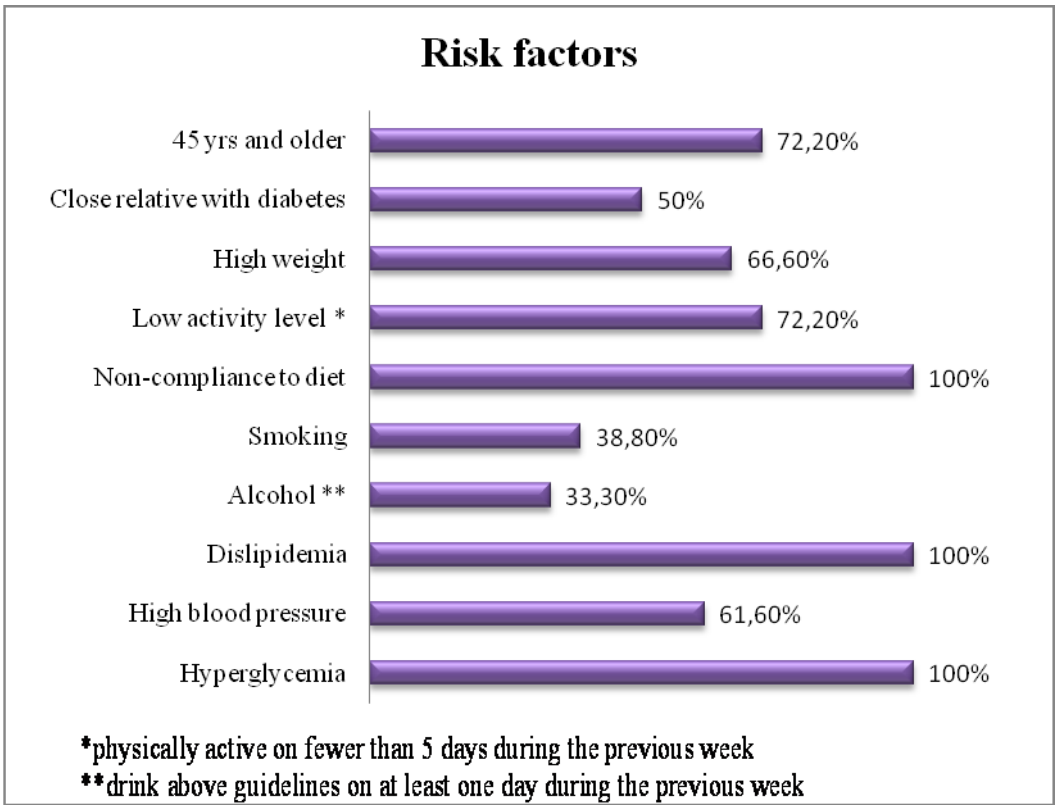

Figure 2. Influence of treatment on changes in the desquamations of the endothelium

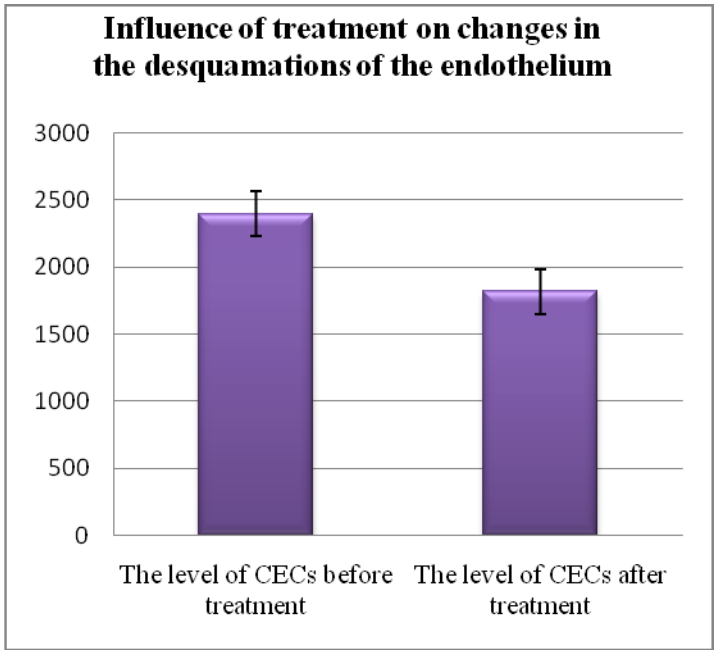

Table 1. Indices of blood pressure and glucose level before and after 10 day treatment

Indices	Before treatment, n=18	After treatment, n=18
Fasting plasma glucose, mmol/L	12,2 ± 0,71	5,8 ± 0,24
2-h postprandial plasma glucose, mmol/L	13,5 ± 0,77	6,4 ± 0,27
Fructosamine, mmol/L	5,2 ± 0,35	3,7 ±0,22
Systolic blood pressure, mm Hg	130,5 ± 5,2	120 ± 1,61
Diastolic blood pressure, mm Hg	76,1 ± 3,46	74,7 ± 1,28

Figure 3. The role of hyperglycemia in the desquamation of the endothelium