ROLE OF IMMUNE RESPONSE CYTOKINE REGULATION DISORDERS IN THE DEVELOPMENT OF ENDOTHELIAL DYSFUNCTION IN DIABETES MELLITUS TYPE 2

N.V.Pashkovska, O.A.Olenovych

Department of Clinical Immunology, Allergology and Endocrinology, Bukovinian State Medical University, Chernivtsy, Ukraine

Summary

With the aim to define the role of disorders of the immune response cytokine regulation in the development of endothelial dysfunction in patients with DM type 2, the degree of insulin resistance, functional state of the endothelium, blood serum level of pro-inflammatory cytokines were investigated. The processes of vascular endothelium desquamation were found to be enhanced in diabetes mellitus type 2, followed by a reliable increase of endotheliocytemia, reduction of endothelium-dependent vasodilation, elevation of blood serum level of endothelin-1 and decrease of stable nitrogen monoxide metabolites in the blood. The degree of endothelial dysfunction manifestation in diabetes mellitus type 2 depends on the indices of insulin resistance (waist circumference, insulinemia, HOMA-IR index) and is connected with the enhanced expression of pro-inflammatory cytokines (tumor necrosis factor-α, interleukin-1-β, interleukin-6, interleukin-8).

Key words: diabetes mellitus, pro-inflammatory cytokines, endothelial dysfunction, functional state of endothelium

Address for correspondence: olcka76@mail.ru.

Introduction

Nowadays diabetes mellitus (DM) is featured as «non-infectious epidemic» worldwide, involving more than a quarter of billion of planet population. In Ukraine for the last 15 years the incidence of this endocrine-metabolic disease has been increased twice. The number of patients with DM crossed the million baseline in 2006, whereas, according to the epidemiologic studies findings, the real level is at least three-fold higher [1].

Furthermore, the number of DM complications dramatically grows, and diabetic angiopathies take an important place among them. The majority of investigators consider the mechanism of vessel endothelium alteration in DM to be extremely complicated and multifactorial, with a leading role, given to hyperglycemia, processes of non-enzymatic protein glycation, dislipidemia etc. [2].

At the same time, the contribution of pro-inflammatory cytokines expression disorders into the formation of morphofunctional endothelium disorders in patients with DM type 2 remains disputable, and existing literary data – contradictory, that is indicative of topicality of further investigation in this field.

Objective of the research was to determine the role of disturbances of the immune response cytokine regulation in the development of endothelial dysfunction in patients with DM type 2.

Materials and Methods

43 patients with DM type 2 (46,5% men and 53,5% women, mean age – 55,3±8,59 years, average duration of DM – 8,2±4,29 years) and 20 healthy individuals, who served as control group, were examined. The investigation was performed on the base of Chernivtsi Regional Endocrinological Center.

The level of glycemia was tested by glucose oxidase method with the usage of kits by НПП "Филисит діагностика" (Ukraine), glycated hemoglobin (HbA_{1c}) – on the automatic analyzer of glycated hemoglobin D10 «Bio-Rad Laboratories Inc.» (France). Immunoreactive insulin (IRI) was measured by enzyme immunoassay using reagents by DRG International Inc. (USA). The degree of insulin resistance was established according to body mass index (BMI) and Caro, HOMA-IR indices [3]. Functional condition of endothelium was studied by measurement of endothelium–dependent vasodilatation of the brachial artery (EDVD) [4], blood level of stable nitrogen monoxide (NO) metabolites according to Green L.C. [5], and endothelin-1 (ET-1) («Bender MedSystems», Austria) by enzyme immunoassay. The amount of blood circulating endotheliocytes was detected according to J. Hladovec technique in N.N.Petryshev and co-authors modification [6].

The content of cytokines in the blood serum, tumor necrosis factor-α (TNF-α), interleukin-1-β (IL-1-β), interleukin-6 (IL-6), interleukin-8 (IL-8), soluble intercellular adhesion molecule type 1 (ICAM-1) in particular, were determined by enzyme immunoassay using commercial test-systems by «Diaclone» (France) and «Bender MedSystems» (Austria).

Statistical analysis of the results was performed by means of special software, using parametric (Student's t-criterion) and non-parametric (Mann-Whitney rank criterion, paired Wilcoxon T-test) methods of variance statistics, compared with adequate control groups. Mathematic analysis of the obtained data was conducted by means of Primer of Biostatistics software. Version 4.03 (Glantz S., США), SPSS 16.0, Excel.

Results and Discussion

The first step of our investigation was to determine the degree of insulin resistance in patients with DM type 2.

Anthropometric findings are presented in *Table 1*. According to the obtained findings, BMI in basic group was by 18,2% higher that of control, waist circumference – by 6,3% in men and by 17,6% in women respectively.

Normal body weight was observed only in 18,6% of patients, whereas in 41,9% of them overweight was diagnosed, in 25,6% – obesity of I degree, in 9,3% – II degree, in 4,6% – III degree correspondingly. Abdominal obesity was established nearly in third (32,6%) of patients. These

results are indicative of a considerable risk to develop insulin resistance and metabolic disturbances in the examined patients.

Table 1. **Characteristics of anthropometric and lipid metabolism indices in patients with diabetes mellitus type 2**

Indices	Control group, n=20 (M±m)	Diabetes mellitus type 2, n=43 (M±m)
Body mass index, kg/м^2	24,04±0,298	28,57±0,508 *
Waist circumference, sm: *men*	90,21+0,673	95,83±0,614*
women	74,06+0,565	88,00±1,042*
Insulin, mkU/l	9,66±0,427	30,39±0,906*
HbA$_{1c}$, %	5,56±0,098	8,86±0,225*
HOMA-IR index	2,15±0,110	13,42±0,846*
Caro index (glucose/insulin)	0,54±0,028	0,33±0,022*

Note: * – reliable changes in comparison with control;
– number of cases.

According to the laboratory findings, a reliable increase of HbA$_{1c}$ by 57,9% was observed. The level of serum insulin and HOMA-IR index reliably exceeded control indices by 3,2 and 6,2 times respectively, Caro index was lower by 24,5%.

Insulin resistance is known to be followed by enhance of pro-inflammatory cytokines expression, that, in its turn, leads to the development and progression of changes in various organs and systems, the cardio-vascular one in particular.

As the results of investigation showed, DM type 2 is accompanied by the development of a significant endothelial dysfunction (Table 2).

Table 2. **Characteristics of indices of endothelium functional state and cytokine regulation in patients with diabetes mellitus type 2**

Indices	Groups, number of cases, reliability of changes	
	Control group n=20 (M±m)	Diabetes mellitus type 2 n=43 (M±m)
EDVD, %	10,86±0,406	4,93±0,098*
Endothelin-1, pmol/l	6,34±0,319	14,10±0,446*
NO metabolites, mkmol/l	16,80±0,906	7,20±0,259*
Endotheliocytes, 10^4/l	3,20±0,138	17,24±0,546*
TNF-α, pg/ml	24,19±1,46	106,50±3,940*
IL-1-β, pg/ml	30,14±2,281	115,40±6,477*
IL-6, pg/ml	20,06±1,382	76,97±3,841*
IL- 8, pg/ml	34,74±2,370	112,40±3,618*

Note: * – reliable changes in comparison with control;
– number of cases.

The index of EDVD, in particular, decreased by 54,6% with high probability degree (P<0,001). On this background statistically evident increase of endothelial vasoconstrictive peptide ET-1 was found to be 2,2-fold elevated. Substantial degree of endothelium alteration in DM type 2 was also evidenced by a significant decrease of stable nitrogen monoxide metabolites in the blood (by 57%).

The index of desquamated endotheliocytes blood level underwent the most noticeable changes, reliably raising by 5,3 times.

The correlation analysis data showed (Table 3), that functional state of the endothelium was connected with the degree of insulin resistance, particularly, with BMI, waist circumference, insulinemia, HOMA-IR index and the level of HbA_{1c} as well.

Table 3. **Linear-regression analysis of correlations between the indices of endothelial function and carbohydrate metabolism in patients with diabetes mellitus type 2**

Indices	EDVD	Endothelin-1	NO metabolites	Endotheliocytes
HbA_{1c}	R=-0,517*	R=0,298	R=-0,464*	R=0,585*
Insulin	R=-0,539*	R=-0,589*	R=-0,421*	R=0,652*
HOMA-IR index	R=-0,545*	R=-0,488*	R=-0,309	R=0,622*
BMI	R=-0,475	R=0,569*	R=-0,401*	R=0,487
Waist circumference	R=-0,574*	R=0,597*	R=-0,503*	R=0,576*

Note: * – reliability of correlation;
– number of pairs in regression analysis.

Cytokines are known to play an important role in the formation of diabetic angiopathies, providing cellular reactions, contributed not only into vascular endothelium alteration, but also into the formation, progression and further «destiny» of atherosclerotic plaque. Thereby, proliferation pool develops in the area of inflammation and, in fact, entire construction of the latest is formed [7]. That's why the next step of our study was to determine peculiarities of cytokine regulation indices in DM type 2.

Among all pro-inflammatory agents, implicated to the damage of the vascular wall, TNF-α is of a special attention. Its elevation in the blood is found even at the early stages of pathological process in response to endothelium alteration by modified lipoproteins. This pro-inflammatory cytokine enhances the expression of adhesion molecules, activates macrophages, neutrophils, promotes the secretion of prostaglandins, provides chemotaxis effect upon various cells, stipulates synthesis of acute-phase inflammation proteins [8]. Reliable 4,4-fold increase of blood serum TNF-α level was revealed in the examined patients (Table 4).

Activated endothelium is known to secrete not only TNF-α, but also such substances, as IL-1 and IL-2, able to activate intima macrophages. Activated macrophages, in their turn, produce a set of cytokines into the subendothelial space, resulting in further endothelium activation, particularly IL-1, IL-6, IL-8 etc. [9]. According to the results of our research, in DM type 2 the expression of IL-1 and IL-6 was 3,8 times elevated.

IL-8, being a chemokine, is known to have considerable pro-inflammatory properties, causes the expression of adhesion molecules and accelerates the adherence of neutrophils to the endothelial cells and matrix proteins, that indicates it's principal role in the mediation of inflammatory response in atherosclerosis [10]. In patients with DM type 2 a reliable 3,2-fold increase of IL-8 serum content was registered.

Thus, in DM type 2 the disturbance of cytokine regulation is characterized by the increased expression of cytokines TNF-α, IL-1, IL-6, IL-8.

Current correlation analysis (Table 4) showed, that expression of studied cytikines in diabetic patients is closely connected with the indices of carbohydrate metabolism, insulin resistance, and

functional state of endothelium as well, that proves the implication of cytokine regulation disturbances to mechanisms of the development of vascular disorders in DM.

Table 4. **Linear-regression analysis of correlations between the indices of cytokine regulation, endothelial function and carbohydrate metabolism in patients with diabetes mellitus type 2**

Показники	TNF-α	IL-1-β	IL-6	IL-8
HbA$_{1c}$	R=0,672*	R=0,584*	R=0,488*	R=0,561*
Insulin	R=0,619*	R=0,575*	R=0,421*	R=0,455*
HOMA-IR index	R=0,645*	R=0,500*	R=0,465*	R=0,298*
BMI	R=0,505*	R=0,345	R=0,408*	R=0,475*
Waist circumference	R=0,687*	R=0,511*	R=0,623*	R=0,398
EDVD	R=-0,571*	R=-0,524*	R=-0,254	R=-0,441*
Endothelin-1	R=0,487*	R=0,380*	R=0,462*	R=0,416*
NO metabolites	R=-0,620*	R=-0,431*	R=-0,389	R=-0,516*
Endotheliocytes	R=0,741*	R=0,585*	R=0,498*	R=0,632*

Note: * – reliability of correlation;
          – number of pairs in regression analysis.

Thus, in patients with DM type 2 the processes of vascular endothelium desquamation are enhanced with simultaneous reduction of endothelium-dependent vasodilatation, elevation of vasoconstrictive factors expression and decrease of stable nitrogen monoxide metabolites blood content, connected with the augmented expression of cytokines TNF-α, IL-1, IL-6, IL-8.

Literary review and the analysis of own findings evidence, that, despite the mechanism of endothelium damage in DM is complicated and multicomponent, the primary role in it belongs to hyperglycemia and glucose toxicity phenomenon, related to it. Non-enzymatic glycation of proteins and other substances, containing amino groups, glucose autooxidation with accelerated production of free radicals, direct glucose toxicity activate the cascade of reactions of the immune, coagulation, fibrinolytic and other systems. This is followed by acceleration of the expression of adhesion molecules by leucocytes, trombocytes and endothelial cells [11].

Substantial degree of endothelial dysfunction in patients with DM type 2, on our opinion, is stipulated, besides glucose toxicity phenomenon, by the presence of other pathogenetical mechanisms as well, related to hyperinsulinemia and insulin resistance.

Conclusions

1. In patients with diabetes mellitus type 2 the processes of vascular endothelium desquamation enhances, followed by a reliable increase of endotheliocytemia, reduction of endothelium-dependent vasodilation, elevation of blood serum level of endothelin-1 and decrease of stable nitrogen monoxide metabolites in the blood with high probability degree.
2. In case of diabetes mellius type 2 the expression of pro-inflammatory cytokines − tumor necrosis factor-α, interleukin-1-β, interleukin-6, interleukin-8 is significantly increased.
3. The degree of endothelial dysfunction manifestation in diabetes mellitus type 2 depends on the indices of insulin resistance (waist circumference, insulinemia, HOMA-IR index) and is connected with the expression of pro-inflammatory cytokines (tumor necrosis factor-α, interleukin-1-β, interleukin-6, interleukin-8).

References

1. Маньковский БН. Лечение артериальной гипертензии у больных сахарным діабетом. Ліки України 2009; 5(131):9-13.
2. Башкирова ЮВ, Рудницкая ТА, Хапаев РС и др. Эндотелиальная дисфункция и микроциркуляторые нарушения у больных сахарным диабетом типа II. Бюллетень РАМН 2008; 6(134):182-186.
3. Gao L, Matthews FE, Sargeant LA. An investigation of the population impact of variation in HbA1c levels in older people in England and Wales: from a population based multi-centre longitudinal study. MRC CFAS BMC Public Health 2008; 11(8):54.
4. Sorensen KE, Celermajer DS, Spiegelhaltcr DJ et al. Noninvasive measurement of human endothelium-dependent arterial responses: accuracy and reproducibility. Brit Heart J 1995; 74:247-253.
5. Green LC, Wagner DA, Glogolwski G et al. Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Analyt Biochem 1982; 126:131-138.
6. Петрищев НН, Беркович ОА, Власов ТД и др. Диагностическая ценность определения десквамированных эндотелиальных клеток крови. Клин лаб диагностика 2001; 1:50–52.
7. Glaros T, Larsen M, Li L. Macrophages and fibroblasts during inflammation, tissue damage and organ injury. Front Biosci 2009; 14:3988-3993.
8. Zhang H, Park Y, Wu J et al. Role of TNF-alpha in vascular dysfunction. Clin Sci 2009; 116(3):219-230.
9. Osborn O, Gram H, Zorrilla EP et al. Insights into the roles of the inflammatory mediators IL-1, IL-18 and PGE2 in obesity and insulin resistance. Swiss Med Wkly 2008; 138(45-46):665-673.
10. Fisman EZ, Adler Y, Tenenbaum A. Biomarkers in cardiovascular diabetology: interleukins and matrixins. Adv Cardiol 2008; 45:44-64.
11. Wittchen ES. Endothelial signaling in paracellular and transcellular leukocyte transmigration. Front Biosci 2009; 1(14):2522-2545.