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Quantitative changes in serum IL-8, TNF-α and TGF-β_1 levels depending on compensation stage in type 2 diabetic patients

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Abstract
Aim: To evaluate serum levels of cytokines, IL-8, TNF-α and TGF-β_1, as well as HbA1c, total protein, albumin, urea, creatinine, IgG, IgA, IgM according to compensation stages in type 2 diabetic patients. **Methods:** All patients (n = 76), depending on the level of glycemia and disease duration were divided into 3 groups: in the stage of compensation (n=28), subcompensation (n=20) and decompensation (n=28). Cytokines were measured by ELISA method. Control group included 20 healthy subjects. **Results:** Cytokine values increased in all three stages of the disease, especially in the stage of decompensation in comparison with control subjects. **Conclusions:** IL-8, TNF-α and TGF-β_1 may participate in the development and progression of diabetic complications. Further study of these immune dates may open new perspective opportunities in prevention and treatment of late complications of diabetes.

Keywords: *Type 2 diabetes mellitus, cytokines, IL-8, TNF-α, TGF-β_1*

Introduction

Despite more than 100 million patients affected worldwide and a dramatic socioeconomic burden due to vascular complications, the etiology of type 2 diabetes mellitus (DM) is not yet completely understood.[1]

Although it is well established that insulin resistance and impaired insulin secretion are central to the pathogenesis of type 2 DM, it is unclear how these abnormalities arise and how they are related to the many different clinical and biochemical features common in type 2 DM. Activation of innate immunity provides a new model for the pathogenesis of type 2 DM and the metabolic syndrome, which may explain some or all of these features, and points to research directions that might result in new therapeutic approaches for managing and predicting type 2 diabetes and its complications.[2]

Studies in recent years have shown that inflammation, and more specifically inflammatory cytokines, are determinant in the development of microvascular diabetic complications, including neuropathy, retinopathy, and nephropathy.[3]

Cytokines provide important signals in the pathophysiology of a range of diseases, including diabetes mellitus. Chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of diabetes and its microvascular complications.[4-8]

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The aim of this study was to evaluate serum levels of cytokines-IL-8, TNF-α and TGF-β_1 according to compensation stages in type 2 diabetic patients.

Patients and methods

About 76 type 2 diabetic patients who visited the Biochemical Laboratory of Azerbaijan Medical University were enrolled for the present study. All subjects gave consent to the study. Type 2 DM was diagnosed based on the criteria of European Bureau of International Diabetes Federation and European Bureau of WHO accepted in 1998.[9] In accordance with disease duration and compensation level patients were divided into three groups: under one year- stage of compensation (n = 28), from six to ten years-stage of subcompensation (n = 20), over ten years-stage of decompensation (n =28). Results of the patients were compared with the results of the 20 age-matched and sex-matched healthy control subjects.

Glucose level was determined by enzymatic glucose-oxidase method using reactive set "Human" (Germany).[10] HbA1c was measured using the thiobarbituric acid colorimetric reaction. The absorbance was measured at 443 nm. The color intensity of the complex is proportional to the concentration of the hemoglobin-glucose adduct present in the sample.[11]

Quantity of total protein was defined by the biuret method using kits "Human" (Germany).[12]

Measurement of albumin level was based on that, albumin binds quantitatively with bromcresol green at pH 4.15 resulting in the formation of a green color which can be measured at 630nm/red filter "Human" (Germany).[12]

Creatinine concentration was quantified by Jaffe's method with reactive set of firm "Lachema" (Czechia).[13]

For determination of urea diacetyl monoxime method was carried out ("Lachema", Czechia).[13]

Value of immunoglobulines was measured through immunoturbidometric method using "Pars Azmun" test systems.

Serum level of cytokines including, IL-8, TNF-α and TGF-β_1 was determined by ELISA (enzyme-linked immunosorbent assay) used commercially available kits purchased from Bender Medsystems (Austria). Principles of the test include: A monoclonal anti-IL-8/NAP-1 coating antibody is adsorbed onto microwells. IL-8 present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated IL-8/NAP-1 antibody is added and binds to IL-8/NAP-1 captured by the first antibody. Following incubation unbound biotin conjugated anti-IL-8-NAP-1 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-IL-8/NAP-1. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of IL-8/NAP-1 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven IL-8/NAP-1 standard dilutions and IL-8/NAP-1 sample concentration determined. The test principles for TNF-α and TGF-β_1 are identical to that of IL-8.

Statistical analysis was performed using STATISTICA 6 program. Data are presented as mean ± standard error (SE). Mann-Whitney U-test was used to compare differences between control and patient groups. A p-value < 0.05 was considered as statistically significant.

Results

We studied general biochemical parameters and some cytokines in the blood serum of type 2 diabetic patients. Glucose (10.87 ± 0.42 mmol/l vs. 5.24 ± 0.06 mmol/l and 3.84 ± 0.13 mmol/l) and HbA1c (11.4 ± 0.32% vs. 6.1 ± 0.05 mmol/l and 5.5 ± 0.07 mmol/l) levels were very high in decompensation stage compared with compensation stage and control. Results are given in Table 1.

Table 2 shows levels of protein metabolism parameters. In the stage of compensation, measured data did not significantly differ compared to control. In the stages of subcompensation and decompensation the levels of creatinine (118.8 ± 3.89 µmol/l and 433.2 ± 24.7 µmol/l vs. 72.7 ± 1.66 µmol/l) and urea (8.98 ± 0.14 mmol/l and 14.43 ± 0.7 mmol/l vs. 5.82 ± 0.08 mmol/l) were significantly increased and quantity of total protein and albumin decreased.

Table1: Glucose and HbA1c levels in type 2 DM patients depending on compensation stage

Patient groups	Number	Glucose (mmol/l)	HbA1c (%)
Compensation	28	5.24 ±0.06*	6.1±0.05*
Subcompensation	20	6.97±0.11 *	6.8±0.05*
Decompensation	28	10.87±0.42*	11.4±0.32*
Control	20	3.84±0.13	5.5±0.07

* p< 0,001 compared with control

As shown in Table 3, increased serum levels of cytokines, IL-8, TNF-α and TGF-β_1 were observed in all groups compared with control. Obtained figures were especially high in the stage of decompensation (IL-8: 249.54 ± 38.99 pg/ml versus 13.27 ± 0.93 pg/ml; TNF-α: 6.19 ± 0.34 pg/ml versus 1.14 ± 0.19 pg/ml; TGF-β_1: 82.43 ± 3.42 versus 2.0 ± 0.23 ng/ml).

Also the level of immunoglobulins increased in the blood serum of patients. The most significant were IgA and IgG which increased in all groups of patients. The level of IgA increased to 230.54 ± 8.13 mg/dl, 320.550 ± 6.01 mg/dl and 536.986 ± 22.92 mg/dl in compensated, subcompensated and decompensated groups, respectively. In a similar way, IgG increased to 1266.73 ± 46.25 mg/dl, 2054.54 ± 68.09 mg/dl, 2704.193 ± 61.14 mg/dl) compensated, subcompensated and decompensated groups, respectively when compared to control subjects (Table 3).

Discussion

In a long and permanent derangement of carbohydrate metabolism, that is, in decompensation stage of diabetes mellitus and in the absence of adequate treatment, the level of HbA1c increases. At the same time, hemoglobin and other body proteins undergo unenzymatic glycosylation. These can cause receptor dysfunction, thickening of membranes and metabolic disorders, which are typical for progression of diabetes mellitus.[14]

Raised globulin levels have been reported in studies that described abnormalities in serum immunoglobulin concentrations in patients with diabetes. It has been hypothesized that elements of the innate immune system, such as cytokines or the acute phase reactants that they may stimulate, may contribute to the development of obesity and type 2 diabetes mellitus.[15]

Studies of the past few years have shown that, in the pathogenesis of type 2 DM leading role play various types of cytokines. Cytokines are a group of pharmacologically active, low molecular weight polypeptides that possess autocrine, paracrine, and juxtacrine effects.[16] They provide important signals in the pathophysiology of a range of diseases, including diabetes mellitus.

At the present time it is recognized that chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of diabetes mellitus.[17,18] Increasing evidence suggests that individuals who progress to diabetes mellitus display features of inflammation years before the disease onset.[19] The main cytokines involved in the pathogenesis of diabetes are IL-1, TNF-α, and IL-6.[20] In addition, studies in recent years have shown that inflammation, and more specifically inflammatory cytokines are determinant in the development of microvascular diabetic complications, including neuropathy, retinopathy, and nephropathy.[3,5]

Table 2: Biochemical parameters of type 2 DM patients depending on compensation stage

Groups	Total protein g/l	Albumin g/l	Creatinine mcmol/l	Urea mmol/l
Compensation	75±0.92	46.7±0.39	87.95±0.82*	5.3±0,21
Subcompensation	70.3±0.51	41.0±0.51*	118.8±3.89*	8.98±0.14*
Decompensation	51.3±0.6*	35.1±0.63*	433.2±24.75*	14.43±0.7*
Control	75.9±0.83	46.1±0.94	72.7±1.66	5.82±0.08

* p< 0.001

Table 3: Serum levels of cytokines and immunoglobulins in type 2 DM patients depending on compensation stage

Parameters	Control n=20	Compensation n=28	Subcompensation n=20	Decompensation N=28
IL-8 (pg/ml)	13.27±0.93	12.48±0.41	25.04±1.76*	249.54±38.99*
TNF-α (pg/ml)	1.14±0.19	1.08±0.08	2.01±0.08**	6.19±0.34*
TGF-β_1(ng/ml)	2.0±0.23	2.43±0.22	5.56±0.66*	82.43±3.42*
IgG (mg/dl)	1151.65±36.8	1266.73±46.25	2054.54±68.09*	2704.19±61.14*
IgM (mg/dl)	151.95±7.49	256.0±9.43*	253.51±8.47*	299.30±10.6*
IgA (mg/dl)	187.25±4.95	230.54±8.13*	320.55±6.01*	536.99±22.92*

* p< 0.001; ** p= 0.001783

TNF-α plays a critical role in the pathogenesis of vascular injury in diabetic patients.[21] Increased circulating levels of TNF-α have been reported in diabetic patients.[22] Hyperglycemia stimulates TNF-α secreted from monocytes and endothelial cells.[23] Moreover, TNF-α may cause vascular injury by affecting the balance between coagulation and fibrinolysis. For example, TNF-α stimulates the expression of tissue factor that is the initiator of blood coagulation activation and the secretion of plasminogen activator inhibitor-1 that inhibits fibrinolysis.[22] In our study increased level of TNF-α in the stage of decompensation may show presence of deep inflammation processes in this group of patients.

TNF-α is also an inductor of IL-8. IL-8 is an important cytokine in the inflammatory process. Its main sources are macrophages, endothelial and epidermal cells. Initially it was suggested that IL-8 inhibits polymorphonuclear neutrophils (PMN) adhesion and aggregation. Some authors noticed later, however, that IL-8 is a strong stimulator of PMN. It seems that the observed PMN activation in diabetic patients is an important pathogenic link in long-term complications of the disease. Endothelial derived IL-8 secreted into subendothelial matrix or bound to the surface of the endothelium promotes adherence and migration neutrophils. IL-8 is stimulated by high glucose concentrations in endothelial cells in vitro and has a chemotactic activity for PMN, lymphocyte T and smooth muscle cells[24-26] and a high expression of IL-8 in the atherosclerotic lesions was shown.[25] IL-8 could have an atherogenic role. Moreover, it was suggested that IL-8 participates in the pathogenesis of diabetic retinopathy because an increase in IL-8 concentrations in the vitreous of patients with diabetic retinopathy was observed.[26]

The result of our study indicate that the serum IL-8 concentration in type 2 diabetic patients is markedly increased in comparison with healthy subjects and that it varies depending on the metabolic control of diabetes. These results might support other authors opinion as described above on the important role of IL-8 in the development of late diabetic complications. But we must mention that the evaluation of this non-specific acute phase variable could be also affected by subclinical infections.

We conclude that serum levels of TGF-β_1 are elevated in type 2 diabetic patients and may be related to average blood glucose. They are associated with the occurrence of diabetic micro- and macrovascular complications. TGF-β_1 is a profibrogenic cytokine that promotes cell growth and regulates extracellular matrix production. Preliminary data also suggest that TGF-β_1 may contribute to the occurrence of diabetic complications. Metwally et al suggest that TGF-β_1 may play a key role in the development and progression of diabetic nephropathy.[27] Accordingly, it may also be directly implicated in the functional deterioration of kidney functions seen in patients with diabetic nephropathy. Therefore beside proper glycemic control, strategies aiming at antagonizing TGF-β_1 such as the use of specific antibodies or a specific inhibitor of TGF-β_1 may help to prevent the development or attenuate the progression of nephropathy in diabetic patients.[27,28] These data support the hypothesis that TGF-β_1 is an important mediator of diabetic renal hypertrophy and extracellular matrix expansion in diabetic nephropathy.

Our results have demonstrated increased serum IL-8, TNF-α and TGF-β_1 levels in type 2 diabetic patients with a significant increase in the stage of decompensation. These data suggest that these cytokines may participate in the development and progression of diabetic complications.

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