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Markers of inflammation and microvascular complications in type 1 diabetes

Research Article

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Abstract: Aims. Long-term hyperglycemia, characteristic for type 1 diabetes, causes enhanced oxidative stress, chronic inflammation and endothelial dysfunction which are the key events in the development of vascular complications. On the other hand, some data shows that existence of chronic degenerative complications may cause increased inflammatory marker levels in diabetic patients, mainly as a repercussion of malfunctioned endothelial repair process. Our study aims to determinate a degree of inflammation in type 1 diabetes patients and to investigate its relation to development of the chronic microvascular complications. Methods: General information, anthropometric, glucose metabolism, lipid and lipoprotein parameters, levels of C reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were analyzed and screening tests for detection of the chronic microvascular complications were conducted in 76 type 1 diabetes patients. Results: Forty six patients had at least one of the complications. They were older and had longer duration of diabetes ($p=0.015$; $p<0.0001$) and higher values of total ($p=0.021$), LDL-cholesterol ($p=0.048$) and triglycerides ($p=0.002$). Levels of CRP ($p=0.004$) and TNF-α ($p=0.048$) were higher in patients with chronic microvascular complications in regard to patients without diagnosed microangiopathy. Conclusion: Low grade chronic inflammation is characteristic for type 1 diabetes patients with developed chronic microvascular complications.

Keywords: *Type 1 diabetes mellitus • Diabetes complications • Inflammation*

1. Introduction

Diabetes mellitus type 1 (DMT1) is autoimmune disease characterized by immune mediated β cell destruction and consequential insulin deficiency. This leads to metabolism disorders with chronic hyperglycemia as the main feature. Chronic hyperglycemia causes exceeded production of Advanced Glycosilation End-products (AGEs) which lead to macrophage activation, increased oxidative stress and production of inflammatory cytokines. Chronic inflammation causes endothelial dysfunction which is the key event in the development of microvascular and macrovascular complications in diabetic patients [1]. Damage in blood vessels of microcirculation of eye, kidney and peripheral nerves are the most common and they are the cause of diabetic retinopathy, nephropathy and neuropathy. These microvascular complications increase morbidity and mortality of DMT1 patients by causing increased incidence of blindness, terminal kidney failure, arthropathy, foot trauma, foot ulceration and infection and lower limb amputation. On the other hand, macrovascular complications are caused by cardiovascular, cerebrovascular and peripheral artery damage leading to heart attack, stroke and peripheral artery occlusion also followed by increased incidence of lower limb amputation. Macrovascular complications of diabetes are also multiplying morbidity and mortality of DMT1 patients.

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There is growing amount of evidence that DMT1 patients, especially those with developed microvascular and macrovascular complications, have increased inflammatory activity expressed through elevated levels of inflammatory cytokines, mainly C reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [2-5]. This is, probably, the consequence of above mentioned mechanisms but some data shows that existence of chronic degenerative complications may cause increased inflammatory marker levels in diabetic patients, mainly as a repercussion of endothelial dysfunction which is associated with malfunction of endothelial repair, characteristic for DMT1 patients [6].

CRP is reactant of acute phase response produced by liver. CRP production is induced by cytokines (interleukin-1 (IL-1), IL-6, TNF-α) and its level is increased in infections, inflammatory and malignant diseases, in tissue injuries and necrosis [7]. IL-6 is cytokine produced mainly by T cells and macrophages and contributes in immune response and acute phase reaction. Some other cells like adipocytes and osteoblasts also secrete IL-6 [8]. TNF-α is cytokine that is involved in systematic inflammation and stimulates the acute phase reaction. It is mainly produced by activated macrophages, but it can be produced by many other cells as CD4+ lymphocytes, natural killer (NK) cells and neurons [9].

Aims of our study are two. Firstly, comparison of age, gender, disease duration, anthropometric measurements, glucose metabolism and lipid and lipoprotein parameters in DMT1 patients with developed at least one of the chronic microvascular complications in regard to DMT1 patients without diagnosed chronic microvascular complications. Secondly, assessment of differences in inflammatory marker (CRP, IL-6 and TNF-α) levels in these two groups of patients and analysis of differences in determined inflammatory marker levels considering every analyzed microvascular complication of diabetes (retinopathy, nephropathy and polyneuropathy) separately.

2. Methods

Cross sectional study included 76 DMT1 patients examined at our clinic. Study has been approved by the local ethics committee and it is performed according to Helsinki Declaration. All participants gave written consent for their partaking in the study.

First, patient's general information and medical history were noted and physical examination was performed. After that, we measured anthropometric parameters (body mass index (BMI) and waist circumference). BMI was calculated as a quotient of a body weight and the square of body height expressed in meters. Waist circumference was measured in the middle of the line connecting the anterior superior iliac crest bone and arch ribs with a centimeter tape with a precision of 0.1 cm. Blood was sampled for analysis of glucose metabolism parameters (fasting and two hour postprandial glucose and HbA_1c), lipid and lipoprotein parameters (total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides) and inflammatory cytokines (CRP, IL-6 and TNF-α). CRP, IL-6 and TNF-α serum samples were kept at -80°C before being analyzed. CRP was determinate by electrochemiluminescence, while IL-6 and TNF-α were determinate by commercial ELISA plates according to the standard procedure recommended by the manufacturer. Standard screening tests for detection of chronic microvascular complications were conducted (24 hour albuminuria measurement, classical fundoscopy, diabetic polyneuropathy questionnaire and neurological examination) and 46 patients were diagnosed with at least one of the chronic microvascular complications.

Data analysis methods included descriptive and inferential statistics. For numerical parameters we used mean and median value, interquartile range of values or a value range (depending on the nature of the data). Comparison of mean values of numerical characteristics between the two groups was performed using t-test and for comparison of proportions we used χ_2-test. For analysis of difference in the distribution of inhomogeneous numerical characteristics we used the nonparametric Mann-Whitney test (two groups) or the Kruskal-Wallis test (three groups).

3. Results

Table 1 shows that DMT1 patients who developed at least one of the microvascular complications were older ($p=0.015$) and had a longer duration of the disease ($p<0.0001$) than DMT1 patients without diagnosed microangiopathy, while there was no statistically significant difference in gender, anthropometric measurements and glucose metabolism parameters. CRP ($p=0.004$) and TNF-α ($p=0.048$) had higher values in the group of DMT1 patients with microvascular complications, while IL-6 level wasn't statistically different between two groups ($p=0.706$). Also, patients who developed complications had a more adverse lipid profile with higher total cholesterol ($p=0.021$), LDL cholesterol ($p=0.048$) and triglycerides ($p=0.002$).

In Figure 1 data shows that there wasn't significant difference in values of CRP ($p=0.301$), IL-6 ($p=0.396$) and TNF-α ($p=0.582$) between patients with and without

Table 1. General, anthropometric, glucose metabolism, lipid and lipoprotein parameters and inflammatory cytokine levels in DMT1 patients with at least one of the microvascular complications and DMT1 patients without chronic microvascular complications

Parameters	DMT1 CG	DMT1 NCG	p value
Gender (male/female)	20/26	12/18	0.764
Age (years)	38.76 ± 11.48	29.83 ± 7.97	**0.015**
Duration of diabetes (years)	22.60 ± 8.00	16.03 ± 8.44	**<0.0001**
Nutritional status (normal/overweight and obese)	28/18	23/7	0.152
Waist circumference (non-risk/risk)	35/11	27/3	0.126
Fasting blood glucose (mmol/l)	10.65 ± 4.50	11.12 ± 5.38	0.682
Postprandial blood glucose (mmol/l)	11.30 ± 5.24	11.32 ± 5.58	0.988
HbA_1c (%)	9.28 ± 1.65	9.05 ± 1.79	0.564
Total cholesterol (mmol/l)	5.44 ± 1.09	4.89 ± 0.80	**0.021**
HDL cholesterol (mmol/l)	1.40 ± 0.38	1.45 ± 0.30	0.271
LDL cholesterol (mmol/l)	3.43 ± 0.96	3.03 ± 0.62	**0.048**
Triglycerides (mmol/l)	1.13 (0.76–1.67)	0.76 (0.61–0.99)	**0.002**
C reactive protein (mg/l)	1.55 (1.00–3.60)	0.80 (0.30–2.08)	**0.004**
Interleukin-6 (pg/ml)	0.74 (0.38–1.31)	0.83 (0.31–1.26)	0.706
Tumor necrosis factor-α (pg/ml)	0.75 (0.40–1.78)	0.33 (0.00–1.88)	**0.048**

DMT1-diabetes mellitus type 1; CG-complications group; NCG-non complications group; Nutritional status (normal: BMI<25 kg/m^2; overweight: 25 kg/m^2≤BMI<30 kg/m^2; obese: BMI≥30 kg/m^2); Waist circumference (non-risk: <80 cm for females and <94 cm for males; risk: ≥80 cm for females and ≥94 cm for males);

developed diabetic nephropathy. Even after we divided patients in the groups with normoalbuminuria, microalbuminuria and macroalbuminuria there were no statistically significant differences in values of CRP (p=0.342), IL-6 (p=0.696) and TNF-α (p=0.704).

As it is shown in Figure 2, patients with diagnosed diabetic retinopathy had higher values of CRP (p=0.039). Considering the fact that there was no statistical difference in values of IL-6 (p=0.727) and TNF-α (p=0.070) between the groups of patients with and without diabetic retinopathy, we decided to analyze their values in different stages of this complications (patients without retinopathy, with non proliferative retinopathy and with proliferative retinopathy) and found that the degree of retinopathy have statistically significant influence on levels of IL-6 (p=0.039) and TNF-α (p=0.045).

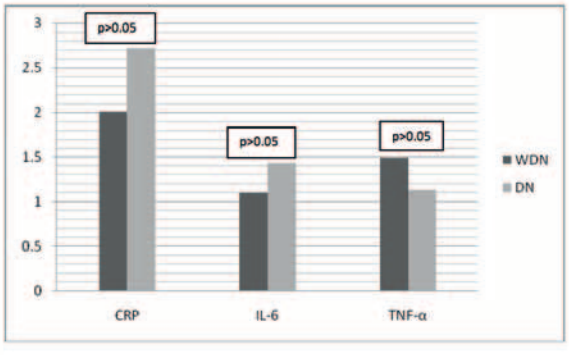

Figure 1. Inflammatory cytokines and diabetic nephropathy. WDN – without diabetic nephropathy; DN – diabetic nephropathy

Figure 3 shows that patients with diabetic polyneuropathy had higher values of CRP (p=0.009) than patients without this microangiopathy, while the presence of this complication didn't have influence on values of IL-6 (p=0.779) and TNF-α (p=0.475).

4. Discussion

The greatest lack of our study is the relatively small number of participants and modest set of analyzed inflammatory markers.

Nevertheless, our study shows that DMT1 patients who developed at least one of the microvascular complications are older and have longer duration of diabetes, which is in accordance with other authors [10].

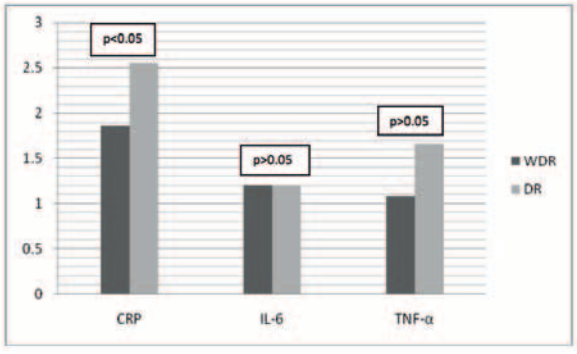

Figure 2. IInflammatory cytokines and diabetic retinopathy WDR – without diabetic retinopathy; DR – diabetic retinopathy;

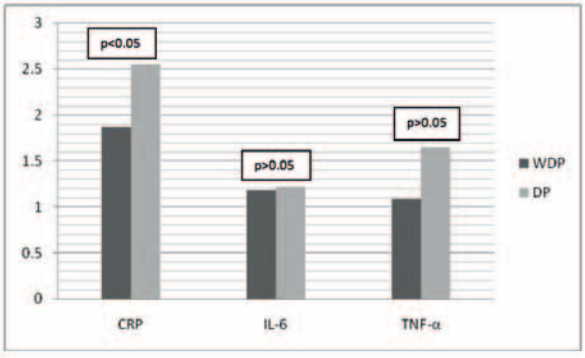

Figure 3. Inflammatory cytokines and diabetic polyneuropathy. WDP – without diabetic polyneuropathy; DR – diabetic polyneuropathy

Inflammation and oxidative stress play an important role in vascular damage and atherosclerosis, and DMT1 patients are at a higher risk of microvascular and macrovascular morbidity and mortality. Although, in recent years, most of the studies dealing with this issue were related to diabetes mellitus type 2 (DMT2) patients, the results of studies that included DMT1 patients have clearly demonstrated that among them also exists an increased inflammatory activity [3,4,11,12]. Our data suggest higher values of CRP and TNF-α in DMT1 patients with developed microangiopathy in regard to DMT1 patients without microvascular complications. That fact is in accordance with literature data which indicate that higher value of inflammatory cytokines correlates with microvascular and macrovascular damage [13-17].

Many studies indicated that there is connection between increased inflammatory activity and diabetic nephropathy [18]. Our data shows no difference in values of inflammatory cytokines in patients with and without developed diabetic nephropathy, which is in accordance with results of some authors [5,19].

Large number of studies indicated the role of inflammation and endothelial dysfunction in the pathogenesis of diabetic retinopathy [20-22]. Inflammatory cytokines may increase retinal vascular permeability which leads to changes in the contact of endothelial cells with pericytes and loss of pericytes followed by development of microaneurysms [20]. Our results show higher values of CRP in patients with diabetic retinopathy than in those without retinopathy. Statistically significant differences also exists between other inflammatory markers, IL-6 and TNF-α, but only in case of considering different degrees of retinopathy.

In recent years, there are studies that support the role of inflammation in the development of diabetic polyneuropathy. Inflammatory cytokines exhibit pleiotropic effects on glial cells and neurons. Cytokines regulate the process of degeneration and regeneration of peripheral nerves, which is essential in the pathogenesis of polyneuropathy [23]. Our results show that value of CRP is higher in patients with diabetic polyneuropathy in regard to patients without this complication and that is in accordance with results of other authors [24].

In DMT1 patients, dyslipidemia develops mainly due to a lack of metabolic regulation of disease, with a subsequent increase in triglyceride level and a decrease in HDL cholesterol level. Also, the development and progression of nephropathy in DMT1 contributes to the occurrence of dyslipidemia, with an increase in total cholesterol, LDL cholesterol, triglycerides and decrease in the protective HDL cholesterol [25]. At the same time hyperlipidemia, especially hypercholesterolemia, may be a risk factor for the progression of diabetic nephropathy, as it is confirmed in numerous studies that included DMT1 patients [26-28]. In our study, values of total and LDL cholesterol and triglycerides were statistically significant higher in the group of DMT1 patients with developed microvascular complications than in the group of DMT1 patients without microvascular complications. Such undesirable lipid profile favors the accumulation of fat cells in the arterial wall and consequently may increase inflammatory activity in DMT1 [29]. These results are consistent with the results of other studies that have examined the lipid and lipoprotein parameters in DMT1 patients and their role in the development of micro- and macrovascular complications [30,31].

DMT1 patients with developed microvascular complications are older, have longer duration of disease and more undesirable lipid profile than patients without diagnosed chronic microvascular complications. Also, they have higher levels of some of the analyzed inflammatory cytokines, which points out the question whether the chronic inflammation is the cause or the consequence of existence of small blood vessel damage. Hopefully, future prospective studies with large number of participants which will follow levels of inflammatory cytokines simultaneously with microangiopathy development will give the answer to this question. In the future novel specific anti-inflammatory drugs that will have a positive effect on delaying of development of chronic microvascular complications should be implemented.

Conflict of interest

The authors have no financial or other relationship to disclose as potential conflicts of interest.

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Earlier publications

Results shown in this study were not published before except congress abstracts. The paper is not under consideration for publishing by other journals.

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