C-reactive protein correlates with markers of endothelial dysfunction in type 1 diabetic patients

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Abstract

The interaction between inflammatory and endothelial cells seem to play a crucial role in the development of late vascular diabetic complications. The aim of our study was to evaluate the level of CRP and some markers of endothelial function in type 1 diabetic patients. Moreover, we assessed the relationship between CRP and those markers: endothelin-1 (ET-1), metabolites of nitric oxide (NO_2^-), soluble form of Intercellular Cell Adhesion Molecule-1 (sICAM-1), Vascular Cell Adhesion Molecule-1 (sVCAM-1), sE-selectin, fibronectin and von Willebrand factor (vWF).

The study was performed in 98 type 1 diabetic patients (57 women and 41 men), aged 32.1±9.8 years, duration of disease 12.8±7.4 years and HbA1c 7.8±2.6%. Serum levels of CRP, ET-1, sICAM-1, sVCAM-1, sE-selectin, fibronectin, metabolite of NO and vWF were estimated using ELISA commercial test.

CRP, ET-1, NO_2^-, sICAM-1, sVCAM-1, vWF, sE-selectin and fibronectin were significantly higher in diabetic patients in comparison with healthy subjects ($p<0.05$). CRP and NO_2^- were markedly higher in diabetic patients with microangiopathy (CRP: 4.91±0.28 vs 2.33±0.28 mg/l, $p<0.05$; NO_2^-: 85.49±1.71 vs 55.48±3.59 µmol/l, $p<0.05$). Moreover, we noticed positive correlation between CRP and markers of endothelial dysfunction, except the level of sVCAM-1 and sE-selectin ($p<0.05$).

The results of this study might suggest association between inflammatory process and endothelial dysfunction in diabetes.

Key words: *C-reactive protein, endothelial dysfunction, microagiopathy, type 1 diabetes*

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Background

The interaction between inflammatory and endothelial cells seem to play a crucial role in the development of late vascular diabetic complications. The cascade of phenomena leading to damage of endothelium is indirectly and directly related to hyperglycaemia. Diabetes Control and Complications Trial (DCCT) study in Type 1 and United Kingdom Prospective Diabetes Study (UKPDS) in Type 2 diabetes have clearly shown that hyperglycaemia is an independent risk factor for the development of microangiopathy [1, 2]. There is good evidence that hyperglycaemia leads to disturbances between endothelial and inflammatory cells [3]. High glucose level increases de novo diacyloglicerol (DAG) synthesis and activates Protein Kinase C (PKC). Abnormal activation of PKC leads to a number of pathogenic consequences such as affecting endothelial cells, increased oxidative stress, overexpression of the different genes [4]. Moreover, PKC activation is responsible for enhanced platelet and leukocytes adhesion and aggregation [5]. All of these disturbances observed in diabetes resemble the cascade of changes characteristic of inflammation. It has been shown that various markers reflecting low-grade inflammatory process and endothelial dysfunction are the risk factors for macrovascular diabetic complications [6].

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Table 1. Clinical characteristics of study groups (group A: diabetic patients without late diabetic complications; group B: diabetic patients with late diabetic complications). Means ±SD. *p<0.05 group A *vs* group B

	Healthy	Group A	Group B
N	20	31	67
Sex (Female/Male)	11/9	18/13	39/28
Age (years)	30.2±4.6	32.7±10.2*	31.7±9.2
Duration of diabetes (years)	–	9.4±3.6*	17.4±6.2
FPG (mmol/l)	4.2±0.6	7.7±2.9*	9.4±3.1
MBG (mmol/l)	–	9.2±1.7*	9.9±2.2
Fructosamine (µmol/l)	–	342.5±115.1*	421.7±129.2
HbA1c (%)	3.6±0.4	7.5±3.2*	8.5±2.1
Total cholesterol (mmol/l)	4.7±0.9	5.1±1.8*	5.1±2.1
HDL cholesterol (mmol/l)	1.2±0.3	1.1±0.4*	1.1±0.6
LDL cholesterol (mmol/l)	2.4±0.3	3.1±1.7*	3.0±1.2
Triglyceride (mmol/l)	1.2±0.4	1.4±1.0*	1.5±0.9
sBP (mmHg)	126.2±18.4	126.8±20.4*	138.2±24.6
dBP (mmHg)	68.8±14.6	62.6±12.8*	88.4±18.6
Insulin (U/kg/d)	–	0.7±0.2	0.6±0.1
ACE inhibitors	–	–	32

sBP – systolic Blood Pressure, dBP – diastolic Blood Pressure

These „inflammatory" markers that have reported associations with coronary heart disease in type 2 diabetes are: leukocyte count, fibrinogen, C-reactive protein and low serum albumin concentration. The results of prospective studies have demonstrated that these inflammatory markers may be regarded as non-traditional risk factors for vascular diabetic complications in type 2 diabetic patients [7, 8]. Moreover, some data may confirm the involvement of inflammatory reactions not only in the development but also in the progression of atherosclerosis [9]. Inflammation is regarded to play a particularly critical role in the destabilization of the fibrous cap, predisposing to the plaque rupture and thrombus formation [10]. The evidence suggesting a causal relation between inflammation and endothelial dysfunction and clinical events of atherosclerosis in general and type 2 diabetic populations was proved [11]. However, there are still a lot of questions concerning the place of inflammatory process in the pathogenesis of vascular complications, especially microangiopathy in type 1 diabetic patients.

The aim of our study was to evaluate the level of CRP and some selected markers of endothelial function in Type 1 diabetic adults with or without microangiopathy and without symptoms of macroangiopathy. Moreover, we assessed the relationship between serum levels of C-reactive protein and markers of endothelial dysfunction. These markers included: endothelin-1 (ET-1), metabolites of nitric oxide (NO_2^-), soluble form of Intercellular Cell Adhesion Molecule-1 (sICAM-1), Vascular Cell Adhesion Molecule-1 (sVCAM-1), sE-selectin, fibronectin and von Willebrand factor (vWF).

Material and methods

The study was performed in 98 type 1 diabetic patients (57 women and 41 men) recruited from Poznań Diabetic Center, aged 32.1±9.8 years, with a mean duration of disease 12.8±7.4 years and HbA1c level 7.8±2.6%. The study subjects were divided into two subgroups according to the absence (group A) or presence (group B) of microvascular diabetic complications. All patients in the group B had clinical evidence of diabetic retinopathy and/or nephropathy and/or neuropathy. 32 patients had microalbuminuria, defined as a urinary albumin excretion between 30 and 300 mg/24 h and 10 had overt proteinuria. On fundoscopic examination, background retinopathy was found in 28 patients, praeproliferative in 18 patients and proliferative retinopathy in 14 patients. There were 15 patients with diabetic neuropathy detected by clinical measurements. Current smokers, patients with renal failure, symptoms of macroangiopathy, acute or latent inflammatory focuses, were excluded from the study. 32 patients with late diabetic complications were treated with angiotensin converting enzyme (ACE) inhibitors. Twenty healthy workers of

Table 2. Serum levels of CRP and markers of vascular dysfunction in healthy subjects, type-1 diabetic patients without late complications (group A) and with complications (group B). Means ±SD. *p<0.05 in comparison with healthy subjects; # p<0.05 in comparison with group A

	Healthy	Group A	Group B
CRP (mg/l)	1.10±0.07	2.33±0.28*	4.91±0.28*#
ET-1 (pg/ml)	0.60±0.05	2.54±0.49*	2.35±0.24*
NO_2^- (µmol/l)	16.68±0.48	55.48±3.59*	85.49±1.71*#
SICAM-1 (ng/ml)	210.60±7.16	252.22±4.55*	241.42±9.12*
SVCAM-1 (ng/ml)	553.02±10.50	762.87±47.78*	696.82±30.53*
sE-selectin (ng/ml)	46.25±0.21	38.71±3.55*	52.17±3.41*
Fibronectin (ng/ml)	297.50±4.65	355.87±10.56*	384.76±3.39*
vWF (%)	110.00±3.96	157.1±6.12*	156.92±4.20*

Table 3. Pearson correlation coefficients, between serum levels of CRP and markers of endothelial dysfunction. *p<0.05

	ET-1	NO_2^-	sICAM-1	vWF	sVCAM-1	sE-selectin	fibronectin
CRP	r=0.58*	r=0.86*	r=0.60*	r=0.47	r=-0.20*	r=-0.49*	r=0.30*

Department of Diabetology served as a control group.

All subjects were informed about the aim of the study and gave their consent. The study was approved by the Ethical Committee. A clinical characteristic of subjects is presented in table 1.

Blood samples were drawn in the fasting state after a period of rest with minimal occlusion of the vein. Blood was collected using Vacutainer tubes. Plasma or serum was stored approximately 2 months at less than -20°C until assayed.

Serum levels of ET-1, sICAM-1, sVCAM-1, sE-selectin, were estimated with the use of ELISA tests (R&D system), fibronectin (Chemicon International) and vWF (Diagnostica Stago; Roche). CRP serum concentration was measured latex-enhanced immunoturbidimetric method. The assay determines total nitric oxide based on the enzymatic conversion of nitrate to nitrite reductase. The reaction is followed by a colorimetric detection of nitrite as azo dye product of the Griess reaction.

Statistical analysis

Results are expressed as means ±SD. Variables were checked for normal distribution by the Kolmogorov-Smirnov test. Statistical analyses between three study groups were performed using ANOVA test and between two groups of diabetic patients using unpaired t-test. Differences with a probability value p<0.05 were considered statistically significant. The correlation between CRP concentration and markers of vascular dysfunction were estimated according to Pearson and expressed as correlation coefficient (r).

Results

Serum levels of CRP, ET-1, NO_2^-, sICAM-1, sVCAM-1, vWF, sE-selectin and fibronectin were significantly higher in type 1 diabetic patients in comparison with healthy subjects (p<0.05) (table 2). The concentrations of CRP and NO_2^- were markedly higher in type 1 diabetic patients with microangiopathy in comparison with subjects without it (CRP: 4.91±0.28 *vs* 2.33±0.28 mg/l, p<0.05; NO_2^-: 85.49±1.71 *vs* 55.48±3.59 µmol/l, p<0.05). We did not observe any differences in the levels of ET-1, sICAM-1, sVCAM-1, vWF, sE-selectin and fibronectin between diabetic patients with and without microangiopathy. Moreover, we noticed positive correlation between serum concentrations of C-reactive protein and markers of endothelial dysfunction, except the level of sVCAM-1 and sE-selectin (p<0.05) (table 3).

Discussion

Our study shows elevated plasma levels of CRP and markers of endothelial dysfunction in type 1 diabetic patients. Diabetes mellitus is closely associated with the development of vascular complications caused by hyperglycaemia. Higher glucose levels may alter the properties of endothelial and inflammatory cells, thus leading to diabetic angiopathy [12]. Indeed, the concentrations of markers of endothelial dysfunction were elevated especially in patients with late complications of diabetes. Schmidt et al. noticed increased levels of VCAM-1 in diabetic patients with microalbuminuria [13]. Significantly higher levels of ICAM-1 in type 1 diabetic patients with proliferative retinopathy in comparison with

subjects without any changes in the eye were shown in the works of Limb et al. [14]. In our study we did not notice any significant differences in the levels of „low-grade inflammatory" markers and endothelial dysfunction between patients with or without microangiopathy, except the levels of CRP and nitric oxide metabolite (NO_2^-). It may indicate the presence of vascular perturbation and inflammatory reactions long before the late diabetic complications reveal. In this regard, measurements of the levels of markers of endothelial dysfunction may be useful to detect the risk of developing diabetic angiopathy.

The inflammatory process is considered to be one of the potential mechanisms of late diabetic complications. C-reactive protein, a hepatic acute phase protein, regulated by many circulating cytokines such as interleukin-6, is regarded to be a marker of systemic inflammation [15]. The levels of CRP are found significantly higher in our study group. These results are consistent with a number of studies, which reveal a link between diabetes mellitus and the levels of C-reactive protein [8]. According to Schmidt et al., it is related mainly to the beginning of the disease and may predict the development of diabetes mellitus [16]. Other authors find elevated levels of CRP in patients with diabetic vascular complications. There is an association between microalbuminuria and concentrations of C-reactive protein and fibrinogen in the works of Festa et al. [17]. The potential role of C-reactive protein in the development of diabetic complications has mostly been described in type 2 diabetic patients. Increased concentrations of CRP have been shown to be associated with an increased risk of cardiovascular disease [7]. However, the levels of C-reactive protein and its correlation with endothelial function have not been systematically investigated in type 1 diabetic patients. In the light of these facts, the levels of CRP noticed in our study may confirm the hypothesis of the potential role of inflammation also in type 1 diabetes mellitus and in late diabetic complications, including microangiopathy. It is emphasized additionally by a considerable difference in the levels of C-reactive protein between our two groups of diabetic patients.

Moreover, the study revealed a succinct association between inflammatory process expressed by the levels of C-reactive protein, and vascular perturbation. Some authors have already noticed this relationship. Romano et al. describe an association between inflammatory reaction and endothelial dysfunction. However, the authors state that it represents an early stage in type 1 diabetes mellitus [12]. On the other hand, there is a positive correlation between CRP levels and von Willebrand factor, Vascular Cell Adhesion Molecule-1 and urinary albumin excretion in the diabetic group shown in the works of Schalkwijk et al. [18]. It is confirmed also by Yudkin et al. who noticed a relationship between concentrations of CRP and plasma levels of von Willebrand factor, tissue plasminogen factor and fibronectin [15]. Considered together, our findings seem to confirm a significant correlation between chronic low-grade inflammation and impaired function of endothelial cells. Moreover, the study suggests that most of the markers of vascular dysfunction might also serve as markers of inflammatory process.

The role of hyperglycemia in the pathogenesis of vascular dysfunction in type 1 diabetic patients has been widely described. Some scientists claim that higher glucose levels could be the factor linking inflammatory process and endothelial perturbation, which result in late diabetic complications. Rodriguez et al. reveal an association between CRP levels and hyperglycaemia suggesting that higher glucose levels increase oxidative stress resulting in inflammation and dysfunction of endothelium [19].

In conclusion, the results of this study might suggest that the inflammatory process is associated with endothelial dysfunction in type 1 diabetes mellitus. Moreover, elevated levels of low-grade inflammation and endothelial dysfunction markers seem to precede the occurrence of diabetic microangiopathy that might have a good prognostic value in type 1 diabetic patients. However, in spite of a great progress in the knowledge concerning pathogenesis of vascular complications in diabetes mellitus made in the recent years, the relationship between inflammation and vascular dysfunction remains uncertain and should still be investigated.

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