Oxidative stress, inflammation and autoimmune reaction in type 1 and type 2 diabetes mellitus

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Abstract: Diabetes mellitus (DM) is associated with oxidative stress, elevation of inflammatory markers and other mechanisms, which may contribute to accelerated atherosclerosis. The aim of the study was to determine prominent factors of these pathogenic processes in patients with DM, to examine their relationship in serum, and to find out the differences between DM1 and DM2. Advanced oxidation protein products (AOPP), C-reactive protein (CRP), pregnancy-associated plasma protein-A (PAPP-A), anticardiolipin antibodies (ACA) and anti-β2-glycoprotein-I antibodies (anti-β2-GPI) were determined in 27 patients with DM1, 27 patients with DM2 and 23 healthy subjects. AOPP, CRP and anti-β2-GPI were significantly elevated in DM2 in comparison with healthy subjects ($p<0.01$, $p<0.0001$, $p<0.0001$, respectively). In DM1, anti-β2-GPI were elevated ($p<0.0001$) as well, but there was no increase of either AOPP or CRP. There was no difference in PAPP-A levels in DM1 or DM2 and healthy subjects. In DM 1, AOPP correlate significantly with anti-β2-GPI ($r = 0.68$, $p<0.05$). In DM2, there is a significant correlation between anti-β2-GPI and PAPP-A ($r=0.45$, $p<0.05$). Oxidative stress and inflammation are more expressed in DM2 and they are partly related. In DM1, oxidative stress seems to be in closer link to autoimmune reaction than to inflammation.

Key words: Diabetes mellitus – Oxidative stress – Inflammation – Advanced oxidation protein products – Pregnancy-associated plasma protein A.

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Introduction

Diabetes mellitus is associated with oxidative and carbonyl stress, microinflammation and other mechanisms, which may contribute to diabetic complications, accelerated atherosclerosis, higher rate of infectious complications, and to the increased morbidity and mortality of diabetic patients [1–3].

Oxidative stress (imbalance between free radicals and antioxidants in favour of free radicals) [4] and carbonyl stress (increase of reactive carbonyl compounds caused by their increased formation and/or decreased degradation or excretion) [1,5] can modify biological structures (lipids, proteins, sugars and nucleic acids) and cause their damage [4,5].

New compounds, formed via these mechanisms, can in vitro experiments directly influence formation of cytokines and growth factors and indicate a relationship to the inflammatory response [6–9].

Additionally, structural changes of biological structures and disclosure of new epitopes might trigger autoimmune response, which results in the formation of auto-antibodies [10].

In vitro studies document well these pathogenic processes, however the response of the whole organism may be more complex. We were interested, how the whole organism reacts on this situation and what can be observed in standard and specialized clinical-chemical examinations. The aim of the study was to determine prominent parameters of oxidative stress (advanced oxidation protein products (AOPP)), inflammation/acute phase reaction (CRP as a classical markers, and pregnancy-associated protein A (PAPP-A), possibly a new acute phase reactant and marker of unstable atherosclerotic plaques) and auto-antibodies (anticardiolipin antibodies (ACA) and anti β2-glycoprotein I antibodies (anti β2-GPI)) in patients with diabetes mellitus, to examine their relationship in serum and to find out the differences between patients with type 1 and type 2 diabetes mellitus (DM).

Material and Methods

Subjects

The studied group consisted of 54 patients with diabetes mellitus.

Patients with type 1 diabetes mellitus (27 patients – 10 men and 17 women), mean age 40±13 years, were treated for diabetes mellitus for 15±13 (1–44) years. Their biochemical parameters were as follows: blood glucose concentration: 9.9±4.1 mmol/l, HbA1c 8.7±1.5 %, cholesterol 4.6±0.8 mmol/l, HDL-cholesterol 1.5±0.4 mmol/l, LDL-cholesterol 2.6±0.7 mmol/l, triglycerides 1.1±0.6 mmol/l, total protein 75.4±6.0 g/l.

Patients with type 2 diabetes mellitus (27 patients – 21 men and 6 women), mean age 61±9 years, were treated for diabetes mellitus for 9±6 (1–28) years. Their biochemical parameters were as follows: blood glucose concentration: 10.9±4.7 mmol/l, HbA1c 8.4±2.1 %, cholesterol 5.3±1.0 mmol/l,

HDL-cholesterol 1.4±0.3 mmol/l, LDL-cholesterol 3.1±0.8 mmol/l, triglycerides 1.9±0.9 mmol/l, total protein 76.0±4.4 g/l.

All patients had serum creatinine level less than 110 mmol/l, were in stable clinical status without signs of acute infection and were not treated with antioxidants.

The control group consisted of 23 healthy subjects (9 men and 14 women), mean age 51±17 years. At the time of the study none of them was taking any antioxidants.

The study was approved by the local Institutional Ethical committee. All patients and controls gave their informed consent prior to entering this study.

Methods

Blood samples: Blood was collected after overnight fasting via venepuncture of the cubital vein. Blood was centrifuged at 1450 g (4 °C) for 10 minutes. Serum was stored at –80 °C and processed within 3 months.

AOPP assay: Determination of AOPP is based on spectrophotometric detection according to Witko-Sarsat [11]. 200 ml of serum diluted 1:5 with PBS, pH 7.4, 200 ml of chloramin T (0–100 μmol/l) for calibration and 200 ml of PBS as blank was applied on a microtitre plate. 10 ml of 1.16 mol/l KI and 20 ml of acetic acid were added and absorbance at 340 nm was measured immediately (photometer Multiskan Ascent, Labsystems, Finland). Concentration of AOPP is expressed in μmol/l (in reference to the calibrator).

Determination of inflammatory markers/acute phase reactants: CRP was determined with turbidimetry (Modular, Roche, Germany). PAPP-A was assessed immunochemically with TRACE (time resolved amplified cryptate emission) method (Kryptor, Brahms, Germany and standard kits Cezanne, France).

Auto-antibodies: Anticardiolipin antibodies and antibodies against β2-GPI were measured with standard ELISA kits (Orgentec, Germany).

Statistics

Results are expressed as mean ± standard deviation (SD). CRP is due to high interindividual variability described with median and interquartile range. Differences among groups were evaluated using unpaired t-test and Mann-Whitney U test. Correlation coefficients (Pearson, Spearman) were used for the examination of the relationship between parameters. All results were considered as statistically significant at $p<0.05$.

Results

Results are shown in Table 1.

Advanced oxidation protein products (AOPP) are elevated in patients DM2 ($p<0.01$ vs controls, borderline significance vs DM1). Similarly, CRP is elevated in patients with DM2 as well ($p<0.0001$ vs controls and DM1), while in DM1, there is no difference in comparison with the control group. PAPP-A has similar serum levels in patients with both types of DM and in healthy subjects.

Elevation of anti β2-GPI antibodies is typical for both types of DM without differences between DM1 and DM2 (2.5 fold elevation, $p<0.0001$ vs controls for both DM1 and DM2). Surprisingly, anticardiolipin antibodies – neither IgG nor IgM were found to be increased in patients with diabetes mellitus, on contrary, they were even decreased in comparison with healthy subjects, however still in normal range.

In DM 1, AOPP correlate significantly with anti β2-GPI ($r=0.68$, $p<0.05$). This correlation is significant also in healthy subjects ($r=0.53$, $p<0.05$). In DM2, there is a significant correlation between anti β2-GPI and PAPP-A ($r=0.45$, $p<0.05$). We did not find any other correlation between studied parameters either in DM1 or in DM2.

Both oxidative stress and (micro)inflammation are more expressed in patients with type 2 diabetes mellitus and are partially related. In DM1, oxidative stress seems to be in closer link to autoimmune reaction than to inflammation.

Discussion

Patients with diabetes mellitus have higher incidence of cardiovascular complications. In diabetic patients, similarly as e.g. in chronic renal failure patients, several mechanisms might contribute to accelerated atherosclerosis [12,13]. In our study, we observed elevation of several non-traditional risk factors of atherosclerosis, which is more pronounced in DM2. Elevation of auto-antibodies against β2-GPI was found in both types of DM, while significant increase of inflammatory parameter CRP and oxidative stress marker AOPP is typical only for DM2. Differences between DM1 and DM2 cannot be explained with higher age of patients with DM2 only (given by prevalence of both diseases in population), they could result from the different etiopathogenesis of both diseases (DM2 as a more complex metabolic disorder with involvement of lipid metabolism disorder).

Table 1 – Selected parameters of oxidative stress, inflammation/acute phase reaction and auto-antibodies in patients with type 1 and type 2 diabetes mellitus and in healthy subjects

	Patients with type 1 diabetes mellitus (n=27)	Patients with type 2 diabetes mellitus (n=27)	Healthy subjects (n=23)
AOPP (μmol/l)	90.5±43.14	106.7±49.9$^{\#\#}$	73.0±16.8
CRP (mg/l)	2 (4)	7 (9)$^{\#\#\#,\dagger}$	2 (1)
PAPP-A (mU/l)	7.9±1.7	7.9±1.7	8.2±2.8
Anti β2-GPI (U/ml)	6.1±3.5**	5.9±2.9$^{\#\#\#}$	2.5±0.8
ACA IgG (U/ml)	2.8±1.2*	3.1±1.2$^{\#\#}$	4.3±1.3
ACA IgM (U/ml)	1.6±0.8**	1.7±1.9$^{\#}$	3.3±1.6

Results are expressed as mean ± standard deviation (CRP as median and interquartile range).
**$p<0.0001$, *$p<0.0005$ DM1 vs controls
$^{\#\#\#}p<0.0001$, $^{\#\#}p<0.005$, $^{\#}p<0.01$ DM2 vs controls
$^{\dagger}p<0.0001$ DM2 vs DM1

CRP is described as risk factor of cardiovascular diseases. Its elevation was often observed mainly in hemodialysed patients, who are at several fold higher risks of both cardiovascular and overall morbidity and mortality [12]. The increase of CRP was found both in DM1 [2,14,15] and DM2 [3], however no study has focused on the differences between DM1 and DM2 so far. We would like to point out the elevation of CRP only in DM2, while patients with DM1 in our study had physiological serum concentrations of CRP. Elevation of other inflammatory markers, acute phase reactants, cytokines and complement activation were found in diabetic patients as well [16], e.g. Arnalich measured higher levels of both IL-6 and sIL-6R in patients with DM2 [3] and Romano refers increased levels of TNF-α in DM1 [14]. Increased urinary excretion of another protein – orosomucoid (acidic glycoprotein) was described as a new risk for cardiovascular and over-all mortality in patients with type 2 DM [17].

Pregnancy-associated plasma protein-A (PAPP-A) assessed by supersensitive methods is an outstanding marker of acute myocardial infarction and non-stable angina pectoris as shown recently [18]. Moreover, this high-molecular-weight protein is elevated in patients with chronic renal failure, mainly treated with hemodialysis, and it shows association with oxidative stress and inflammation [13]. However, despite marked differences between patients with DM1 and DM2 in inflammatory and oxidative stress markers, we did not observe any difference in serum levels of PAPP-A in both groups of diabetic patients. Surprisingly, there is no elevation either in DM2 in comparison with healthy controls, although these patients are at higher risk of cardiovascular complications. Additionally, PAPP-A was shown as a potential marker of echogenic atherosclerotic plaques in asymptomatic hyperlipidemic subjects at high cardiovascular risk [19]. Stable clinical status, normal renal function and lack of any acute cardiovascular complication of our patients could explain our findings.

Oxidative stress is typical for both types of diabetes mellitus, but it is more pronounced in DM2. Patients with type 2 DM have higher levels of oxidative and carbonyl stress products – advanced oxidation protein products (AOPP), advanced glycation (glycoxidation) end products (AGEs) [20,21] and lipoperoxidation product malondialdehyde (MDA) [22,23]. Due to higher damage caused by oxidative stress and probably for other reasons, patients with DM2 are at higher risk for accelerated atherosclerosis, i.e. macrovascular complications. Diabetes mellitus plays also an important role in enhancement of oxidative and carbonyl stress in chronic renal failure patients – both uremic and diabetic milieu are synergistic and contribute to formation of glycoxidation products [24] with several biological toxic effects [6].

Production of auto-antibodies in patients with diabetes mellitus can have various explanations: DM1 is an autoimmune disorder often connected with other autoimmune diseases (polyglandular syndrome, celiac disease) and the presence of various auto-antibodies can just belong to the picture of the disease.

On the other hand, both DM1 and DM2 are connected with oxidative and carbonyl stress (even if of a different degree) that leads to modification of biological structures. Formation of auto-antibodies could be the natural response of organism to those changes. However, the presence of auto-antibodies can result from a non-specific reaction. Our finding of normal levels of anticardiolipin antibodies in patients with both types of DM was surprising as well as the PAPP-A in DM2. Similarly, Arnalich found a correlation of TBARS (thiobarbituric acid reacting substances) with CRP and IL-6 in DM2 [3] and Romano observed a direct correlation of inflammatory markers with parameters defining endothelial perturbation [14]. Additionally, there is a possible synergism between generation of auto-antibodies and endothelial activation [2]. We failed to demonstrate a correlation between PAPP-A and CRP, which is typical for both acute myocardial infarction [18] and renal failure patients[28]. It could be explained by only slight elevation of CRP and PAPP-A within the normal range in diabetic patients (and more pronounced elevation and differences in other states mentioned above).

Both oxidative stress and (micro)inflammation are more expressed in patients with type 2 diabetes mellitus. In DM1, oxidative stress seems to be in closer link to autoimmune reaction than to inflammation. All these mechanisms might contribute to the acceleration of atherosclerosis, mainly in DM2, which is a more complex metabolic disorder with more pronounced reaction of the whole organism.

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