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sVCAM-1 levels in patients with type 2 diabetes mellitus with micro- and macrovascular complications

Poziomy sVCAM-1 u pacjentów z cukrzycą typu 2 z powikłaniami mikro- i makronaczyniowymi

INTRODUCTION

Type 2 diabetes is a disease with a long latency period and for this reason it is often not diagnosed at early stage. In most cases, the is detected at the time of it first complications occurrence. Complications may also result from poorly treated diabetes. Late complications of diabetes are a major problem in diabetes care. They are a major mortality factor. Currently, 75% of diabetic patients die of cardiovascular disease. Late complications include micro and macroangiopathy, among them the most important being: diabetic nephro- and neuro-pathy, retinopathy, diabetic foot, hypertension, atherosclerosis, stroke, ischemic heart disease, myocardial infarction.

In the course of prolonged hyperglycemia changes similar to chronic inflammation occur in the organism, with increased phagocytic cell transition (neutrophils, monocytes) by the endothelium towards the sites of inflammation [16]. An important role in these processes is played by some of the protein structures found on the cell surface which serve to allow interaction among cells and between cells and the extracellular matrix. Those molecules are called adhesion molecules (cell adhesion molecules – CAM) [14]. So far, the known cell adhesion molecules have been classified into the following groups: integrins, immunoglobulin molecules (immunoglobulin supergene family), selectins, cadherins, and unclassified molecules- antigen CD44 [4,10].

Vascular cell adhesion molecule, or immunoglobulin related molecules, plays a special role in the controlling of oriented cell migration process (chemotaxis) to the extravasular space (outside the intravascular space) [14]. Vascular adhesion molecule-1 VCAM-1 (CD106) included into this group is a glycoprotein with a molecular weight of 110 kDa. VCAM is involved particularly in monocytes and endothelial cells adhesion and their passage through the endothelial barrier [14].

The soluble form of VCAM-1 (sVCAM-1) is considered an indicator of endothelial activation, as an early marker of immune activation and inflammation. Elevated sVCAM-1 concentrations were

found in the course of many neoplasms, including ovarian, stomach, intestines, bladder cancers, immune diseases: multiple sclerosis, lupus erythematosus, as well as in patients with type 1 and 2 diabetes mellitus [9]. Therefore, the aim of this study was to evaluate the concentration of soluble vascular cell adhesion molecules (sVCAM) in serum of patients with type 2 diabetes with associated micro-and macro-vascular complications.

MATERIAL AND METHODS

The study was conducted in 51 patients with type 2 diabetes (mean age 62.3 ± 9.3 years). Among the enrolled subjects were: 26 women (51%) with the mean age 40.3 ± 17.3 years and 25 men (49%) with the mean age 45.8 ± 18.0 years. The patients were treated in the Endocrinology Clinic of the Independent Public Clinical Hospital No. 4 (SPSK 4) in Lublin The average *disease* duration *since diagnosis was* 133.4 ± 84.2 months.

In the medical history records of the studied diabetic patients macro - (58.8%), micro-vascular (37.3%) and concomitantly (micro + macro) (76.5%) complications were found; mainly arterial hypertension (80.4%), coronary artery disease (53%), myocardial infarction (25.5 %), heart failure (17.7%), stroke (9.8%), diabetic nephropathy (9.8%), retinopathy (13.7%), polineuropathy (9.6%), diabetic foot syndrome (2%), metabolic syndrome (63.3%), and overweight based on BMI (65.3 %).

The control group was composed of healthy subjects (n=30) with the mean age of 55.1 13.2 years, attending the periodic health checks at the Department of Laboratory Diagnostics of the Independent Public Clinical Hospital No. 1 in Lublin.

The biochemical parameters were measured using a biochemical analyser Konelab (B*ioMérieux)* with dedicated reagents from the same company based on methods routinely used in clinical laboratories. The concentration of sVCAM was measured with immunoenzymatic ELISA (*enzym linked immunosorbent assay*) from DIACLONE. The reference range was 80–1502 ng/ml.

The clinical data and biochemical determinations were expressed with the use of descriptive statistics (mean – X, standard deviation – SD, median – Me). Distributions of the analysed variables were tested using the Shapiro-Wilk test. For a comparison of independent variables between patients with and without diabetes the Student t or U Mann-Whitney tests were used. Correlations between variables were investigated using Pearson's or Spearman's test. A p value ≤ 0.05 was considered as statistically significant in all analyses.

RESULTS

Table 1 shows the results the selected biochemical parameters in patients with diabetes mellitus and healthy controls. The median of sVCAM-1 concentrations in the group with diabetes was 847.8 ng/ml and was significantly higher than in control group (572 ng/ml).

Table 1. The concentrations of chosen biochemical parameters in the diabetic patients and in the control group

	Control Group	Study Group	P
sVCAM-1	604.0 ± 170.0 572.0 (334.7 – 996.8)	939.4 ± 307.4 847.8 (508.5 – 1750.0)	< 0.001
Glucose	89.0 ± 11.5 89.0 (70.0 – 113.0)	149.2 ± 41.6 137.0 (94.0 – 246.0)	< 0.001
HbA1c	-	8.72 ± 1.86 8.60 (5.10 – 14.00)	-
Urea	36.8 ± 11.5 35.0 (19.2 – 73.8)	41.3 ± 16.6 39.8 (10.6 – 107.4)	NS
Creatinine	0.85 ± 0.20 0.81 (0.60 – 1.30)	1.10 ± 0.28 1.05 (0.7 – 2.2)	< 0.001
Total protein	6.9 ± 0.7 7.0 (5.2 – 8.5)	6.9 ± 0.6 7.1 (5.7 – 8.0)	NS
Fibrynogen	3.41 ± 0.42 3.36 (2.2 – 4.8)	4.19 ± 0.94 4.12 (2.68 – 7.05)	< 0.05
CRP	3.25 ± 0.57 3.00 (2.99 – 4.10)	9.96 ± 16.7 4.41 (0.40 – 71.80)	NS
Total cholesterol	207.2 ± 38.5 196.5 (130.0 – 300.0)	194.1 ± 53.5 193.0 (88.0 – 344.0)	NS
HDL cholesterol	60.3 ± 17.4 56.5 (30.0 – 105.0)	40.9 ± 10.2 40.0 (25.0 – 77.9)	< 0.001
LDL cholesterol	124.6 ± 30.5 121.0 (64.0 – 181.0)	119.9 ± 46.4 117.0 (54.0 – 293.8)	NS
Triglycerides	112.1 ± 46.9 108.5 (26.0 – 203.0)	173.8 ± 119.3 149.5 (42.0 – 796.0)	< 0.01

Moreover, in diabetic patients we also found significantly higher levels of blood glucose (149.2 ± 41.6 mg/dl), creatinine (1.10 ± 0.28 mg/dl), fibrinogen (4.19 ± 0.94 g/l) and triglycerides (173.8 ± 119.3 mg/dl) in comparison with control subjects (respectively: 89.0 ± 11.5 mg/dl, 0.85 ± 0.20 mg/dl, 3.41 ± 0.42 g/L and 112.1 ± 46.9 mg/dl). However, the level of HDL cholesterol in the study group was significantly lower than in the control group (40.9 ± 10.2 mg/dl vs. 60.3 ± 17.4 mg/dl).

Table 2. sVCAM-1 concentrations in the study group with and without complications of diabetes

	Diabetic complications	
	present	absent
hypertension	933.2 ± 317.8 787.1 (508.5 – 1507.0)	964.7 ± 274.2 936.1 (599.5 – 1750.0)
Ischemic heart disease	899.9 ± 283.8 787.1 (508.5 – 1471.0)	983.8 ± 332.3 907.2 (547.1 – 1750.0)
Heart infarct	843.3 ± 250.2 784.4 (514.0 – 1416.0)	972.2 ± 321.0 919.6 (508.5 – 1750.0)
Stroke	862.1 ± 274.1 847.8 (514.0 – 1179.0)	947.8 ± 312.4 856.1 (508.5 – 1750.0)
Heart failure	986.9 ± 328.5 894.7 (514.0 – 1507.0)	929.2 ± 305.9 845.1 (508.5 – 1750.0)
Retinopathy	810.5 ± 182.3 762.3 (643.7 – 1160.0)	959.9 ± 319.5 882.3 (508.5 – 1750.0)
Nephropaty	899.1 ± 323.2 787.1 (514.0 – 1286.0)	943.8 ± 309.0 858.9 (508.5 – 1750.0)
Metabolic syndrome	940.2 ± 324.9 784.4 (508.5 – 1507.0)	948.3 ± 278.4 922.3 (552.6 – 750.0)
Microangipathic complications	876.7 ± 229.6 787.1 (514.0 – 1286.0)	976.6 ± 343.4 924.2 (508.5 – 1750.0)
Macroangiopayhic complications	831.3 ± 293.0 786.3 (508.5 – 1507.0)	991.1 ± 333.9 942.3 (547.1 – 1750.0)

The highest median of sVCAM-1 levels were found in patients with diabetes who showed macrovascular complications (Table 2). In these patients, the median level of sVCAM-1 was 942.3 ng/ml and it was significantly higher than that observed in patients without these complications (786.3 ng/ml). In this group, patients with myocardial infarct (919.6 ng/ml) and coronary heart disease (907.2 ng/ml) had significantly higher levels of sVCAM-1 compared with the group without complications. In patients with stroke and congestive heart failure sVACM-1 levels were comparable with the levels found in the group without complications.

In patients with microvascular complications, the median of sVCAM-1 concentration was 922.3 ng/ml and was also significantly higher than in patients without these complications (787.1 ng/ml). In this group diabetic retinopathy was the dominant type of diabetes complications and the concentrations of sVCAM-1 were 882.3 ng/ml.

Hypertension was the most common among the other complications of diabetes. In the patients group with hypertension the median level of sVCAM was 936.1 ng/ml and was significantly higher than in the control group (787.1 ng/ml).

The concomitant presence of type 2 diabetes and metabolic syndrome also significantly increased the concentrations of sVCAM-1. These patients had a significantly higher median concentration of sVCAM in comparison to the control group (784.4 ng/ml and 922.3 ng/ml; respectively).

In the study group a significant correlation between the concentration of sVCAM-1 and glycated hemoglobin was found (Figure 1). However, no significant relationships were found among sVCAM-1 concentrations and other parameters (age, height, weight, BMI, blood urea, creatinine, CRP, fibrinogen, total cholesterol, HDL cholesterol and triglycerides).

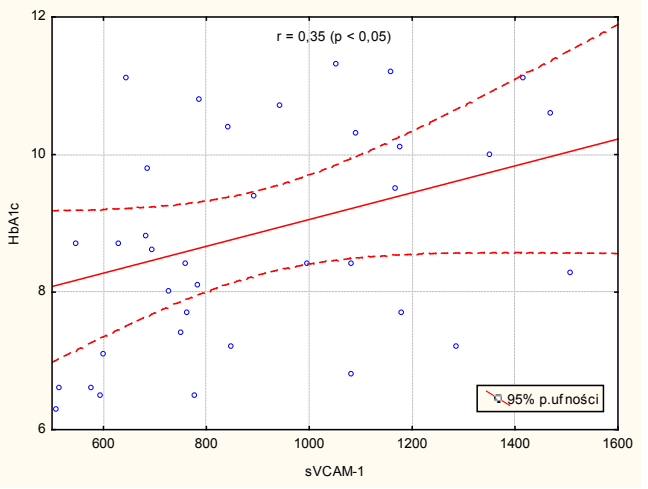

Fig. 1. Correlation of sVCAM-1 concentration with glycated hemoglobin in patients with type2 diabetes mellitus

DISCUSSION

Recently, a huge interest in adhesion molecules has been observed. It is related to the growing knowledge about their role in the pathogenesis of inflammatory and immunological diseases as well as in the development of type 2 diabetes complications [6].

Nowadays it is believed that endothelial cell dysfunction is not only a marker of vascular injury, but it plays an important role in the initiation, progression and the occurrence of vascular disease clinical symptoms due to atherosclerosis. The factors that stimulate endothelial cell dysfunction in atherosclerosis include: diabetes, oxygen free radicals from cigarette smoke, hemodynamic disturbances, as well as elevated LDL-cholesterol and homocysteine levels [17]. A high circulating level of serum adhesion molecules reflects their own expression on the surface of endothelial cells and may be an early marker of vascular lesions [17].

Results of this study showed an increase of soluble vascular cell adhesion molecules concentrations in patients with type 2 diabetes. This may indicate that in patients with macrovacular complications, hyperglycemia is a significant impact on these molecules expression and the presence of atherosclerotic lesions. Many other researchers confirmed elevated levels of sVCAM-1 in the blood of patients with atherosclerosis [13,17]; additionally, O'Brien et al. [12] showed a correlation of blood sVCAM-1 levels with the severity of atherosclerosis.

Results of the study by Otsuki et al. [11] conducted in patients with atherosclerosis with and without concomitant diabetes, revealed higher sVCAM-1 levels in patients with simultaneous diseases presence. Otsuki et al. [11] in their study conducted in patients with atherosclerosis with and without diabetes, noted higher levels of sVCAM-1 in patients with simultaneous diseases presence. Therefore, our results as well as numerous literature reports confirm that atherosclerosis is blood vessels' chronic inflammatory process, during which damage of endothelium and expression of adhesion molecules occurs.

Moreover, we found significantly higher levels of sVCAM-1(> 900 ng/ml) in patients with macrovascular complications compared with the group without these complications within the group of patients with myocardial infarction and coronary heart disease. Our results are accordance with other authors reports.

Jarosz and Nowicki [7] in their study compared the levels of sVCAM-1 between groups of men with and without coronary artery disease. They found very high levels of serum sVCAM-l which exceeded 800 ng/ml in patients with ischemic heart disease. These results may suggest that molecules as VCAM-1 could be a helpful parameter in assessing the probability of sudden vascular events in patients with coronary heart disease. Furthermore, they may be useful in the decision making in regards to therapy intensification in patients with high VCAM-1 concentrations.

A similar study was conducted by Drobniak-Hełdak et al. [3], who assessed the levels of sVCAM-1 in the group of 40 males with stable coronary artery disease without diabetes. These results showed increased levels of sVCAM-1 in patients with coronary artery disease compared with healthy subjects.

Furthermore, it was found that in the course of myocardial infarct (MI), due to muscle cells ischaemia (hypoxia), increased expression of adhesion molecules on the surface of endothelial cells in contact with the changed necrotic cardiomyocytes is observed. As a result, the number of molecules both on the surface of endothelial cells as well as their soluble forms in plasma increase. In patients with acute cardiac events an increased expression of sVCAM-1 soluble forms was detected [19].

Thus, on the basis of these results and available literature it could be concluded that both patients with type 2 diabetes and myocardial infarct, as well as patients with MI without diabetes demonstrate changes in the vascular endothelium, increased levels of adhesion molecules and acute phase proteins such as CRP and fibrinogen. This may indicate the presence of inflammatory endothelial changes in these patients.

In our study conducted in patients with type 2 diabetes we showed increased concentrations of adhesion molecules as a result of the history of stroke. In a stroke accompanied by inflammatory response, locally synthesized pro-inflammatory cytokines induce expression of adhesion molecules such as immunoglobulin like molecules, i.e. VCAM-1 at the surface of endothelial cells, increasing their concentration in the blood. This was confirmed in other studies, i.e. by Zareba and Losy [18]. Similarly, Fedorowicz and Chlopicki [5] demonstrated endothelial cells failure in terms of PGI, NO production and activation of endothelial inflammation in patients with hypertension, which manifests itself in increased soluble VCAM-1 concentrations.

In our study we found high levels of sVCAM-1 in the blood of patients with microvascular complications in whom the dominant pathology was diabetic retinopathy. Similar results were

obtained by Adamiec and Oficjalska-Mlynczak [1,2], in the group of 46 subjects with type 2 diabetes mellitus. They showed that the serum and ocular levels of sVCAM-1 and proinflammatory cytokines (IL-6, TNF-α) in diabetic patients significantly exceeded the values observed in the control group. At the same time, the obtained results confirmed the correlation of HbA1c with intraocular levels of VCAM-1. These results revealed directly proportional increase in the concentration of VCAM-1 and TNFα in the vitreous depending on the degree of compensation (evaluated by HbA1c)

Total cholesterol and LDL cholesterol concentrations remained at a comparable level between patients with diabetes and the control group, while triglyceride levels were higher, and HDL cholesterol lower in patients with type 2 diabetes. Similar results were found by Winiarska et al. [15]. Moreover, in the group of patients with type 2 diabetes we also found a significantly increased plasma fibrinogen. In contrast, Kolcowa et al. [33] found elevated levels of fibrinogen in patients with type 2 diabetes. Additionally, they found a positive correlation between concentrations of adhesion molecules and the concentration of fibrinogen. Since both proteins are markers of inflammation these results may indicate the presence of inflammation in these group of patients. They also found a positive correlation of adhesion molecules with LDL cholesterol concentration, and a negative one with HDL cholesterol is serum of patients with diabetes mellitus type 2.

CONCLUSIONS

Taking into account the available literature reports as well as the results obtained during this study we can conclude that the metabolic abnormalities characteristic of type 2 diabetes, i.e. hyperglycemia, are the results of inflammation and may be responsible for their intensification.

Markers of inflammation as adhesion molecules may be useful predictive indicators of cardiovascular complications of diabetes. More studies are needed to understand the sequence of events leading to abnormal vascular function in subjects with type 2 diabetes.

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SUMMARY

In the course of prolonged hyperglycemia, changes similar to chronic inflammation occur in the organism, with increased phagocytic cell transition (neutrophils, monocytes) by the endothelium towards sites of inflammation. An important role in these processes is played by some of the protein structures found on the cell surface which serve to allow interaction among cells and between cells and the extracellular matrix. Those molecules are called adhesion molecules (cell adhesion molecules – CAM). Therefore, the aim of this study was to evaluate the concentration of soluble vascular cell adhesion molecules (sVCAM) in serum of patients with type 2 diabetes with associated micro-and macro-vascular complications. The highest median sVCAM-1 levels were found in patients with diabetes who showed macrovascular complications. In this group, patients with myocardial infarct (919.6 ng/ml) and coronary heart disease (907.2 ng/ml) had significantly higher levels of sVCAM-1 compared with the group without complications. Taking into account the available literature reports

as well as the results obtained during this study we can conclude that the metabolic abnormalities characteristic of type 2 diabetes i.e. hyperglycemia, are the results of inflammation and may be responsible for their intensification. Markers of inflammation as adhesion molecules may be useful predictive indicators of cardiovascular complications of diabetes. More studies are needed to understand the sequence of events leading to abnormal vascular function in subjects with type 2 diabetes.

Keywords: diabetes, microangiopathy, macroangiopathy, sVCAM-1, cardiovascular complications

STRESZCZENIE

Zmiany zachodzące w organizmie człowieka w warunkach długotrwałej hiperglikemii przypominają przewlekły odczyn zapalny z nasilonym przechodzeniem komórek fagocytujących (granulocyty obojętnochłonne, monocyty) przez śródbłonek naczyniowy w kierunku miejsca zapalenia. Istotną rolę w tych procesach odgrywają struktury białkowe znajdujące się zwykle na powierzchni błony komórkowej i umożliwiające interakcje pomiędzy komórkami oraz komórkami i macierzą pozakomórkową, nazywane cząsteczkami adhezyjnymi (*cell adhesion molecules* – CAM). Celem niniejszej pracy była ocena stężenia rozpuszczalnych cząstek adhezji komórkowej naczyń (sVCAM) w surowicy krwi chorych na cukrzycę typu 2 ze współistniejącymi powikłaniami mikro- i mikronaczyniowymi. Najwyższe stężenie cząstek sVCAM-1 stwierdzono u osób chorych na cukrzycę, u których wykazano występowanie powikłań makronaczyniowych. W tej grupie chorych istotnie wyższe stężenia sVCAM-1 w porównaniu z grupą bez powikłań zaobserwowano u osób z zawałem mięśnia sercowego (919,6 ng/ml) oraz chorobą niedokrwienną serca (907,2 ng/ml). Biorąc pod uwagę doniesienia literatury jak również wyniki tej pracy można stwierdzić, iż zaburzenia metaboliczne, charakterystyczne dla cukrzycy typu 2, takie jak hiperglikemia, zarówno nasilają toczący się proces zapalny, jak i są jego rezultatem. Markery reakcji zapalnej, takie jak molekuły adhezyjne, mogą służyć jako predykatory powikłań sercowo-naczyniowych.

Słowa kluczowe: cukrzyca, mikroangiopatia, makroangiopatia, sVCAM-1, powikłania sercowo-naczyniowe