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VASCULAR CELL ADHESİON MOLECULE-1, INTERCELLULAR ADHESİON MOLECULE-1 AND CD 146 LEVELS IN PATİENTS WİTH TYPE 2 DİABETES WİTH COMPLİCATİONS

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Abstract

BACKGROUND: Type 2 Diabetes Mellitus (T2DM) is a multisistemic and chronic disease accompanied with microvascular complications with various complicated mechanisms that exacerbate the prognosis, as the life duration with the diasease lengthens.

Intercellular adhesion molecule-1 (ICAM-1), vasculary cell adhesion molecule-1 (VCAM-1) CD146 are mainly expressed by endothelial cells and facilitate adhesion and transmigration of immun cells, leading to inflammation. In the present study, we evaluated the levels of soluble adhesion molecules in the patients with microvascular complications of T2DM.

METHODS: Serum and whole blood samples were collected from 58 T2DM patients with microvascular complications and 20 age matched healthy subjects. Biochemical assays were performed using ELISA for sICAM1 and sVCAM1 and flowcytometer for CD146.

RESULTS: Serum sICAM1 levels were lower in T2DM patients with microvascular complications than healthy controls (p<0.05). No significant difference was found between sVCAM1 and CD146 levels between the study and the control group.

While patients were subdivided into groups considering the types of microvascular complications, cell adhesion molecule levels were not correlated with the complication subtypes.

CONCLUSIONS: Decreased or unchanged levels of solubl forms of cell adhesion molecules might be a result of local inflammation and insulin and ACE inhibitor mediated therapy in the patients with T2DM.

Key Words: Cell adhesion molecules, CD146, Diabetes Mellitus, ICAM-1, microvascular complications, VCAM-1.

Introduction

Type 2 diabetes mellitus (T2DM) characterized by hyperglycemia is a metabolic disease with an increasing rate worldwide. In uncontrolled diabetic patients, hyperglycemia leads oxidative stress and thus, endothelial dysfunction via inflammation. Long-term course of diabetes induces systemic endothelial dysfunction and chronic inflammation, which is responsible for microvascular complications as diabetic kidney diasease (DKD), retinopathy (DR) and neuropathy (DN) (1).

DR is characterized by loss of pericytes, endothelial cell dysfunction, blood-retinal barrier breakdown, capillary non-perfusion, microaneurysm, haemorrhage and neovascularization (2).

DKD is recognized when vascular, post-inflammatory changes, diffuse nodular or less frequently exudative glomerulosclerosis and hyalinizing alterations are observed in the blood vessels along with gradual reduction in glomerular filtration rate (GFR) (3).

Proinflammatory cytokine TNF-□ stimulates endothelial cells and the induced endothelium expresses adhesion molecules such as intercellular, vascular cellular and melanoma cell adhesion (also known as CD146) molecules (ICAM-1, VCAM-1, MCAM-1) (4). These adhesion molecules are found on the walls of vessels, and they mediate rolling and transendothelial migration of these inflammatory cells into the intima by anchoring circulating leucocytes (5).

Prior studies have shown soluble forms of these adhesion molecules in the sera of diabetic patients, suggesting a role for diabetic endothelial activation (6-8).

Thus, vascular endothelium is not only affected but also contributes to development of microvascular complications through these processes.

Four cellular adhesion molecules (CAM) families have been identified: the cadherins, the selectins, the integrins, and the immunoglobulin CAM superfamily (IgSF-CAM). ICAM-1, VCAM-1 and CD146 are normally expressed on the surface of endothelial and epithelial cells at low levels under physiological conditions (9).

On a properly functioning endothelium, inflammatory cells do not adhere to the vessel wall as a result of the balance between pro- and anti-inflammatory phases. On the other hand, under hyperglycemic conditions, excess glucose molecules are non-enzymatically coupled with the lateral chains of lysine in proteins -the mechanism how glycosylation end products occur-, and with the contribution of oxidative stress, adhesion molecules are produced in higher amounts on the surface of activated endothelial cells (7).

Leukocytes bind to activated endothelium via CAMs and their receptors (LFA-1, VLA-4) and transmigrate into tissues, then initiate the inflammatory process (10,11).

With the further induction of these pathways, vascular endothelium is impaired and capillary permeability is increased, substantially leading to microvascular damage and thus, complications observed in T2DM patients (7).

Numerous studies suggested important role for CAMs in the development of diabetic complications. Soluble forms of CAMs can be detected in circulation as the potential markers of endothelial dysfunction.

Aim

In this study, we aim to evaluate the concentrations of soluble forms of selected CAMs, since their concentration might reflect their expression on endothelial cells in patients with microvascular complications of uncontrolled T2DM. We attempted to find out if these molecules are related to the state of endothelial dysfunction, systemic inflammation and severity of T2DM.

Materıals and Methods

Study group consists of 58 T2DM patients with microvascular complications, either DKD, DR, DN and 23 healthy age-matched volunteers. All subjects were clinically stable without an acute or chronic infection.

DKD was present in all of the diabetic patients with microvascular complications, and accompanied with DN in three and DR in 27 and both DR and DN in 10 patients.

A total of 44 subjects were receiving insulin therapy, 32 oral medication of hypoglicemic agents (sulfonylureas or sulfonylureas plus biguanides) and 18 combination of insulin and oral hypoglycemic agents. Forty-two of the diabetic patients were treated with angiotensin converting enzyme (ACE) inhibitors. Control groups were not on medications affecting carbohydrate or lipid metabolism or any vitamin and mineral support.

The presence of a microvascular complication related to T2DM was identified if patients suffered from DKD, DR or DN and diagnoses were made by expert nephrologist, neurologist and ophtalmologists with detailed examination recruited by fundoscopy and electromyelography.

DKD was defined using urine albumin/creatinine ratio (ACR) and graded as microalbuminuria while ACR is between of 30.0–299 mg/g and macroalbuminuria while ACR is $\geq$ 300 mg/g.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of Istanbul Education and Research Hospital. Written informed consent was obtained from all subjects.

Demographic (gender, age), anthropometric data (weight, height, BMI) and clinical data (diasease duration, medications) were verified by face-to-face interview with the subjects.

Routine analyses

Venous blood samples and spot urine samples were obtained following overnight fasting for analysis. All icteric, lypemic or haemolytic blood samples were discarded.

Clinical chemistry analyses (HDL-C, LDL-C, Triglycerides, Total cholesterol, glucose, Insulin, C-peptide, hs-CRP, BUN, Creatinine, Hb A1c, urinary albumin/creatinine ratio) were performed on the same day of sample withdrawal, using Siemens Advia 2400 and Centaur XP autoanalyzers by enzymatic and immunoassay methods using commercial kits (Siemens Diagnostics, Siemens Healthcare GmbH, Erlangen, Germany).

Serum sICAM and sICAM levels

For the measurement of ICAM1 and VCAM1 levels, serum samples were stored at -80ºC until the analysis day.

Serum ICAM-1 and VCAM-1 levels were measured using human solubl ICAM-1 (RayBiotech Inc., USA) and human solubl VCAM-1 (RayBiotech Inc., USA) reagents by Enzyme-linked immunoassay (ELISA) method. For absorbance measurements, Synergy HT Multi-Mode Microplate Reader (Bio-Tek, USA) was used.

Flowcytometric analyses

Flowcytometric analysis was performed in fresh whole blood samples in order to obtain the most possible viable cell count.

For flowcytometric analysis of CD 146 levels, PE Mouse Anti-Human CD146 (BD Pharmingen, USA) antibody was used. Peripheral blood cells were dyed using 7-aminoactinomycin D, fluorescent isothiocyanide marked anti-CD31, phycoerythrin marked anti-CD146 and PC5 marked anti-CD45 antibodies.

Following the erythrolysis, cells were evaluated using BD FACSCanto II flowcytometer.

First, cells were grouped according to their expression of CD 45 (lymphocyte common antigen). While CD45 negative cells, in other words, non-lymphocytic cells were sub-grouped according to CD31 (platelet-endothelial cell adhesion molecule-1 (PECAM-1)) and CD146 expression on the surface, three different sub-populations (CD31+ CD146+, CD31− CD146+ and CD31+ CD146−) were obtained.

The percentage of these populations were compared between the study group and the control group and within the study group. Peripheral endothelial cells were defined as the cells with the CD45− CD31+ CD146+ expression pattern.

Statistical analyses

Statistical analyses were obtained using SPSS (Statistical Package for Social Sciences) for Windows 10.0 program. Besides presentation of the data as the mean and standard deviation (SD), Student-t and Mann Whitney U tests were recruited for the comparison of quantitative data. Ki-square test was done on qualitative data. A p-value $p<0.05$ was considered statistically significant within the 95% confidence interval. Correlations between parameters were tested using Pearson's and Spearman's correlation coefficients.

Results

Table 1 summarizes demographic and biochemical values of the T2DM patients and the control group. Comparison of the age, gender and BMI revealed no significant difference between the groups ($p>0.05$).

Blood lipid profile, including total cholesterol, LDL-cholesterol, HDL-cholesterol were not significantly different between the groups, however, triglyceride levels were higher in the T2DM group ($p<0.05$).

Glucose, BUN, creatinine, HbA1c levels and urinary Albumin/Creatinine ratio were signficantly higher in patients with T2DM in comparison with the control group ($p<0.001$ except for HbA1c).

There were no differences between the C-peptide and hsCRP levels among the groups.

Endothelial ICAM1, VCAM1, CD31-CD146+, CD31+CD146+ and CD31+CD146- parameters of control and patient groups were shown in Table 2. ICAM levels were significantly lower in the T2DM group in comparison with the controls ($p<0.05$). However, there were no significant differences in VCAM and CD31-CD146+, CD31+CD146+ and CD31+CD146 levels between the groups.

While complications were investigated solely, glucose and HbA1c levels were significantly higher in the neuropathy group when compared with the T2DM patients without neuropathy($p<0.05$). On the other hand, demographical, clinical and biochemical variables did not differ as well as the tested endothelial parameters ($p>0.05$) (Table 3).

While T2DM patients were subdivided into two groups regarding presence of DR, no significant difference was observed between the two groups for the levels of neither biochemical nor the study parameters ($p>0.05$) (Table 4).

Table 5 shows T2DM patients with only DKD and DKD accompanied with other microvascular complications. There was no significant difference between any of the parameters tested between the groups.

sICAM1 and sVCAM1 levels were significantly positively correlated in the T2DM group (Figure 1). However, when the study group was subdivided considering the types of microvascular complications, no correlation was found. Similarly, no correlation was present between the endothelial markers and other clinical and biochemical parameters.

Table 1. Demographic and biochemical values of diabetic and control subjects.

	T2DM		Control		
	Mean	SD	Mean	SD	**p**
Age (years)	63,45	12,35	60,00	5,85	**NS**
BMI (kg/m2)	32,131	5,612	30,975	5,013	**NS**
Sex	n	%	n	%	**p**
M	23	39,6	6	30,0	**NS**
F	35	60,4	14	70,0	
Glucose (mg/dL)	164	66	87	7	**,000*****
BUN (mg/dL)	43	16	16	5	**,000*****
hsCRP (mg/dL)	1,028	2,328	,737	1,057	**NS**
Creatinine (mg/dL)	2,2	1	,8	,3	**,000*****
Total Cholesterol (mg/dL)	194	41	204	49	**NS**
Triglycerides (mg/dL)	182	84	137	54	**,039***
HDL-C (mg/dL)	48	10	53	13	**NS**
LDL-C (mg/dL)	109	28	124	45	**NS**
C-peptide (ng/mL)	2.25	0.86	2.60	1.2	**NS**
HbA1c (%)	7.5	1.6	5.3	0.6	**,002****
Urinary Albumin/Creatinine ratio (mg/g)	310	287	3.9	1.8	**,000*****

BMI: body mass index; BUN: Blood urea nitrogen; hsCRP: high sensitive C-reactive protein; NS: not significant.

Table 2. Comparison of ICAM 1, VCAM 1 and CD146 levels between the T2DM patients and the control group.

	T2DM		Control		
	Mean	SD	Mean	SD	**p**
VCAM1 (ng/mL)	24.81	9.92	32.00	16.02	**NS**
ICAM1 (ng/mL)	3.639	0.786	4.269	0.907	**,013***
CD31-CD146+ (%)	,05	,05	,01	,01	**NS**
CD31+CD146+	,12	,17	,01	,02	**NS**

(%)					
CD31+CD146- (%)	1,87	6,51	,16	,12	**NS**

Table 3. Comparison of demographic, biochemical and endothelial parameters between the T2DM patients with and without neuropathy.

T2DM Patients	**Without neuropathy**		**With neuropathy**		
	Mean	SD	Mean	SD	p
Age (years)	65,80	11,39	58,22	13,45	**NS**
BMI (kg/m2)	32,220	5,136	31,933	6,893	**NS**
hsCRP (mg/dL)	,504	,451	2,193	4,034	**NS**
Glucose (mg/dL)	141	44	215	81	**,004****
BUN (mg/dL)	45	17	39	16	**NS**
Creatinine (mg/dL)	2,4	1,1	1,7	,6	**NS**
Total Cholesterol (mg/dL)	199	42	182	38	**NS**
Triglycerides (mg/dL)	183	87	180	83	**NS**
HDL-C (mg/dL)	48	9	48	13	**NS**
LDL-C(mg/dL)	114	27	98	28	**NS**
Insulin (pmol/L)	45,560	45,220	53,093	54,645	**NS**
C-peptid (ng/mL)	1,7	1	1,6	,85	**NS**
Urinary Albumin/Creatinine ratio (mg/g)	220	250	300	310	**NS**
HbA1c (%)	7,1	1	8,7	2,1	**,015***
Diabetes Duration (years)	11,89	8,76	15,11	6,81	**NS**
VCAM1 (ng/mL)	25.14	10.21	24.10	9.79	**NS**
ICAM (ng/mL)	3.73	8.63	3.43	0.57	**NS**
CD31-CD146+ (%)	,05	,06	,04	,05	**NS**
CD31+CD146+ (%)	,11	,16	,14	,21	**NS**
CD31+CD146- (%)	2,45	7,81	,56	,73	**NS**

Table 4. Comparison of demographic, biochemical and endothelial parameters between the T2DM patients with and without DR.

T2DM patients	**Without retinopathy**		**With retinopathy**		
	Mean	SD	Mean	SD	p
Age (years)	65,00	11,85	62,35	12,93	**NS**
BMI (kg/m2)	32,775	4,981	31,676	6,126	**NS**
hsCRP (mg/dL)	,565	,413	1,355	3,015	**NS**
Glucose (mg/dL)	143,75	62,75	179,18	67,17	**NS**
BUN (mg/dL)	41	20	46	15	**NS**
Creatinine (mg/dL)	2,233	1,065	2,206	1,069	**NS**
Total Cholesterol	189,92	30,53	197,12	48,25	**NS**

(mg/dL)					
Triglycerides (mg/dL)	185,00	89,81	181,18	83,75	**NS**
HDL-C (mg/dL)	48,08	8,71	48,59	12,40	**NS**
LDL-C (mg/dL)	104,92	21,43	112,29	33,16	**NS**
Insulin (pmol/L)	18,030	17,410	59,650	49,260	**NS**
C-peptide (ng/mL)	1,477	,532	1,756	1,055	**NS**
Urinary Albumin/Creatinine ratio (mg/g)	225	265	257	285	**NS**
HbA1c (%)	7,508	1,917	7,800	1,457	**NS**
Diabetes Duration (years)	9,73	9,09	15,00	7,08	**NS**
VCAM1 (ng/mL)	26.06	9.16	23.93	10.61	**NS**
ICAM (ng/mL)	3.66	062	3.62	0.90	**NS**
CD31-CD146+ (%)	,03	,02	,06	,07	**NS**
CD31+CD146+ (%)	,05	,08	,16	,20	**NS**
CD31+CD146- (%)	,76	1,69	2,65	8,40	**NS**

Table 5. Comparison of demographic, biochemical and endothelial parameters between the T2DM patients with only DKD vs complications accompanied by DKD.

T2DM patients	**With nephropathy only**		**Nephropathy accompanied with microvascular complications**		
	Mean	SD	Mean	SD	**p**
Age (years)	65	11.1	63	12.1	**NS**
BMI (kg/m2)	32.57	4.46	31.75	6.20	**NS**
hsCRP (mg/dL)	0.74	0.78	1.13	2.1	**NS**
Glucose (mg/dL)	140.1	51.9	163.7	65.8	**NS**
BUN (mg/dL)	46	19	42	20	**NS**
Creatinine	2.34	0.97	2.03	1.11	**NS**

(mg/dL)					
Total Cholesterol (mg/dL)	208.1	64.1	194.3	45.5	**NS**
Triglycerides (mg/dL)	166.6	89.6	188.7	90.3	**NS**
HDL-C (mg/dL)	52.3	26.9	48.5	14.4	**NS**
LDL-C (mg/dL)	124.6	45.2	108.7	33.9	**NS**
Insulin (pmol/L)	30.1	22.9	45.6	42.9	**NS**
C-peptide (ng/mL)	2	1.28	2.2	1.32	**NS**
Urinary Albumin/Creatinine ratio (mg/g)	275	258	206	322	**NS**
HbA1c (%)	7.33	1.33	7.59	1.46	**NS**
Diabetes Duration (years)	8.6	10.0	14.1	7.0	**NS**
VCAM1 (ng/mL)	25.57	12.7	22.22	13.8	**NS**
ICAM1 (ng/mL)	3.90	0.59	3.76	0.77	**NS**
CD31-CD146+ (%)	0.049	0.038	0.039	0.050	**NS**
CD31+CD146+ (%)	0.087	0.18	0.087	0.13	**NS**
CD31+CD146- (%)	0.83	1.61	1.33	5.82	**NS**

Dıscussıon

In the present study, we investigated whether circulating levels of ICAM-1, VCAM-1 and CD146 are correlated with endothelial damage leading to diabetic microvascular complications. According to our results, sICAM-1 levels are

considerably lower in T2DM patients with microvascular complications compared with age-matched healthy controls irrespective of the type of diabetic complications. The levels of other sCAMs did not differ between the groups. As a result of long term hyperglycemia and having complications via inflammatory processes, glucose, Hb A1c, creatinine and BUN leves were significantly higher in the T2DM group. We did not detect any significant difference between lipoprotein levels, however, triglyceride levels were notably higher in the T2DM group.

DKD was present in all study group, whereas it is accompanied with/by DN in 13 and DR in 37 of the patients. When patients were divided into groups according to type of microvascular complications, there was not difference between the levels of circulating CAMs. However, glucose and HbA1c levels were significantly higher in the DN group, as a natural consequence of uncontrolled, long duration of T2DM.

When compared with healthy controls, decreased levels of sICAM-1 in the study group might be a cause of late-stage diabetic complications. Supporting this hypothesis, studies have shown decreased or stabilized levels of sCAMs in unstable diabetic patients (12, 13).

Additional evidence comes from recent studies showing the concentrations of soluble CAMs reflect the degree of endothelial damage in the progression of diabetic complications (14-16).

The conflicting evidence about the levels of sCAMs in the circulation suggests different CAMs may play different roles in different stages of microangiopathy in the course of T2DM since endothelial cells isolated from diabetic patients express higher amounts of sVCAM-1 compared to sICAM-1 when stimulated by cytokines in a high-glucose mediated microenvironment (17, 18). In a study of patients with T2DM, the sICAM-1 and sVCAM-1 concentrations were found to be higher in the study group. However, plasma VCAM-1, but not ICAM-1, was independently associated with DKD suggesting that ICAM-1 and VCAM-1 may play different roles in different stages of the same disease (19).

Increased levels of ICAM-1 have been reported in the diabetic retina in the early stages of retinopathy suggesting that ICAM-1 mediates the adhesion and transendothelial migration of circulating leukocytes through retinal vessel walls, one of the earliest pathological changes observed in the development of diabetic retinopathy (19). Further research has shown that the plasma CD146 levels are elevated at the early stage of diabetic complications (20).

Additional data showed the increased expression of adhesion molecules in kidneys during the progression of DKD in T2DM duration (21). This is consistent with our findings that, the elevated levels of sCAMs were observed in the

early stages of diabetic complications, and the excess amount of CAMs might be localized on the inflammation sites such as microvessels and thus, in the late stages, their levels could be decreased or diminished in the circulation.

Yet, the study by Guler et al. and Lu et al. comparing patients with T2DM with and without DKD revealed higher mean levels of sICAM-1 in the patients with DKD, compared to the patients without nephropathy.

According to the theory of Kanasaki K et al., the binding between CAMs and their receptors can further aggravate tissue damage by the aggregation of leukocytes on endothelial cells. Also, impaired blood flow increase glomerular capillary pressure and stimulates the ICAM-1 expression by endothelial cells resulting in chronic inflammation (22).

In order to prevent mentioned damages, ACE inhibitors are used in the treatment of the patients. There are numerous studies reporting Angiotensin II to increase endothelial cell death (7).

A vasodilator molecule, NO has anti-inflammatory properties and decreases VCAM expression (23). It has been shown that inhibition of ACE stimulates nitric oxide (NO) production by increasing bradykinin (24). Furthermore, ACE inhibitors have been shown to reduce circulating levels of CAM in T2DM patients (25).

Insulin has been shown to be an arterial and venous vasodilator via stimulation of NO release by increasing endothelial nitric oxide synthase (e-NOS) expression and inhibitor of ICAM-1 expression through the induction of NOS and NO generation (26). Supporting this hypothesis, majority of our patients in the study group were on therapy with insülin and ACE inhibitors. However, why only the ICAM-1 levels are found to be decreased in a correlation with the presence of diabetic microvascular complications needs more investigation. It is also worth mentioning that, CRP levels did not differ between groups, offering a non-inflammatory microenvironment and in this lack of association, it is not expected to find increased levels of inflammatory substances. In summary, our study shows that, in late-stage T2DM with complications, circulating ICAM levels are decreased, whereas other components of our study panel, VCAM and MCAM levels were not found to be different between all groups suggesting a local inflammatory model for these molecules. Also, wide use of ACE inhibitors in our study group could be a reason for these findings, by their vasodilator and anti-inflammatory properties. In conclusion, our data suggest that circulating levels of CAMs are not associated with chronic and late-stage complications in T2DM patients, but, their role in different stages of inflammatory process in diabetic patients should further be studied.

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