The Levels of the Circulating Cellular Adhesion Molecules ICAM-1, VCAM-1 and Endothelin-1 and the Flow-mediated Vasodilatation Values in Patients with Type 1 Diabetes Mellitus with Early-stage Diabetic Retinopathy

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Objective Endothelial dysfunction plays an important role in the development of diabetic retinopathy. The aim of this study was to evaluate endothelial dysfunction using different approaches in patients with type 1 diabetes mellitus with early stages of diabetic retinopathy. For this purpose, we investigated the serum levels of cellular adhesion molecules, including intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1) and endothelin-1 (ET-1), which have emerged as specific markers of endothelial dysfunction, and measured the flow-mediated dilatation (FMD), a noninvasive technique used to evaluate endothelial dysfunction.

Methods The study group included 59 patients with type 1 diabetes mellitus (DM) and 30 age-matched healthy control subjects. The diabetic patients were divided into two groups according to the ophthalmoscopic findings: Group 1, composed of type 1 diabetic patients having no signs of diabetic retinopathy (DRP), and Group 2, composed of type 1 diabetic patients having findings of the early stages of nonproliferative diabetic retinopathy (NPDR).

Results The serum levels of ET-1 (fmol/mL), ICAM-1 (ng/mL) and VCAM-1 (ng/mL) were 8.52±0.699 vs. 478.39±46.22 vs. 728.64±35.081 in the patients without retinopathy, 8.91±1.354 vs. 451.79±48.262 vs. 863.59±62.37 in the diabetic patients with NPDR and 10.73±1.04 vs. 608.15±74.92 vs. 872.95±57.63 in the control group. There were no significant differences in the serum levels of the three molecules between the groups. The FMD values were 6.51±0.46% in the diabetic patients without retinopathy, 6.66±0.29% in the diabetic patients with NPDR and 6.68±0.51% in the control group. No significant differences were found between the groups.

Conclusion The early stages of diabetic retinopathy cannot be considered in the evaluation of systemic markers of endothelial dysfunction.

Key words: endothelial dysfunction, FMD, diabetic retinopathy


Introduction

Diabetic retinopathy (DRP) is one of the most common and potentially sight-threatening microvascular complications of diabetes mellitus (DM). It is the most common cause of preventable vision loss among individuals 20 to 74 years of age worldwide (1). Many basic and clinical studies...
have been conducted to elucidate the pathophysiologic mechanisms underlying the development of diabetic retinopathy and determine the risk factors in order to decrease the prevalence of the disease (2). There is a considerable body of evidence demonstrating that endothelial dysfunction plays an important role in the pathogenesis of DRP development (3).

The endothelium forms the inner layer of blood vessels and exhibits a diverse spectrum of actions, such as regulating vascular tone, permeability and the balance between coagulation and fibrinolysis and controlling the adhesion and extravasation of leukocytes. Endothelial dysfunction is defined as the alteration of the properties of the endothelium. Due to its multifactorial nature, endothelial dysfunction can be evaluated using many different approaches (4-7). The measurement of the serum levels of endothelium-derived cellular adhesion molecules (CAMs), such as endothelin-1 (ET-1), intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), E-selectin and von Willebrand factor, and endothelium-dependent vasodilation in addition to widely used indirect methods are employed to estimate the degree of endothelial dysfunction (6).

ICAM-1, VCAM-1 and ET-1 are important markers of endothelial dysfunction that have been demonstrated to play important roles in the development of DRP. ICAM-1 and VCAM-1 mediate leukocyte adhesion to the retinal vasculature, one of the earliest pathological changes in DRP (8, 9). The ICAM-1 level has been shown to be increased in the diabetic retina, even in the early stages of retinopathy (10). Moreover, the administration of neutralizing anti-ICAM-1 antibodies causes a dramatic reduction in the incidence of leukocyte-related pathologies in newly diabetic animals (8).

ET-1 is one of the most potent vasoconstrictor molecules causing abnormalities in retinal hemodynamics, thereby contributing to the development of DRP (11, 12). The role of ET-1 in the pathogenesis of DRP has been demonstrated in many studies investigating streptozocin-induced diabetic rats (13).

However, in spite of the consensus about the roles of these molecules in the development of diabetic retinopathy, findings regarding the serum ICAM-1 and VCAM-1 levels in DRP patients are conflicting. Some studies have reported significant increases in the serum concentrations in patients with DRP compared with that observed in controls or diabetic groups without retinopathy (14-16). In contrast, other studies have reported no significant differences in these levels between diabetic patients with and without DRP and control groups (17, 18).

Flow-mediated endothelial dependent vasodilation (FMD) of the brachial artery is a noninvasive ultrasound method capable of reflecting an impaired endothelial function dependent on the decreased release of nitric oxide. For more than two decades, it has been used to assess endothelial dysfunction in patients with DM and other vascular diseases (6, 7).

There is debate in the literature regarding the FMD values of diabetic patients with or without diabetic retinopathy. Some studies have reported statistically significant differences in the FMD values of diabetic patients with retinopathy compared with those observed in both diabetic patients without diabetic retinopathy and healthy control subjects (19, 20), while other studies have reported no significant differences between these groups (21, 22).

This is the first study reported in the literature to evaluate the degree of endothelial dysfunction in the early stages of diabetic retinopathy in type 1 diabetic patients. Subjects with type 2 diabetes were not evaluated in this study since they have comorbidities, such as obesity, hypertension and antihypertensive medication use, that may affect endothelial dysfunction, in contrast to patients with type 1 diabetes.

The aim of this study was to investigate the degree of endothelial dysfunction using different approaches and to evaluate the value of these approaches as early indicators of DRP.

Materials and Methods

The study was conducted at the Department of Ophthalmology, Ankara Atatürk Research and Training Hospital. The study protocol was approved by the Ethics Committee of the Atatürk Research and Training Hospital and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice Guidelines. The participants were informed of the nature of the study, and informed consent was obtained from each patient.

Subjects

A total of 59 patients with type 1 DM and 30 aged-matched healthy control subjects were included in this study. Detailed medical histories were obtained from all participants. Associated diseases, the duration of diabetes, cigarette smoking and medications were recorded. A general physical examination was carried out in all participants. The subjects continued their insulin regimen of multiple dose injections (MDI) or continuous subcutaneous insulin infusion (CSII). The subjects treated with MDI received basal insulin injections the night prior to the study, and the subjects treated with CSII continued with their basal insulin infusion over-night. In order to limit confounding effects on the endothelial function, subjects with a BP >95th percentile, uncorrected hypothyroidism, overt nephropathy or early renal failure (random urine microalbumin/creatinine >0.02 mg albumin/mg creatinine; serum creatinine >1.0 mg/dL) and those who were pregnant were excluded.

All subjects underwent detailed ophthalmologic examinations, including an assessment of the best-corrected visual acuity, applanation tonometry, anterior segment slit lamp biomicroscopy and a dilated fundus examination. Colored fundus photographs were obtained of all subjects using a non-mydriatic retinal camera (CR2-45 NM Non-mydriatic fundus Camera; Canon Inc., Japan). Patients with early findings of DRP without diabetic retinopathy were recruited. Early findings of DRP were accepted as mild and moderate
retinopathy findings, according to the American Academy of Ophthalmology (AAO) classification system based on the Early Treatment Diabetic Retinopathy study (23). The diabetic subjects were divided into two groups according to the ophthalmoscopic findings: Group 1 included diabetic patients with no diabetic retinopathy findings and Group 2 included diabetic patients with the early stages of DRP non-proliferative diabetic retinopathy (NPDR). Group 3 included healthy control subjects.

Biochemical tests

Blood samples were drawn into plain vacutainers from the antecubital vein in the healthy controls and patients after 12 hours of fasting. The blood was allowed to clot for 30 minutes, then the serum specimens were centrifuged for 10 minutes at 4,000 rpm, transferred to Eppendorf tubes and stored at -80°C until the analysis. The serum levels of fasting glucose, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDLC) cholesterol, triglycerides and creatinine were measured using enzymatic methods. The levels of glycosylated hemoglobin (HbA1c) were measured using high-performance liquid chromatography.

The concentrations of the adhesion molecules were detected using commercial ELISA kits (Endothelin-1 Biomedica Gruppe, SICAM-1 and SVCAM-1 Bender medsystems GmbH, Vienna, Austria). The measurements were calculated on a micro-ELISA reader KHB ST-360, washer KHB ST-36W. The intra-assay and interassay precision coefficients of variation (%) were as follows: Endothelin-1 4.0/6.0, sICAM-1 4.1/7.7, sVCAM-1 3.1/5.2.

Evaluation of the endothelial function

The endothelial dysfunction of the brachial artery was evaluated using a flow-mediated vasodilatation test. The test was performed according to the guidelines defined by Corretti et al. (24) in a quiet room at a temperature of 22-25°C in the morning (between 8:00-9:00 AM) after 12 hours of fasting. The use of alcohol, caffeine or vasoactive drugs and smoking were restricted during the fasting period. The left brachial artery was used in all subjects. The transducer was placed 5 cm above the antecubital fossa and the artery was longitudinally viewed at the best region along its route. The amplification and focusing properties of ultrasonography were used to enlarge the images. The artery segment 5-7 cm superior to a point 5 cm above the antecubital fossa was used to standardize the region of measurement in all subjects. During the measurements, real-time electrocardiogram (ECG) recordings were obtained via an ECG connected to the ultrasonography equipment. The lumen of the artery was measured at the segment between the T wave and the Q wave on the ECG recordings. The lumen size was accepted as the distance between the lumen-intima reflections of the anterior and posterior walls. The diameter of the brachial artery was measured three times (the distance between two intima), and the average was accepted as the basal diameter. The flow rate was recorded during the peak of systole via pulse Doppler at the area in which the diameter was measured. After the basal value was obtained, the cuff of the sphygmomomanometer was placed above the brachial artery segment for blood pressure measurement. The cuff was inflated to a pressure of 250 mmHg or 50 mmHg higher than the systolic blood pressure level and maintained at this pressure. Then, the cuff was deflated, and the flow rate was measured for 15 seconds via pulse Doppler and recorded (hyperemic response). The brachial artery segment diameter was measured at 1, 2 and 3 minutes. The highest value was accepted as the reference value for the maximum dilatation capacity and used to calculate the flow-mediated dilatation. The flow-mediated vasodilatation value was accepted as the percent change of the vessel width compared to the basal value. The flow-mediated dilatation was calculated using the formula 

\[ \text{Flow-mediated dilatation} = \left( \frac{\text{Diameter max} - \text{Diameter basal}}{\text{Diameter basal}} \right) \times 100 \]

Statistical analysis

An analysis of variance (one-way ANOVA) was performed to evaluate the differences in continuous variables between the groups. Tukey’s test was also used to evaluate the differences between the groups. A p value of less than 0.05 was considered to be statistically significant. The statistical analyses were performed using the SPSS software package (version 10.0; SPSS Inc., Chicago, IL).

Results

The general characteristics of the study groups are demonstrated in Table 1. There were no significant differences in the age or gender distribution between the groups. Among the diabetic patients, the mean HgbA1c was 9.03±1.3% and the mean duration of diabetes was 9.7±6.12 years. No significant differences were observed in the HbA1c levels or the duration of diabetes between the diabetic patients without retinopathy and the diabetic patients with NPDR. The serum LDL cholesterol levels were significantly reduced in the diabetic patients without retinopathy compared with those observed in the diabetic patients with NPDR (92.13±6.75 vs. 117.4±7.35, p=0.047). No differences were observed in the serum levels of HDL cholesterol, triglycerides or C-reactive protein [CRP, (p=0.110, p=0.25, p=0.129, respectively), Table 1].

The degree of endothelial dysfunction was evaluated using two different approaches in the present study. The ET-1 levels (fmol/mL) were 8.52±0.699 the diabetic patients without retinopathy, 8.91±1.35 in the diabetic patients with NPDR and 10.73±0.04 in the control group. There were no significant differences between the groups (p=0.239). In addition, no significant differences were observed in the
Table 1. General Characteristics of Study Groups. Values are Mean±SE

<table>
<thead>
<tr>
<th></th>
<th>Patients without retinopathy</th>
<th>Patients with NPDR</th>
<th>Control subjects</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.68 (1.344)</td>
<td>31.83 (2.38)</td>
<td>31.71 (0.73)</td>
<td>0.186</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.11 (0.27)</td>
<td>8.90 (0.24)</td>
<td>-</td>
<td>0.827</td>
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<tr>
<td>LDL cholesterol</td>
<td>92.12 (6.75)</td>
<td>117.4 (7.35)</td>
<td>108.31 (8.43)</td>
<td>0.047</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>56.94 (2.63)</td>
<td>55.55 (3.79)</td>
<td>48.38 (2.24)</td>
<td>0.110</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>107.75 (9.75)</td>
<td>118.05 (13.99)</td>
<td>145.33 (26.05)</td>
<td>0.25</td>
</tr>
<tr>
<td>CRP</td>
<td>0.83 (0.03)</td>
<td>0.96 (0.97)</td>
<td>1.32 (0.36)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Table 2. Serum Levels of Cellular Adhesion Molecules and FMD Measurements in Study Groups. Values are Mean±SE

<table>
<thead>
<tr>
<th></th>
<th>Patients without retinopathy</th>
<th>Patients with NPDR</th>
<th>Control subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endotelin-1 (fmol/mL)</td>
<td>8.52 (0.699)</td>
<td>8.91 (1.35)</td>
<td>10.73 (1.04)</td>
<td>0.239</td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>478.39 (46.22)</td>
<td>451.79 (48.262)</td>
<td>608.15 (74.92)</td>
<td>0.150</td>
</tr>
<tr>
<td>VCAM-1 (ng/mL)</td>
<td>728.64 (35.081)</td>
<td>863.59 (62.37)</td>
<td>872.95 (57.63)</td>
<td>0.055</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>6.51 (0.46)</td>
<td>6.66 (0.29)</td>
<td>6.68 (0.51)</td>
<td>0.959</td>
</tr>
</tbody>
</table>

VCAM-1 or ICAM-1 levels between the groups (p=0.959 and p=0.150, respectively). The serum levels of VCAM-1 and ICAM-1 were 728.64±35.081 ng/mL vs. 478.39±46.22 ng/mL in the diabetic patients without retinopathy, 863.59±62.37 ng/mL vs. 451.79±48.26 ng/mL in the diabetic patients with NPDR and 872.95±57.63 ng/mL vs. 608.15±74.92 ng/mL in the control group (Table 2).

The FMD values were also used to assess the degree of endothelial dysfunction. There were no significant differences in the FMD values between the diabetic patients without retinopathy (6.51±0.46%), the diabetic patients with NPDR (6.66±0.29%) and the subjects in the control group (6.68±0.51%) (Table 2).

Discussion

The results of the present study demonstrated that there are no significant differences in the serum levels of three cellular adhesion molecules, Endothelin-1, ICAM-1 and VCAM-1, between type 1 diabetic patients with or without early findings of DRP and healthy controls. In this study, the FMD measurements were also not significantly different between the groups.

Increased levels of ICAM-1 have been reported in the diabetic retinal vasculature of both humans and rodents (8-10). ICAM-1 mediates the attachment and transendothelial migration of circulating leukocytes into the retinal vasculature. Leukocyte adhesion to the retinal vasculature is one of the earliest pathological changes observed in the development of diabetic retinopathy and leads to enhanced vascular permeability, endothelial cell damage and capillary non-perfusion (8, 9). In addition, the incidence of leukocyte-related changes is reduced by the administration of neutralizing anti-ICAM-1 antibodies in newly diabetic animals. Therefore, increased ICAM-1 levels have been suggested to play a crucial role in the development of DRP. VCAM-1 is also an adhesion molecule that mediates leukocyte adhesion and is considered to play an important role in the development of DRP (25). ET-1 has been demonstrated to play a role in the development of DRP in diabetic animals (13). It has been suggested that autoregulation of the retinal blood flow is impaired by ET-1, leading to hypoperfusion resulting in microaneurysms and edema (11, 12). Moreover, ET-1-mediated vasoconstriction has been suggested to trigger a hypoxic state, which may play a role in the pathological angiogenesis observed in patients with DRP (26).

These findings provide evidence that the upregulation of ICAM, VCAM and ET-1 in the diabetic retina plays an important role in the development of DRP. In addition, the serum levels of these molecules have been assessed in many studies; however, the results are not consistent (14-18). It has been reported that the serum ICAM-1 and VCAM-1 levels are significantly increased in diabetic patients with retinopathy compared with those observed in patients without retinopathy and healthy controls (14, 15). However, it also has been reported that there are no differences in the VCAM-1 and ICAM-1 levels between diabetic patients with or without retinopathy (17, 18). Furthermore, it has been reported that only the serum levels of VCAM-1 differ between diabetic patients with or without retinopathy, while the ICAM-1 levels are similar between these groups (27, 28), or that only the ICAM-1 levels are increased, with similar VCAM-1 levels, in diabetic patients with retinopathy (22). We also observed no significant differences in the serum
levels of ICAM-1, VCAM-1 and ET-1 between the groups in the present study.

There are several reasons for these discrepancies. First, the study groups had different general characteristics that may have affected the serum levels of the adhesion molecules. Moreover, different classifications of retinopathy were selected, and patients with different stages of retinopathy were evaluated, making reliable comparisons difficult. In addition, it should be noted that the retina has its own microcirculation that is controlled by autoregulatory processes. The production of these three molecules is controlled by several local stimuli, including dyslipidemia and the activation of vascular endothelial growth factor (VEGF) and Poly (ADP-ribose) polymerase [PARP (29-31)]. Therefore, the levels observed in retinal tissues may be upregulated by these molecules, which may not be detected when measuring the serum levels, at least in the early stages of DRP.

We also evaluated the degree of endothelial dysfunction based on FMD measurements and found no significant differences in the FMD values between the groups. There are conflicting results regarding FMD values in diabetic patients with or without diabetic retinopathy. Several studies have evaluated the FMD values in diabetic patients with and without retinopathy and reported no significant differences between the groups (21, 22). Other studies, in contrast, have reported significant differences in the FMD values between patients with DRP and patients without DRP (19, 20). However, the important point, as previously stated, is that the selected diabetic retinopathy classification system and selected groups of patients differed in these studies. The study groups consisted of either patients with all stages of DRP or primarily patients with proliferative DRP only. There is a very limited number of studies in the literature assessing the FMD values in patients with mild or moderate DRP (19-22). Sogawa et al. divided patients with DRP into five groups according to the AAO classification system. They evaluated the FMD values in patients with different stages of retinopathy and reported no significant differences in the FMD values between the No Diabetic retinopathy (NDR), mild DRP and moderate DRP groups (19). The results of the present study are consistent with the findings of that study and demonstrated that the FMD values in patients with early-stage DRP are not significantly different from those observed in control groups.

Another important point that should be taken into consideration is that, in all of these studies, the patient groups had a diagnosis of type 2 diabetes with comorbidities that frequently accompany type 2 DM and affect the FMD values, such as hypertension, which is a risk factor for the development of DRP (32) and reduces the FMD values (33). Moreover, antihypertensive medications also affect the FMD values (33). In the present study, all of the patients had type 1 DM and none had hypertension or were using antihypertensive medication. Olsweska et al. evaluated the association between the FMD values and NDRP in young, type 1 DM patients. Although the number of patients was very small (five) the authors reported that they found no significant differences in the FMD values between the patients with type 1 diabetes with or without NPDR and the controls (22). Our findings are consistent with the results of this study, and we evaluated a larger number of patients.

Our findings and previous similar results declaring that no significant associations are found between the FMD values and the presence of early-stage DRP may have several explanations. First, the FMD values measure the degree of endothelial dysfunction in large arteries in a noninvasive manner, and it may be difficult to assess retinal arteriolar endothelial dysfunction using the same approach, especially in the early stages. Second, the FMD measurements may not be sufficiently sensitive to assess levels of endothelial dysfunction that cause only mild retinal disorders. Finally, the retinal microcirculation may have unique additional features that are different from those of the systemic circulation, and no direct correlations may exist between early large vessel disorders and early retinal microvascular disease. Therefore, large arterial endothelial dysfunction cannot be used as an indicator of retinal microvascular endothelial dysfunction in the early stages of diabetic retinopathy.

In conclusion, endothelial dysfunction markers present in the systemic circulation and the FMD values do not appear to be suitable for assessing retinal vascular changes, at least in the early stages of diabetic retinopathy. Markers and tests specific to the retinal microvasculature should be investigated in order to study changes in DRP.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement
This research was supported by the Turkish Ophthalmology Society.

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