Microvascular abnormalities in capillaroscopy correlate with higher serum IL-18 and sE-selectin levels in patients with type 1 diabetes complicated by microangiopathy

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Abstract: Microvascular abnormalities are one of the most important causes of persistent diabetic complications. The aim of this study was to compare microvascular changes examined by nailfold capillaroscopy with serum concentrations of soluble E-selectin (sE-selectin) and IL-18 in type 1 diabetic patients with and without microangiopathy. Serum levels of sE-selectin and IL-18 were determined by an enzyme-linked immunosorbent assay in 106 patients with type 1 diabetes and in 40 healthy controls. All diabetic patients were evaluated by extensive clinical, laboratory and capillaroscopic studies. Morphological changes were observed by nailfold capillaroscopy in 86 out of 106 (81%) diabetic patients. Severe capillaroscopic changes were seen in 32 out of 54 (59%) patients with microangiopathy, but in only seven out of 52 (13%) patients without microangiopathy. Higher serum levels of sE-selectin (p < 0.001) and IL-18 (p < 0.05) were demonstrated in diabetic patients compared to controls. Significant differences of sE-selectin (p < 0.001) and IL-18 (p < 0.01) serum concentrations were observed between diabetic patients with microangiopathy and controls. Moreover, comparison between patients with and without microangiopathic complications showed a significantly higher capillaroscopic score and sE-selectin serum concentration in the group with microangiopathy (p < 0.001). Furthermore, diabetic patients with severe microvascular changes in capillaroscopy showed significantly higher IL-18 (p < 0.001) and sE-selectin (p < 0.05) serum levels than subgroups without changes or with mild abnormalities. Our findings suggest that abnormalities in nailfold capillaroscopy may reflect the extent of microvascular involvement and are associated with higher sE-selectin and IL-18 serum levels, as well as with microangiopathic complications in diabetic patients. (Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 1, pp. 104–110)

Key words: capillaroscopy, IL-18, soluble E-selectin, type 1 diabetes, microangiopathy

Introduction

Microvascular abnormalities are one of the most important causes of persistent diabetic complications. Endothelial dysfunction plays a key role in the development of vascular disease and represents an early stage in the pathogenesis of diabetic microangiopathy [1]. Damaged or activated endothelial cells are a target and the source of various factors responsible for the control of vascular tone, coagulation systems and inflammatory processes, among which endothelial selectin (E-selectin) has attracted attention as a speci-
Capillaroscopy and microcirculation in type 1 diabetes

Material and methods

Patients

The study included 106 patients with type 1 diabetes, treated in the Clinical Department of Endocrinology, Diabetology and Internal Diseases and Diabetic Polyclinic of Clinical Hospital of Medical University in Bialystok. The study was approved by the Ethics Committee of the Medical University of Bialystok (resolution No. R-I-002/96/2008), and informed consent was obtained from each patient.

All patients were evaluated by clinical, laboratory and capillaroscopic studies on the day of blood sample collection. The weight and height of all patients was recorded, their BMI calculated and biochemical parameters, such as HbA1c (HPLC Variant, BIO-RAD Laboratories, Germany), total cholesterol, HDL-cholesterol, triglycerides (TG) (enzyme assay, ANALCO-GBG, Poland) and LDL-cholesterol (calculated from Friedewald formula), were measured.

Based on the medical records, the prevalence and progress of chronic complications were determined. According to clinical and laboratory examinations, the whole group of diabetic patients was subdivided into patients without (Group A, n = 52) and with microangiopathy (Group B, n = 54) i.e. retinopathy (45 patients), nephropathy (29 patients) and/or neuropathy (41 patients). All diabetic patients were on insulin therapy comprising at least three insulin doses per day. Exclusion criteria from our study were: vascular coexistent disorders, hypertension, hepatopathy, smoking, active infection, vascular effective drug use and hand lesions.

The control group consisted of 40 healthy volunteers comparable in terms of age, sex, and body mass index. Table 1 shows the characteristics of the type 1 diabetic patient group and the control group.

Clinical and laboratory analysis

Ophtalmological and neurological examinations, renal ultrasonography, renal function tests, peripheral blood cell counts, hemoglobin concentration, serum creatinine, creatinine clearance, urinalysis, albuminuria, daily urinary protein excretion and erythrocyte sedimentation rate (ESR) were performed for all patients.

Serum specimen preparation

For the determination of serum analyses, venous blood samples were collected, after overnight fasting,
in tubes without additives. Serum was prepared from blood samples by centrifugation at 3,000 g for 10 minutes and then immediately transferred in aliquots into plastic tubes and stored at −80°C.

**Laboratory studies**

The serum levels of sE-selectin and interleukin 18 concentrations were analyzed by ELISA kits (Parameter Human sE-Selectin Immunoassay, R&D Systems and Medical & Biological Laboratories, Freiburg, Germany). The experimental procedures were performed adhering strictly to the manufacturer’s instructions.

**Nailfold capillaroscopy**

All patients underwent a nailfold capillaroscopy examination using a stereomicroscope SZ 4045 with a final magnification of × 200 (Olympus, Germany). A fiber-optic light source and filter provided cold illumination. The optical microscope was connected to a color digital camera and a personal computer. The images taken at the time of examination were analyzed by the same experienced investigator (AKM). Each patient was allowed to acclimatize for 20 minutes at a room temperature of 20–24°C before the examination.

The nailfold of all fingers (not the thumbs) were examined in 10–100 zoom after a drop of immersion oil was placed on the nailfold bed. The mean score for each patient was obtained from the analysis of all studied fingers. The loop density in 1 mm, numbers and morphology of loops with architectural derangement, and the presence of extravasations were evaluated. The intensity of morphological changes was expressed according to the accepted classification [8, 17] and ranged from 0 to 3: a score of 0 indicated no changes (< 13 capillaries/mm, hairpin-shaped loops arranged in parallel rows, absence of hemorrhages); a score of 1 = mild (< 13 capillaries/mm, < 20% capillaries longer than 750 μm, < 50% morphologically changed capillaries, arranged in parallel rows, absence of hemorrhages); a score of 2 = moderate (< 9 capillaries/mm, more than 20% capillaries longer than 750 μm, 50–75% changed loops with irregular distribution of the capillary array, increased visibility of sub-papillary venular plexus, without extravasations); and a score of 3 = severe changes (< 9 capillaries/mm, capillary length variability, more than 75% changed loops such as meandering, ramified and/or disarranged capillaries with the presence of hemorrhages).

**Statistical analysis**

Statistical analysis of the results was carried out using Statistica 8.0 software from StatSoft. Data was analyzed by the Mann–Whitney U-test. The probability of differences in frequency distributions was determined by chi-square test or Fisher’s exact test. In all calculations, p < 0.05 were considered statistically significant.

**Results**

There were no significant differences in age, gender, body mass, growth or BMI between the group of patients with type 1 diabetes and the healthy controls. Biochemical parameters such as HbA1c concentration, total cholesterol, triglycerides, LDL-cholesterol and creatinine were significantly higher, and HDL-cholesterol level was significantly lower, in diabetic patients compared to the control group (Table 1).

The NC patterns of 106 patients with type 1 diabetes and 40 healthy controls were examined in this study. The capillaroscopic feature of the control group showed hairpin capillaries in a parallel arrangement. All healthy controls reached a capillaroscopic score = 0.

Patients with type 1 diabetes had spiral loops with decreased density and dilatation of the apical part and the venous limb. Normal capillaroscopic patterns (score 0) were observed in 54 (29%) patients. Severe morphological changes in nailfold capillaroscopy were observed in 32 out of 54 (59%) patients with microangiopathy but only in seven out of 52 (13%) diabetic patients without microangiopathy.

A comparison between the type 1 diabetic patients with (Group B) and those without microangiopathy (Group A), found there to be a significantly higher capillaroscopic score in patients with (2.3 ± 1.0) than without microangiopathy (1.15 ± 1.0; p<0.001). Severe morphological changes in nailfold capillaroscopy were observed in 32 out of 54 (59%) patients with microangiopathy but only in seven out of 52 (13%) diabetic patients without microangiopathy.
capillaroscopic abnormalities, and in 40 healthy subjects. Higher serum concentrations of sE-selectin (p < 0.001) and IL-18 (p < 0.05) were observed in diabetic patients compared to the control group. Moreover, significant differences of sE-selectin (p < 0.001) and IL-18 (p < 0.01) serum concentrations were observed between diabetic patients with microangiopathy and controls. Although patients with microangiopathy demonstrated higher serum levels of IL-18 and sE-selectin than patients without microangiopathy, significant differences were only found in sE-selectin concentrations (p < 0.05) (Table 2).

To demonstrate the relationships between capillaroscopic abnormalities, sE-selectin and IL-18 serum levels, the four subgroups of diabetic patients were distinguished according to the severity of capillaroscopic changes. Patients with severe capillaroscopic abnormalities showed significantly higher sE-selectin and IL-18 serum levels than patients with normal capillaroscopic pattern or with mild changes (p < 0.05; p < 0.001, respectively) (Table 3).

**Discussion**

Although progress in diagnostic and treatment procedures has dramatically improved the quality of life for young diabetic patients, the mortality rate in this group is still significantly higher than in the general population, mostly due to the development of micro- and macroangiopathic complications. It is claimed that endothelial cell activation and damage play a potentially crucial role in the pathogenesis of microvascular complications in diabetes [1, 10]. Hence, the early diagnosis of vascular pathology is vital for the therapy of diabetic patients. Therefore, growing evidence points to the need for reliable methods of visualizing the morphological changes in microcirculation.

Nailfold capillary microscopy has been used extensively as a non-invasive means of investigating microvascular involvement. Our previous studies reported the diagnostic value of nailfold capillary abnormalities and their correlation with organ systemic

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristic of diabetic patients and controls</th>
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<tbody>
<tr>
<td>Characteristics</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Disease duration (years)</td>
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<tr>
<td>BMI [kg/m²]</td>
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<tr>
<td>HbA1c (%)</td>
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<tr>
<td>Total cholesterol [mg/dl]</td>
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<td>Triglycerides [mg/dl]</td>
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<td>HDL-cholesterol [mg/dl]</td>
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<td>LDL-cholesterol [mg/dl]</td>
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<tr>
<td>Serum creatinine [mg/dl]</td>
</tr>
<tr>
<td>Retinopathy (no. of patients)</td>
</tr>
<tr>
<td>Nephropathy (no. of patients)</td>
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<td>Neupropathy (no. of patients)</td>
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NS — non significant

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<th>Table 2. Serum concentrations of soluble E-selectin (sE-selectin) [ng/ml] and IL-18 [pg/ml] in patients with type 1 diabetes according to the presence of microangiopathy and in healthy controls. Data is shown as mean ± SD</th>
</tr>
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<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>sE-selectin</td>
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<td>IL-18</td>
</tr>
</tbody>
</table>

1Statistical significance: p < 0.001 compared to healthy controls; 2Statistical significance: p < 0.01 compared to healthy controls; 3Statistical significance: p < 0.05 compared to healthy controls; 4Statistical significance: p < 0.05 between both diabetic groups
involvement and immunological parameters of disease activity in patients with connective tissue diseases [8].

There is growing understanding of the microvascular abnormalities in diabetes [17–19], but little is known about the relationship between capillaroscopic abnormalities, immunological parameters and the clinical features of type 1 diabetes. Furthermore, according to recent studies, chronic inflammation leads to the impairment of endothelial function and microvascular complications in the course of diabetes [3, 10]. Our previous report showed a relation between the severity of capillaroscopic changes and metabolic control and the presence of persistent complications in patients with type 1 diabetes [18].

The present study evaluated the associations between nailfold capillaroscopic abnormalities, sE-selectin and IL-18 serum levels and the clinical features of a cohort of 106 patients with type 1 diabetes. The intensity of capillaroscopic changes, expressed according to the established classification, ranged from 0 to 3 [8, 18].

The results of this study confirmed the findings of other authors who have reported a pattern of capillary changes expressed by decreased density, dilatations of venous limb, increased tortuosity of vascular loops and microaneurysms in the apical part of the loop [17].

In the present study, severe capillaroscopic changes were observed frequently in diabetic patients complicated by microangiopathy. The mean capillaroscopic score in diabetic patients with microangiopathy was significantly higher than in the group without complications (p < 0.001).

Therefore, it may be suggested that microvascular abnormalities in NC are an indicator to microangiopathy in diabetic patients, and useful in monitoring the severity of vascular changes. It has been suggested that increased pressure in the capillary vascular bed [17], nonenzymatic glycation of basal membrane proteins [20], endothelial dysfunction and angiogenic activity [1, 10] are implicated in the pathogenesis of microcirculation disturbances in diabetes.

Our present study demonstrated the relationship between the severity of capillaroscopic abnormalities and disease duration. This may confirm our previous findings [18]. In addition, other authors have reported the correlation between microvascular changes in capillaroscopy and the occurrence of retinopathy in 25 patients with type 1 diabetes [21]. Furthermore, in patients with type 2 diabetes, a relationship has been shown between the occurrence of hemostatic loops and disease duration, as well as the presence of persistent complications [20]. However, other authors did not find differences between

### Table 3. Serum concentrations of IL-18 in patients with type 1 diabetes according to the severity of microvascular changes in capillaroscopy and in healthy controls

<table>
<thead>
<tr>
<th>Capillaroscopic abnormalities Score</th>
<th>sE-selectin (mean ± SD) [pg/ml] Together (n = 106)</th>
<th>Controls (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>218.67 ± 63.77</td>
<td>217.41 ± 59.81</td>
</tr>
<tr>
<td>1</td>
<td>212.79 ± 74.56</td>
<td>32.81 ± 10.14</td>
</tr>
<tr>
<td>2</td>
<td>259.12 ± 73.37</td>
<td>312.53 ± 99.99</td>
</tr>
<tr>
<td>3</td>
<td>312.53 ± 99.99</td>
<td>321.00 ± 99.99</td>
</tr>
</tbody>
</table>

1Statistical significance: p < 0.001 compared to healthy controls; 2Statistical significance: p < 0.05 compared to healthy controls; 3Statistical significance: p < 0.001 between diabetic groups (0 and 3); 4Statistical significance: p < 0.05 between diabetic groups (1 and 3)

### Table 4. Serum concentrations of soluble E-selectin (sE-selectin) in patients with type 1 diabetes according to the severity of microvascular changes in capillaroscopy and in healthy controls

<table>
<thead>
<tr>
<th>Capillaroscopic abnormalities Score</th>
<th>IL-18 mean ± SD [pg/ml] Together (n = 106)</th>
<th>Controls (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>212.79 ± 74.56</td>
<td>32.81 ± 10.14</td>
</tr>
<tr>
<td>1</td>
<td>259.12 ± 73.37</td>
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<td>2</td>
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</tr>
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<td>3</td>
<td>321.00 ± 99.99</td>
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</table>

1Statistical significance: p < 0.05 compared to healthy controls; 2Statistical significance: p < 0.001 compared to healthy controls; 3Statistical significance: p < 0.05 between diabetic groups (0 and 3); 4Statistical significance: p < 0.001 between diabetic groups (1 and 3)
the capillaroscopic features of type 1 diabetic patients and those of healthy volunteers [22].

The results of this study confirm that serum sE-selectin was significantly elevated in patients with capillaroscopic abnormalities in diabetic microangiopathy such as retinopathy, nephropathy or neuropathy, compared to a subgroup without microvascular changes. Moreover, we found significant differences in sE-selectin serum concentrations between diabetes patients with severe capillaroscopic changes and the subgroup without or with mild capillaroscopic changes (p < 0.05). Several studies have demonstrated elevated levels of sE-selectin in patients with diabetes [23]. Moreover, there was shown to be a positive correlation between the serum sE-selectin levels and the presence of retinopathy, albuminuria and cardiovascular system diseases in 540 patients with type 1 diabetes in the EURODIAB Prospective Complication Study [24]. By contrast, according to other studies, serum levels of sE-selectin are not related to the presence of diabetic kidney disease in patients with type 1 diabetes [25].

In our study, we demonstrated significantly elevated levels of IL-18 in serum of type 1 diabetes patients, and in all subgroups with capillaroscopic abnormalities, compared to healthy controls. Moreover, the serum concentration of IL-18 was significantly higher in patients with severe microvascular changes in nailfold capillaroscopy (score = 3) compared to subgroups with normal capillaroscopic pattern or with mild abnormalities (p < 0.001). Katakami et al. observed increased levels and positive correlations between IL-18, sE-selectin and sICAM serum levels in 77 patients with type 1 diabetes. In the same study, a correlation between IL-18 concentration and metabolic control of the disease was found [23]. Moreover, elevated IL-18 serum levels were reported in patients with type 1 diabetes complicated by nephropathy [26]. Other authors have also demonstrated increased IL-18 serum levels in relation to the presence of persistent complications in the course of type 2 diabetes [12].

In the present study, we demonstrated significantly higher serum levels of sE-selectin and IL-18 in type 1 diabetic patients compared to healthy controls. Moreover, we found a relationship between elevated serum sE-selectin and IL-18 concentrations and the severity of microvascular capillaroscopic changes in patients with type 1 diabetes.

In conclusion, our findings suggest that abnormalities in nailfold capillaroscopy may reflect the severity of microvascular changes in the course of type 1 diabetes. Therefore, nailfold capillaroscopy may be a useful diagnostic and prognostic tool for the better evaluation and monitoring of microvascular complications in type 1 diabetic patients. Further investigations of the role of microvascular changes in the development of diabetic complications may be crucial for better diagnosis, monitoring and more effective strategies for the treatment of organ dysfunction in diabetic patients.

References


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