Increased Collagen Around Deformed Finger Nailfold Capillaries in Type I Diabetes Mellitus*

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Summary. Quantitative finger nailfold capillary microscopy was performed in 25 patients with type I diabetes and in 27 healthy control subjects. In the last consecutive 6 patients and 7 controls of these populations, finger nailfold biopsies were taken. Measurements of loop width as an in vivo parameter for deformities of the capillary loops showed significantly higher values in diabetic patients than in controls. Histopathological examination showed markedly and significantly increased deposition of collagen in nailfold dermal papillae of the diabetic patients. The deposition of collagen was positively correlated with the number of capillary endothelial cells in the nailfold dermal papillae and with the size of the papillae in diabetic patients. It is concluded that, in addition to deformity of nailfold capillaries, collagen deposition may also be a sign of metabolic disturbance and perhaps of proliferation of capillary endothelial cells in diabetic microangiopathy.

Key words: Finger nailfold – Capillary microscopy – Nailfold biopsy – Capillary deformity – Collagen deposition

Diabetes mellitus may lead to a number of complications, among which microangiopathy is quite prevalent. The structural abnormalities of capillary endothelial cell swelling and proliferation, narrowing of capillary lumen, pericyte degeneration, and particularly basement membrane thickening, have been described in diabetic microangiopathy [3, 5, 19]. Recently, by using quantitative measurements in clinical nailfold capillary microscopy, we observed significant enlargement of finger nailfold capillary loops in diabetic patients as compared with controls [11]. However, few reports on nailfold skin histopathology, in particular with attention to the components of extracapillary structures in diabetes, have appeared in the literature. In this study, we extended the technique of finger skin nailfold biopsy at the site of capillaroscopy diabetic patients. The results of the capillary in vivo microscopy and the biopsy were compared between diabetic patients and healthy control subjects.

Patients and Subjects
In this study 4678 finger nailfold capillaries were measured in 25 patients with type I diabetes (insulin-dependent) and 4478 capillaries in 27 healthy control subjects. In the last consecutive 6 diabetic patients and 7 controls of these populations, finger nailfold biopsies were performed (see Nailfold Biopsy Technique below). The characterizations on the two complete groups and the two subgroups are indicated in Table 1. Diabetic retinopathy was diagnosed by the ophthalmologist as either the proliferative or the non-proliferative type. Nephropathy was assumed in patients with elevated serum creatinin values above 120 μmol/l and/or albuminuria above 30 mg/dl. Polyneuropathy included missing tendon reflexes and vibration perception.

Methods
Nailfold Capillary Microscopy
An apparatus for television capillary microscopy was used [10] to obtain a modified parameter of capillary loop width (LW) measurement as described by Maricq [13]. Examinations were performed with the finger at heart level in sitting position, at a room temperature of 24°C. A linear distance of 5–10 mm of each nailfold was searched

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Table 1. Clinical data in diabetic patients and control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Sex</th>
<th>Age years (range)</th>
<th>Duration years (range)</th>
<th>HbA1 % (range)</th>
<th>LW μm (SD)</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes complete group</td>
<td>25</td>
<td>12/13</td>
<td>45 (19-80)</td>
<td>18.6 (2-40)</td>
<td>12.9 (5.5-13.2)</td>
<td>43.6±5.9</td>
<td>N (%)</td>
</tr>
<tr>
<td>With histology</td>
<td>6</td>
<td>2/4</td>
<td>28± (19-43)</td>
<td>16 (2-40)</td>
<td>8.1± (5.5-11.3)</td>
<td>42.3±5.0</td>
<td>10 (40)</td>
</tr>
<tr>
<td>Control complete group</td>
<td>27</td>
<td>14/13</td>
<td>46 (22-76)</td>
<td>-</td>
<td>-</td>
<td>39.4±5.4</td>
<td>-</td>
</tr>
<tr>
<td>With histology</td>
<td>7</td>
<td>2/5</td>
<td>35 (28-48)</td>
<td>-</td>
<td>-</td>
<td>39.4±4.1</td>
<td>-</td>
</tr>
</tbody>
</table>

m = male; f = female; LW = loop width; N = nephropathy; P = polyneuropathy; R = retinopathy

* Compared with complete control group, p<0.01; " compared with histology subgroup p=0.16; c compared with complete diabetes group p<0.05

systematically by a 10-times objective, producing a final magnification of 270 times on the TV monitor (43 cm diagonal diameter). LW of all visible end-row capillaries was measured as maximum distance from the outer contour of the arteriolar to the venular limb within 100 μm from the center of the transitional limb.

Nailfold Biopsy Technique (12, 13)

The ring finger of the non-dominant hand was chosen for biopsy. The biopsy was performed under sterile precautions. For local anesthesia 1% lidocaine without adrenalin was applied on the right and left side of the nailfold intradermally. A minimal amount of anesthetic was used in order to leave the circulation of the nailfold capillaries uninfluenced by the anesthetic procedure. After the local anesthesia became effective, a spatula was inserted under the proximal nailfold and the tissue loosened from the underlying nail plate. A crescent-shaped section of nailfold was then excised from one junction of the proximal and lateral nailfold to the junction on the other side by a scalpel. The biopsy specimen was approximately 2 mm wide in its middle portion. It was fixed immediately in a solution of 10% of formaldeyde. Bleeding was slight and only a band-aid dressing was applied. Healing took place in about one week in all patients and subjects without any complications.

Light Microscopy

Each biopsy specimen was embedded in epon, cut at a microtome setting of 1 μm across the long axis of the finger so that cross sections of the dermal papilla and capillary lumen were displayed. The sections were stained with hematoxylin and eosin or Masson's trichrome stain after removal of the resin. Twenty serial sections of each subject taken at 5 μm intervals were evaluated with light microscopy. A representative example is shown in Figure 1. The following parameters were estimated:

1. Surface area of papillary space: five papillae complete on cross-section were estimated on each 5 μm level. A 10 x 10 square grid test system contained in an eyepiece was placed over a randomly selected papilla. The crossings of the grid-lines served as test points and those lying on the trans-section of the papilla were counted as area of papilla. At a magnification of 8 x 40 each point planimetrically represented 2.25 μm².

2. Surface areas of collagen within papillary cut surface: test-points falling on hyalinized material shown as dark red or green in the respective stainings outside the capillary basal lamina representing collagen on the connective tissue stains within each papilla were counted as collagen area.

3. Number of capillary endothelial cells: a count of the number of endothelial cells within each papillary area was made, assuming that cells within a closed capillary basal lamina were of endothelial lineage.

4. Number of other connective tissue cells: all connective tissue cells, including mast cells, pericytes, and interstitial fibroblasts, were counted within each papillary cross section.

Statistical Analysis

Results are expressed as mean and SD of surface area or corresponding volumes without correction