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DIAMETERS OF LYMPHATIC CAPILLARIES IN PATIENTS WITH DIFFERENT FORMS OF PRIMARY LYMPHEDEMA

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ABSTRACT

Fluorescence microlymphography was performed near the medial ankle in 12 healthy controls, 12 patients with congenital and 12 with sporadic lymphedema of the lower extremities. Diameters of lymphatic capillaries were determined on the videoscreen by playback of the tape recordings using a morphometric technique.

In the patients with congenital disease (Nonne-Milroy) aplasia of microlymphatics was diagnosed in 8 instances, ectasia in 4. Patients with sporadic lymphedema and manifestation after puberty exhibited initial lymphatics of normal caliber. In controls mean capillary diameter was 56.3±9.0μm, in congenital disease with ectasia 99.3±9.0μm (difference significant at the $p<0.005$ level) and in the sporadic form 49.7±7.7μm.

Congenital lymphedema may be subdivided into a form with aplastic and ectatic microvessels which possibly show different responses to therapy.

The superficial lymphatic capillary network of human skin which is located at the level of the dermal papillae may be visualized by intravital fluorescence microlymphography in an almost atraumatic way (1,2).

In previous publications it has been shown that the sporadic form of primary lymphedema manifesting after puberty is associated with normal morphology of superficial microlymphatics (1,2), whereas in patients with primary congenital lymphedema (Nonne-Milroy's disease) the superficial capillary network is aplastic (3). In the present study we describe patients with congenital lymphedema and ectatic initial lymphatics of ankle skin. Using a morphometric method, lymphatic capillary diameters were measured in different forms of primary lymphedema and compared to the values obtained in a group of healthy controls.

CLINICAL STUDIES

Twenty-four patients with primary lymphedema were studied and the results compared to a group of 12 healthy subjects. The main clinical data are presented in *Table 1.*

The technique of fluorescence microlymphography has been described in detail (1,2). The subjects rested comfortably in the lateral supine position with the region just proximal to the medial ankle under the microscope. 0.01ml 25% FITC-dextran 150,000 was injected subepidermally by a microsyringe and a steel microneedle with an outer diameter of 0.2mm. Above the medial ankle, a section of the superficial network was filled by the dye from the subepidermal depot and visualized under the epi-illumination fluorescence microscope (Wild-Leitz). Dye deposit and capillary filling were recorded by a sensitive videocamera (Cadmium-Selenide Vidicon, Siemens), stored on videotape (Grundig BK 204) and dis-

Table 1
Summary of Clinical Data

Group	n	Sex (M/F)	Age* at examination (years)	Balance onset of edema* (years)
Normal	12	2M/10F	25.6 (19-43)	-
Sporadic primary lymphedema	12	2M/10F	32.8 (19-52)	8.1 (1-33)
Congenital primary lymphedema (Milroy)	12	4M/8F	19.1 (1/4-44)	19.1 (1/4-44)

*Mean and range (in parenthesis)

played on a screen with actual magnification and time. Using 1/0.04 and 2.5/0.08 objectives measurements were performed with a final magnification of 30 and 70 times. The microscope was mounted on the arm of a solid stage (Wild-Leitz, Foba) which permitted micrometric three-dimensional adjustment without moving the extremity (4).

In each patient and healthy subject videotape recordings of the original dye deposit, capillary filling and maximal dye expansion at 10 minutes into proximal, distal, ventral, and dorsal direction were performed. The distances between the depot border and the most distant meshes depicted were measured. Between the injection and the 10 minute recordings (1/0.04 objective) representative images of details of the capillary network were stored with higher magnification (2.5/0.08 objective).

For statistical comparison of the groups studied, the Mann Whitney U-Test was used. The study protocol was approved by the Ethical Committee of the University Hospital. Controls and patients gave their informed consent.

Data evaluation

Evaluation was made off-line by playback of the videotape. The following parameters were evaluated: morphological findings, capillary diameters and expansion of the dye into the superficial network. Measurements were performed on single frames of the television recordings at the times and magnifications defined above. Mean lymphatic capillary diameters of patients and healthy subjects were determined by analyzing 30 representative microvessels with the 70x magnification.

For determination of lymphatic capillary diameters, a morphometric method was used. A grid containing 63 fields was attached to the television monitor displaying a single frame of the television recordings *(Fig. 1)*. The size of a single field was 430x430μm. This size

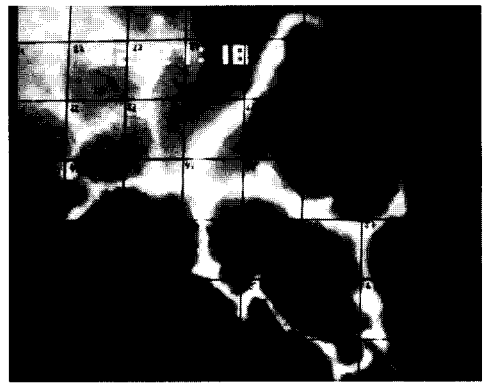

Fig. 1. Fluorescence microlymphography with the grid for evaluation of lymphatic capillary diameters.

fulfilled 2 conditions: the field was larger than the maximal capillary diameter and did not contain more than 2 capillaries.

In each patient and healthy subject, 10 fields selected at random on 3 single frames were evaluated. The mean of the 30 measurements performed in a single control subject or patient was considered as the individual value of capillary diame-

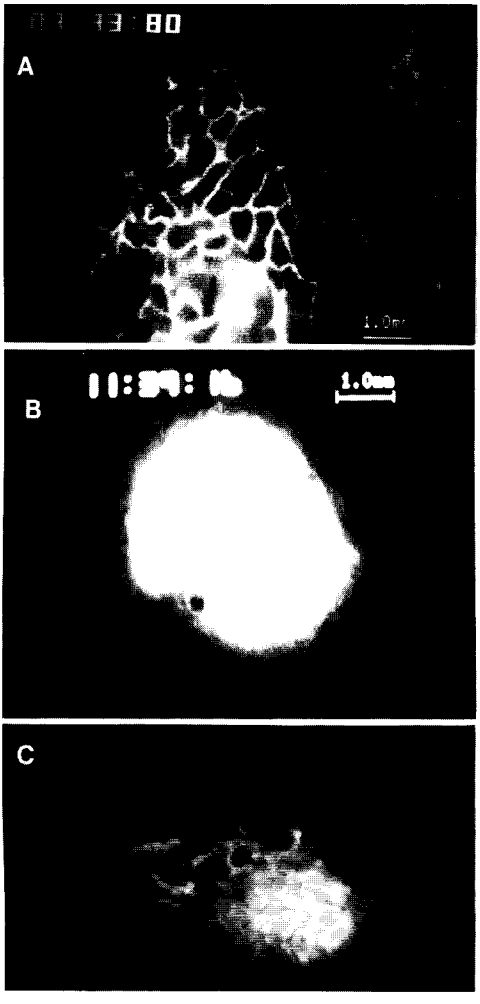

Fig. 2. (A) Lymphatic capillary network at the medial aspect of the ankle in a patient with primary lymphedema and onset of swelling after puberty. An extended part of the network with normal microlymphatics is stained by FITC-dextran. (B) Aplasia of the superficial capillary network in a patient with primary hereditary lymphedema present at birth. No microlymphatics are depicted. The bright fluorescent spot corresponds to the dye depot. (C) Ectatic form of primary congenital lymphedema. The microlymph vessels filling from the depot are enlarged (17s after dye injection and before marked tracer propagation).

ter. In a given field, the capillary site with the lowest scattering of fluorescent light (well delineated vessel wall) was selected for measurement.

RESULTS

Morphological findings

In healthy subjects only a few meshes of the lymphatic capillary network were depicted. In contrast, an extensive superficial network was rapidly filled by the dye in the patients after sporadic form of primary lymphedema and disease onset after puberty *(Fig. 2A)*. The intravital morphology of microvessels appeared to be normal. The meshes were regularly filled and not interrupted by occlusions. The diameter of the initial lymphatics varied within narrow limits.

Based on morphological findings, two subgroups of congenital lymphedema were differentiated. In eight patients no superficial lymphatic microvessels could be visualized in the edematous part of the limb despite placing five dye deposits or more *(Fig. 2B)*. Complete aplasia of the superficial microlymphatics was diagnosed.

In the four remaining patients with congenital lymphedema, fluorescence microlymphography revealed an intact superficial network, but the lymphatics were enlarged *(Fig. 2C)* and the depicted network more extensive than in the controls (see below). In some regions of the network, the caliber of the microvessels was irregular. Saccular enlargements occurred. Cutaneous backflow was observed in one patient. A part of the superficial network was filled far away from the dye depot by FITC-dextran emerging at the surface from deeper invisible channels.

Capillary diameter

The mean diameters of lymphatic capillaries just above the medial aspect of the ankle were 56.3±9.0μm in the 12 healthy controls and 49.7±7.7μm ($p<0.02$) in 12 patients with primary lymphedema and onset of symptoms after puberty. Although the mean width was significantly smaller in patients than in controls, almost all values of the patients were within the range measured in the healthy sub-

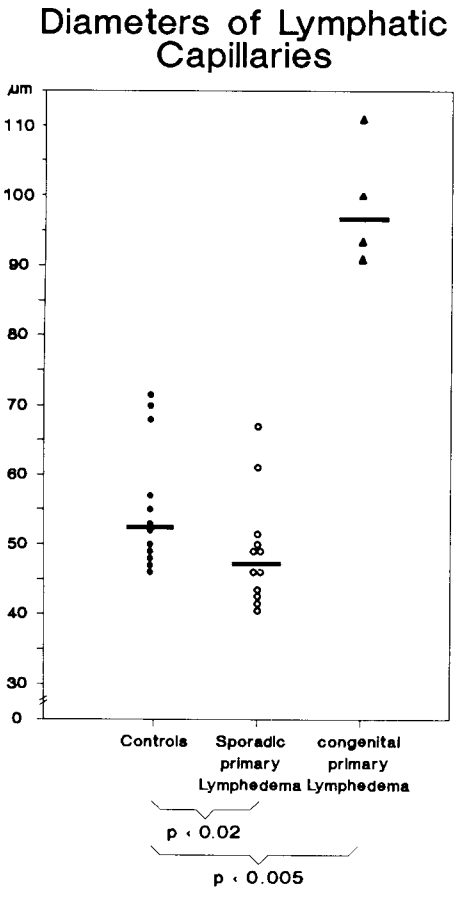

Fig. 3. Individual and median values of lymphatic capillary diameters in the three groups.

jects *(Fig. 3)*. In the 4 patients with congenital primary lymphedema and enlarged microvessels, mean diameter was 99.3±9.0µm. This mean value was significantly greater compared to the healthy subjects ($p<0.005$). The range of the values was 91.9-112µm *(Fig. 3)*. All the diameters determined in controls and primary lymphedema manifesting in teenagers or adults were below 75µm.

Propagation of the fluorescent dye

In the healthy volunteers, FITC-dextran 150,000 did not spread extensively into the superficial network. In contrast, in patients with sporadic primary lymphedema, and in those with congenital lymphedema and ectatic microlymphatic vessels, there was a statistically significant ($p<0.05$ and <0.005, respectively) increase of dye propagation into the superficial capillary network compared to controls *(Table 2)*.

Table 2
Maximal Dye Propagation in Superficial Lymphatic Capillary Network

Group	n	Distance (mm)
Controls	12	8.6±4.0
Sporadic lymphedema	12	14.17±7.4*
Congenital lymphedema (with ectasia)	4	17.25±5.3**

*$p<0.05$; **$p<0.005$ compared with controls

DISCUSSION

Whereas conventional lymphography reveals aplastic or hypoplastic large collectors in sporadic primary lymphedema, it often fails to depict any lymphatics in congenital lymphedema (5,6). Almost atraumatic and simpler fluorescence microlymphography differentiates equally well between the sporadic and congenital form. Moreover, two subclasses of congenital lymphedema may be characterized by the fluorescent technique.

Patients with sporadic primary lymphedema show enhanced propagation of the fluorescent dye into an intact superficial network, findings which confirm earlier results (1,2). Probably, the intact superficial network acts as a compensatory basin when lymph flow into deeper collectors is impeded (hypoplasia or aplasia of large trunks). Although mean lymphatic capillary diameter was significantly smaller compared to healthy controls, individual values varied within the normal range.

Patients with congenital lymphedema can be subdivided into two subgroups:

1) Type I is characterized by complete aplasia of the superficial lymphatic capillaries as described earlier (3). Four additional patients are described in this article.

2) In Type II, the propagation of the fluorescent dye into the superficial network of microlymphatics is enhanced and capillary diameters are enlarged (ectasia). It has to be emphasized that all the diameters of the four patients with Type II exceeded the highest value measured in the healthy controls and in the patients with sporadic primary lymphedema *(Fig. 3)*. A diameter larger than 75μm corresponds to microvessel lymphangiectasia.

Using indirect lymphography with water soluble contrast media which depicts precollectors and collectors, an ectatic and aplastic form of congenital lymphedema has also been diagnosed (7,8). Therefore, typical changes are not confined to the capillary network, but involve precollectors as well.

The distinction of two types of primary congenital lymphedema has potential prognostic implications. Patients with enlarged lymphatic capillaries may respond better to complex physical therapy (9) than those with aplasia of microlymphatic vessels.

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