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Lymphatic Capillary Pressure in Patients with Primary Lymphedema

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Flow and pressure dynamics in minute human lymphatics are unexplored. Lymphatic capillary pressure was measured by the servo-nulling technique at the foot dorsum of 14 patients with primary lymphedema and 15 healthy controls. Glass micropipettes (7–9 μm) were inserted under microscopic control into lymphatic microvessels previously stained by fluorescence microlymphography (FITC–Dextran 150,000). Mean lymphatic capillary pressure was 7.9 ± 3.4 mm Hg in the controls and 15.0 ± 5.1 mm Hg in the patients. The difference was significant at the $P < 0.001$ level. In about half of the patients and control subjects studied pressure fluctuated by more than 3 mm Hg. The mean intralymphatic pressure of lymphedema patients was slightly below mean interstitial pressure measured by J. T. Christensen, N. J. Shaw, M. M. Hamas and H. K. Al Hassan (1985, *Microcirc., Endothelium, Lymphatics* **2**, 267–384) (17.9 mm Hg) in lower leg lymphedema. Microlymphatic hypertension present in patients with primary lymphedema is probably an important factor for edema formation. © 1993 Academic Press, Inc.

INTRODUCTION

Recently, we have published microlymphatic pressure values in the skin of healthy volunteers (Spiegel *et al.*, 1992) after visualizing the lymphatic capillaries by fluorescence microlymphography (Bollinger *et al.*, 1981). The site of measurement was the distal tibial plateau above the ankle. The values obtained in supine position varied between −6.8 and +10.7 mm Hg with a mean value and standard deviation of 4.0 ± 4.5 mm Hg (Spiegel *et al.*, 1992).

In this article we present the first pressure data obtained by a servo-nulling system in patients with primary lymphedema and disease manifestation after puberty. The site of measurement was selected at the distal forefoot proximal to the basis of the first and second toe because lymphatic swelling is preferentially located in this region. Microlymphatic pressure values in 14 patients were compared to the values obtained in 15 healthy controls.

PATIENTS AND METHODS

The network of lymphatic capillaries was depicted by fluorescence microlymphography (Bollinger *et al.*, 1981) at the distal forefoot between the basis of the

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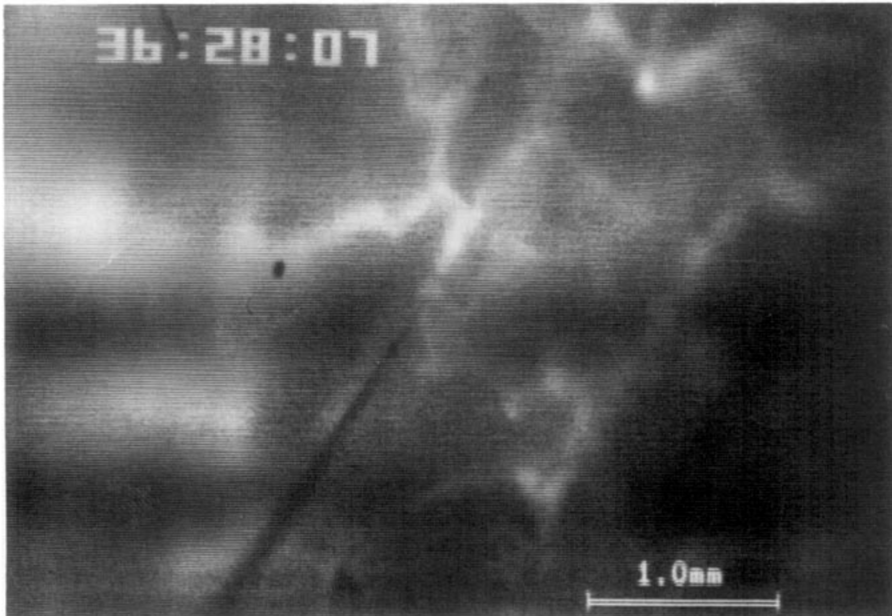

FIG. 1. Network of lymphatic capillaries with inserted glass micropipette appearing as a dark arrow.

first and second toe. FITC–Dextran 150,000 (0.01 ml) was injected into the subepidermal layer. From the original dye depot the lymphatic capillaries were filled by the fluorescent tracer and visualized by fluorescence video microscopy. Maximal dye spread in one of the four directions of the microlymphatic network was measured off-line on television recordings. The reference point was the border of the dye depot. Lymphatic capillary diameters were determined on well visualized segments of the network (Pfister *et al.*, 1990). Individual values are a mean of 5 single measurements.

As described previously in detail (Spiegel *et al.*, 1992) pressure within the lymphatic capillaries was measured by a servo-nulling pressure system (IPM, San Diego). A glass micropipette with a tip diameter of 7–9 μm was inserted by means of a micromanipulator (Leica, Glattbrugg, Switzerland) into one of the lymphatic capillaries visualized (Fig. 1) and intralymphatic pressure measured by the servo-nulling system (Intaglietta *et al.*, 1970; Wiederhielm and Weston, 1973). Pressure was recorded by a Gould–Brush plotter (Model 2600 S, Gould Electronics, USA). Chart speed was 1 mm/sec and changed to 100 mm/sec for a couple of seconds. Intralymphatic position of the glass micropipette was ascertained by some dye entering the tip before connecting the micropressure system. A second method of checking the intravascular position of the micropipette is provided by the servo-nulling system itself. The feedback gain of the servo-controlled counter pressure system can be varied without changing the recorded pressure, provided that the tip of the micropipette is located in a liquid-filled space (lymphatic capillary). Atmospheric (zero) pressure was determined in a 0.9% saline pool before and after cannulation of the microlymphatics. The measurement was performed with the pipette at the same level as the site of cannulation. The system was calibrated

before micropuncture and after each measurement of intralymphatic pressure (two steps of 10 cm H_2O each). If the two zero values differed by more than 1 mm Hg, the cannulation was considered not successful and the data discarded.

Fifteen healthy subjects with a mean age of 26.7 years (range 22–47 years) and 14 patients with primary lymphedema with disease onset after puberty and a mean age of 43.3 years (range 18–62 years) were examined. Primary lymphedema was diagnosed when typical symptoms like white painless swelling of toes, foot dorsum, and/or calf were present. The edema was bilateral in 7 patients. In these cases the more affected leg was selected for study. Mean duration of edema after first manifestation was 10.2 years (range 3 months–40 years). Edema of venous origin was ruled out by clinical means and Doppler sonography of deep leg veins. The maximal expansion of FITC–Dextran in the microvascular network was ≥12 mm in all patients and <12 mm in the healthy controls. The study was performed in supine position with the forefoot placed at heart level and fixed by a vacuum pillow. At least 20 min elapsed before the measurements were started.

In 10 controls and 10 patients more than one capillary was successfully cannulated. The number of microvessels studied in the 15 healthy subjects was 34 and in the 14 patients also 34. Recordings were included only when the measurement lasted more than 20 sec. If microvascular pressure fluctuated the mean value was estimated during the whole period of measurement. A reading was considered as fluctuating if the difference between the lowest and highest pressure exceeded 3 mm Hg. Two procedures were used for calculating mean pressures and standard deviations in controls and patients. First, the values measured in all 34 capillaries were averaged in both groups. The second way of evaluation consisted of calculating the individual value for each subject by averaging the values measured in the different capillaries of this single subject.

The statistical comparison between the pressure values in normals and in patients was performed by the unpaired *t* test and the Mann–Whitney U test. A *P* value of ≤0.05 was considered significant.

The study protocol has been approved by the Ethical Committee of the Department of Internal Medicine, University Hospital (Zurich). Control subjects and patients gave their informed consent to participate in the study.

RESULTS

The mean values and standard deviations of the parameters measured are shown in Table 1. The *mean diameter* of the lymphatic capillaries was not significantly different in the two groups. However, *mean maximal extension* of the depicted microlymphatic network was significantly greater in the patients than in the controls ($P = 0.001$). Intravascular pressure could be monitored during a mean time of 50.5 ± 39.7 sec (range 20–182 sec) in the healthy subjects and of 67.4 ± 76.2 sec (range 20–328 sec) in the patients with lymphedema. The difference was not significant.

The pressure fluctuated in 6 capillaries of 5 controls and in 12 capillaries of 10 patients. In the remaining lymphatic microvessels the pressure differences recorded on the tracing were equal or less than 3 mm Hg. In several controls and patients capillaries with stable and unstable pressure coexisted. Figure 2 shows an original

TABLE 1

MEAN VALUES AND STANDARD DEVIATIONS OF LYMPHATIC CAPILLARY PRESSURE, DURATION OF MEASUREMENT, CAPILLARY DIAMETER, AND NETWORK EXTENSION IN 15 CONTROLS (34 CAPILLARIES) AND 14 PATIENTS (34 CAPILLARIES)

	Controls (n = 34)	Lymphedema (n = 34)	P
Lymphatic capillary pressure	7.9 ± 3.4	15.0 ± 5.1	<0.001
(mm Hg)	(Range 1–14)	(Range 7–24)	
Duration of measurement	50.5 ± 39.7	67.4 ± 76.2	ns.s.
(sec)	(Range 20–182)	(Range 20–328)	
Capillary diameter	59.2 ± 9.3	63.0 ± 10.1	n.s.
(μm)	(Range 46–76)	(Range 50–85)	
Network extension	4.3 ± 1.7	16.3 ± 6.8	<0.001
(mm)	(Range 1–7)	(Range 12–32)	

pressure recording with fluctuating values in a control subject, Fig. 3 a stable recording in a lymphedema patient.

The *individual and mean values of microlymphatic pressure* in 34 capillaries of controls and 34 capillaries of patients with primary lymphedema are plotted in Fig. 4. In the control subjects lymphatic capillary pressure averaged 7.9 ± 3.4 mm Hg (range −1–14 mm Hg). In the patients with primary lymphedema the corresponding mean value was 15.0 ± 5.1 mm Hg (range 7–24 mm Hg). The difference between the two groups was statistically significant ($P < 0.001$). Similar values were obtained if the individual values were calculated as the mean value of two or more capillaries evaluated in the same subject (8.4 ± 3.0 mm Hg in controls and 14.6 ± 5.2 mm Hg in patients, $P < 0.005$).

DISCUSSION

The patients with primary lymphedema included in the study had mild to marked edema at the site of pressure measurement. They exhibited significantly ($P <$ 0.001) enhanced propagation of the macromolecular fluorescent dye FITC–Dextran 150,000 into the superficial microlymphatic network. Increased extension of the depicted network is characteristic for lymphedema, probably because of impeded transport of lymph toward the deeper channels (Bollinger *et al.*, 1981; Isenring *et al.*, 1982; Pfister *et al.*, 1990). In this series patients with maximal

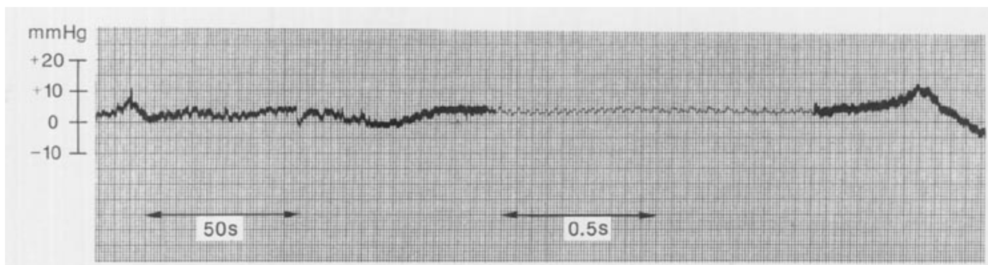

FIG. 2. Original recording of lymphatic capillary pressure in a healthy subject with fluctuations between +12 and −3 mm Hg.

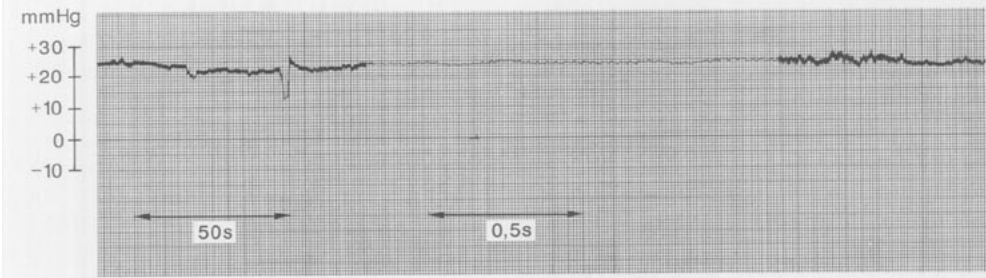

FIG. 3. Original recording of lymphatic capillary pressure in a patient with primary lymphedema. The stable values exceed 20 mm Hg during the observation time of more than 2 min.

network filling into one of the four directions less than 12 mm from the deposit border were excluded. Each individual value was above the maximal value observed in the controls, confirming the clinical diagnosis of lymphedema.

Mean lymphatic capillary diameter was almost identical in patients and controls. It was comparable to earlier values determined at the distal tibial plateau (Spiegel *et al.*, 1992) and at the medial ankle (Pfister *et al.*, 1990). Lymphatic capillary diameter is not enhanced in primary lymphedema with late onset, but only in some patients with familial and congenital lymphedema present at birth (Pfister *et al.*, 1990). Potentially, lymphatic hypertension could induce an increased size of the capillaries. Low compliance of these vessels or stiffening probably prevents dilatation of microlymphatics.

In normal subjects mean lymphatic capillary pressure was somewhat lower at the distal tibial plateau (4.0 ± 4.5 mm Hg) (Spiegel *et al.*, 1992) than at the distal foot dorsum (7.9 ± 3.4 mm Hg). This difference might be explained by the tendency to skin concavity at the tibial plateau which is often associated with negative interstitial pressure (Adair *et al.*, 1991). In lymphatic collectors of healthy

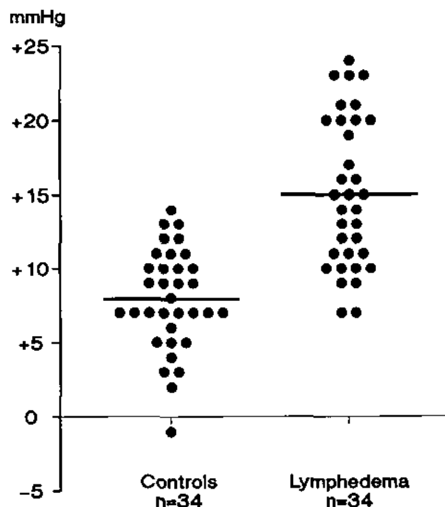

FIG. 4. Individual pressures measured in the 34 capillaries of controls and in the 34 capillaries of patients. The mean pressure are indicated by a bar. The difference between the two groups is significant ($P < 0.001$).

subjects at the proximal foot dorsum mean pressure was 4.1 mm Hg (Quill and Byrne, 1972).

Mean lymphatic capillary pressure was significantly ($P < 0.001$) higher in the patients with primary lymphedema than in the healthy controls. The mean values and standard deviations were 15.0 ± 5.1 and 7.9 ± 3.4 mm Hg, respectively. These values were measured in supine position. Because micropipettes easily move out of microvessels determinations in leg dependency were not possible as in a study on lymphatic collectors (Quill and Byrne, 1972).

Microlymphatic hypertension has not been demonstrated previously in patients with primary lymphedema. In experimental animal preparations pressure of initial lymphatics has only been measured in the absence of lymphedema (Wiederhielm *et al.*, 1964; Zweifach and Prather, 1975; Clough and Smaje, 1978; Taylor, 1990). However, in experimental lymphedema on rabbit ear (Huang and Hsin, 1984) and dog hindleg (Han *et al.*, 1985) data have been obtained in larger lymphatic collectors previously stained by methylene blue. The mean values measured were 16.9 and 10.6 ± 8.6 mm Hg, respectively. In both studies these mean pressures were significantly higher than in control lymphatics without lymphedema.

There are only few data concerning interstitial pressure in lymphedema. Christensen *et al.* (1985) measured a mean value of 17.9 mm Hg in edematous tissue of lymphedema patients. This pressure is slightly more elevated than the intravascular values determined in our study (15.0 mm Hg). Only simultaneous measurements of both interstitial and microlymphatic pressure would allow one to establish the real pressure gradients between the two compartments. In arm lymphedema lower interstitial pressure (1.5 mm Hg) has been reported, probably because of the 10 times greater compliance of the interstitial space at the upper extremity (Bates *et al.*, 1992). Still, these values exceeded significantly those measured at the normal, contralateral arm.

Intralymphatic pressure fluctuated in about one half of controls and patients. Figure 2 illustrates such a recording with fluctuating pressure and peak values of +12 mm Hg and minimal values of −3 mm Hg. Possible explanations of pressure fluctuations observed in microlymphatics include small movements of the leg increasing interstitial pressure around proximal collectors and the well known pumping of proximal segments of large channels (Hall *et al.*, 1965; Olszewski, 1985). The latter mechanism might induce temporal changes of the pressure gradient between interstitial space and microlymphatics.

It is well known from conventional lymphography that the number of perfused subcutaneous collectors at calf and thigh is markedly decreased in number. On the average the number of lymphatic collectors at the thigh (8–15) is reduced by a factor of five (Kinmonth, 1972; Beltz and Picard, 1980) so that drainage capacity of the deep channels is reduced. Probably, a significant amount of lymphatic fluid is removed by the intact higher resistance networks of skin or subcutis. The fact that dye spread into the intact superficial microlymphatics is significantly enhanced in lymphedema speaks in favor of such a hypothesis. If it is correct lymphatic hypertension is the result of prevalent drainage of fluid through the high-resistance lymphatic capillaries and microvessels.

Microlymphatic hypertension is probably one of the determining factors for lymphedema formation. The diagnostic significance of measurements of lymphatic capillary pressure in patients remains to be established. So far no studies are available in patients with different forms of edema.

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