Original Paper

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Study of Dynamic Microcirculatory Problems in 'Blackfoot Disease' – Emphasizing Its Differences from Arteriosclerosis

Key Words
Capillaroscopy
Microcirculation
Blackfoot disease
Arteriosclerosis

Abstract
The cutaneous microcirculation can be divided into thermoregulatory shunt vessels and nutritive skin capillaries. Flux in nonnutritional shunt vessels dominates the signal recorded by the laser Doppler flowmeter. Computerized videophotometric capillaroscopy is a sensitive method for assessing cutaneous nutritive microcirculation. Using patients with blackfoot disease and arteriosclerosis as disease models, we evaluated the sensitivity and clinical usefulness of these relatively new techniques for peripheral vascular disorders. In blackfoot disease, blood flux measured by the laser Doppler flowmeter in the affected toe was lower than that in the nonaffected toe. In symptom-free fingers, blood flow was not significantly different between blackfoot disease and arteriosclerosis. However, blood flow in both diseases was lower than that of the control group. Patients who had the same status of thermoregulatory flow and eyeground arteriosclerotic classification underwent a 1-min arterial occlusion of the digits. The postocclusive reactive hyperemia response (PRH) of nailfold capillary loops was evaluated. All parameters for PRH for the cutaneous nutrient microcirculation including resting capillary blood cell velocity (rCBV), peak capillary blood cell velocity (pCBV) and time to pCBV were more significantly disturbed in the blackfoot disease group than in the arteriosclerotic group. On the basis of the results of this study, dynamic capillaroscopy provides a new approach for the early detection of circulatory disturbances resulting from different mechanisms.

The cutaneous microcirculation can be divided into thermoregulatory shunt vessels and nutritive skin capillaries. Flux in nonnutritional shunt vessels dominates the signal recorded by the laser Doppler flowmeter. At present capillary microscopy is the best choice for studying the nutritional status of a certain skin area [5, 16]. Recently capillary microscopy has been coupled with a videophotometric system and software to analyze the capillary blood cell velocity [6, 12]. Some applications have quickly developed to evaluate the dynamic microcir-

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culatory status in peripheral vascular disorders including arterial occlusive diseases [3, 13, 16], collagen vascular diseases [1, 11] and diabetes [18, 19]. An endemic peripheral vascular disorder called blackfoot disease, resulting from chronic arsenical poisoning, has been noticed in Taiwan [21]. Clinically, the symptoms and signs of blackfoot disease are similar, though much more severe, than those of arteriosclerosis and Buerger's disease [20]. Pathologically, blackfoot disease can be divided into two distinct groups: thromboangiitis obliterans and arteriosclerosis obliterans [22]. Blackfoot disease differs from Buerger's disease in that smoking does not affect the clinical course, and differs from arteriosclerosis in that the destruction of capillary endothelial cells takes place at an early stage [23, 24].

The purpose of this study was to evaluate the deterioration of microcirculation in blackfoot disease and to compare the dynamic microcirculatory differences between blackfoot disease and arteriosclerosis. A third purpose was to demonstrate the usefulness (due to its sensitivity) of dynamic capillaroscopy to analyze the status of peripheral vascular disorders. Vital dynamic capillaroscopy is at present the only available noninvasive and clinically useful method for evaluating the extent of deterioration [5]. In this study, we found that the microcirculatory response to ischemia in blackfoot disease was significantly worse than in arteriosclerosis.

Table 1. Personal characteristics of subjects (n = 14 in each group)

	Age years	Sex (m/f)	BP[1]	Eye-ground	Smoking
BFD	65±1	8/6	145.6±18.7/88.0±7.0	Gr. I–II	7/14
ATS	64±10	9/5	175.5±24.6/94.8±9.4	Gr. I–II	9/14
NC	62±10	9/5	130.0±10.0/80.0±5.0	Negative	4/14

BFD = Blackfoot disease patients; Gr. = Sheie's arteriosclerotic classification; ATS = arteriosclerotic subjects; NC = normal control.
[1] Blood pressure (BP) is shown as systolic/diastolic values (mm Hg).

Subjects and Methods

Selection of Subjects

A total of 14 patients with blackfoot disease were selected from an endemic area. At the time of assessment, all but 3 patients were in the erythematous stage, and 6 patients had previously suffered from ulceration and gangrene. For comparison, 14 age-matched arteriosclerotic patients and 14 healthy volunteers were enrolled in this study. None of the arteriosclerotic patients had clinical manifestations of a peripheral vascular disorder. The upper extremities appeared normal in all of the disease subjects. Ophthalmoscopic examination of the fundus in all patients revealed Sheie's arteriosclerotic classification stages 1–2. None of the studied subjects had received any treatment before participating in this study. The personal data of the subjects are shown in table 1. Cutaneous blood flow in the nailfold areas was studied. For comparison, big toes of the affected and nonaffected sides of the patients with blackfoot disease, the left big toe of both the arteriosclerotic and healthy subjects, and the left ring finger (i.e. the finger next to the smallest finger) of all studied subjects were selected for assessment.

Methods

Microcirculatory assessment was performed after 30 min equilibration in a constant temperature room maintained at 23–25 °C.

Laser Doppler Flowmeter Measurements. Cutaneous thermoregulatory blood flow of the nailfolds was measured by a laser Doppler flowmeter. Blood flux was expressed as the perfusion unit (p.u.), i.e. the product of blood flow velocity and the number of moving red cell corpuscles in the vessels. Skin blood flow was recorded with a laser Doppler flowmeter (PeriFlux, PF3, Perimed, Sweden). The mean

Table 2. Morphologic analysis of nailfold capillary loops in blackfoot disease (BFD), arteriosclerosis (ATS) and normal controls (NC)

	BFD			ATS		NC	
	big toe affected	big toe nonaffected	ringfinger	big toe	ring finger	big toe	ring finger
Capillary loops in 1 mm	9.2±2.5	8.7±1.1	10.5±1.1	8.4±1.2	8.4±1.3	8.5±0.9	8.6±0.9
Intercapillary distance, μm	131.6±30.5	114.2±15.0	102.7±14.5	127.6±23.2	124.3±24.5	102.0±7.4	103.0±8.3
Tortuous capillary loop, %	55.1±9.1**	48.5±5.2**	19.6±11.2	47.8±17.2**	21.6±16.2	33.0±3.5	19.5±12.2
Diameter, μm	18.5±4.0*	16.2±1.8	15.5±2.9	14.5±2.5	13.5±3.5	15.3±3.8	14.0±3.2

* $p < 0.05$; ** $p < 0.01$, vs. normal controls.

value of the perfusion unit in a defined period was analyzed by Perisoft (the Perimed analysis program for PeriFlux).

Dynamic Capillaroscopy. A fully computerized system for capillary blood cell velocity (CBV) measurement was used in this study. This included a microscope, television camera, monitor, and software, to calculate automatically the whole process of CBV by cross-correlation velocimetry [7, 9]. Nailfold was studied by a Leitz Laborlux 12 MES capillary microscope equipped with a TV camera (Ikegami CTC-2110, Japan). A drop of immersion oil was applied to render the nailfold transparent. The finger or toe rested on a plate, and a small bracket was allowed to touch lightly the distal end of the nail. The image was displayed on a TV monitor. All recordings were stored on video tape and analyzed by the CapiFlow computerized analysis system (CapiFlow, Kista, Sweden). The following observations were recorded: number and type of capillaries, intercapillary distance, caliber, visibility of the capillary loops, status of blood flow, and CBV measured and analyzed as described previously [4, 12]. The postocclusive reactive hyperemia response (PRH) of nailfold capillary loops after a 1-min arterial occlusion of the digital artery was also recorded [15]. Blood flow of the digits was completely occluded for 1 min by inflation of a pneumatic cuff placed around the subject's ankle or elbow to 200 mm Hg. Images in the dynamic microscope were recorded for 5 min following release of the cuff. The important parameters for PRH including resting CBV (rCBV), peak CBV (pCBV) and time to pCBV (tpCBV) were assessed.

Statistical Methods. Analysis of variance and paired Student's t test were used to evaluate the results. The mean and standard deviation (SD) are described as mean ± SD. A p value of < 0.05 is considered to be statistically significant.

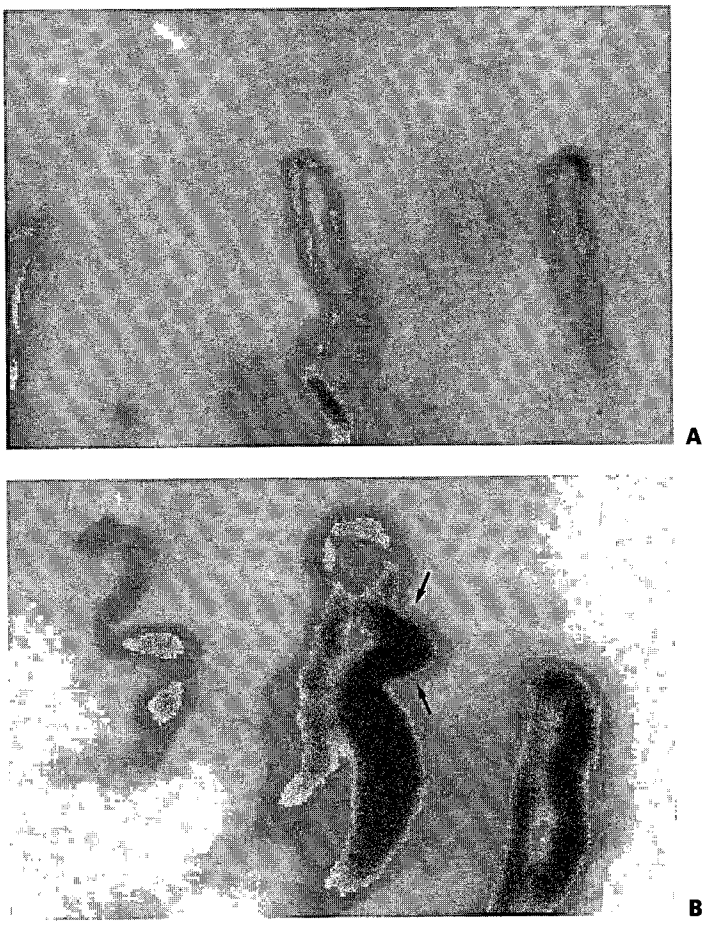


Fig. 1. A Hairpin-like capillary loops observed in the big toe nailfold of a healthy subject. **B** Tortuous, dilated capillary loops (arrows) observed in the big toe nailfold of a blackfoot disease patient.

Results

Laser Doppler Flowmeter Measurements

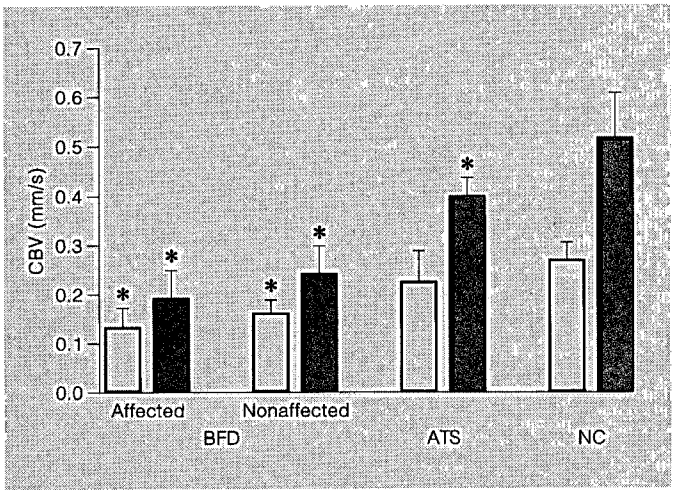
In blackfoot disease, blood flux of the big toe on the affected side (5.3 ± 4.3 p.u.) was lower than that of the nonaffected side (18.3 ± 16.4 p.u.), as measured by laser Doppler flowmeter. Blood flux of the big toe in blackfoot disease (nonaffected side) and arteriosclerosis (25.8 ± 11.9 p.u.) was lower than that of healthy controls (34.1 ± 11.7 p.u.). Blood flow in the left finger did not significantly differ between blackfoot disease (40.4 ± 30.1 p.u.) and arteriosclerosis (40.4 ± 25.6 p.u.). However, it was lower than that of the control group (66.8 ± 9.6 p.u.).

Morphology Analysis

The nailfold capillary loops in healthy volunteers resembled hairpin-like structures with uniform diameter and regular distribution (fig. 1A). In the disease groups, the big toe nailfold capillaries showed an increased number of tortuous loops (fig. 1B). Morphologic analysis of the capillary loops of the left ring finger nailfold revealed no significant differences between the three groups (table 2).

Dynamic Capillaroscopy

Examination of the capillary blood flow in the normal controls revealed a continuous pattern as compared to a granular and/or static pattern in the toe and finger of blackfoot disease or a granular and/or continuous pattern in arteriosclerosis. A continuous bloodstream in a capillary indicates a patent and persistent movement of erythrocytes. A granular pattern reflects an aggregation of erythrocytes and slow blood flow. Stasis of the blood stream is due to high blood viscosity in the capillary loop [10]. All the parameters including rCBV, tpCBV and pCBV assessed in this study were significantly affected in blackfoot disease as shown in tables 3 and 4, including a decrease in rCBV and pCBV and an increase in tpCBV as compared to the normal control (fig. 2, 3). The rCBV of both toes and fingers in arteriosclerosis was within the



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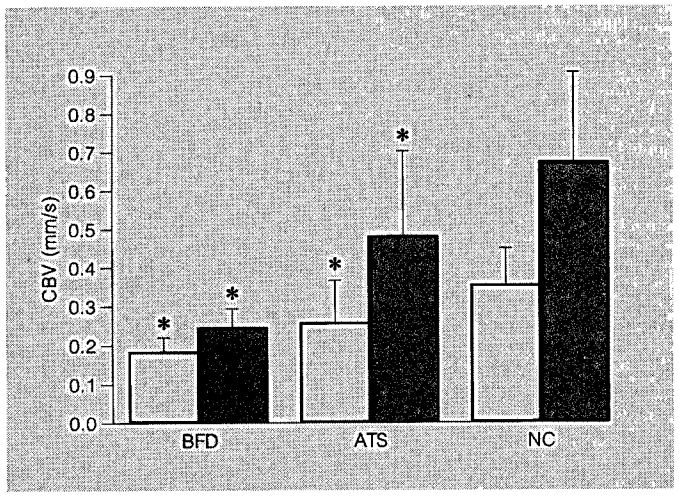

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Fig. 2. CBV before (□; resting) and after (■; peak) reactive hyperemia in the big toe. BFD = Blackfoot disease patients; ATS = arteriosclerotic subjects; NC = normal controls. Affected indicates affected foot, nonaffected indicates nonaffected foot. * $p < 0.01$, vs. NC groups.

Fig. 3. CBV before (□; resting) and after (■; peak) reactive hyperemia in the ring finger. BFD = Blackfoot disease patients; ATS = arteriosclerotic subjects; NC = normal controls. * $p < 0.01$, vs. NC groups.

normal range but there was a significant deterioration in parameters of the postocclusive reactive hyperemia response (a decrease in pCBV and an increase in tpCBV as compared to the normal control; tables 3, 4). Although there were no differences in blood flux of p.u. between the ring fingers of blackfoot disease and arteriosclerosis, all parameters assessed by dynamic capillaroscopy revealed a significant difference between these two groups (a decrease in rCBV and pCBV and an increase in tpCBV were noticed in blackfoot disease as compared to arteriosclerosis; table 4, fig. 3).

Discussion

The aims of this study were to assess the microcirculatory disturbance in blackfoot disease and also to evaluate the usefulness of microcirculatory dynamics in the early detection of various peripheral circulatory disturbances. The most prominent morphologic finding in the diseased group was an increase in the tortuous loops of the big toe (fig. 1B). On the other hand, there were no significant differences between the ring fingers of the three groups. Our data suggest that the morphologic analysis of nutritive blood vessels is not a sensitive method for evaluating peripheral vascular disease at a mild stage.

In blackfoot disease the blood flow in thermoregulatory shunt vessels in the big toe of the affected foot was significantly lower than that in the vessels of the nonaffected limb. However, perfusion unit in the big toe of the nonaffected side and left ring fingers in blackfoot disease showed no difference from that of the left big toe and ring finger in arteriosclerosis. The cutaneous capillary blood vessel velocity is highly dependent on the diameter of the capillary, skin temperature, and vasomotion [2]. In this study, it is reasonable to propose that the impairments of cutaneous microcirculation in the disease groups lie in the dysfunction of regulatory mechanisms for microcirculato-

Table 3. Dynamic capillaroscopy of big toe nailfold capillary loops in blackfoot disease (BFD), arteriosclerosis (ATS) and normal controls (NC)

	BFD (n = 14)		ATS (n = 14)	NC (n = 14)
	affected	nonaffected		
Status of blood flow	granular/stasis	granular/stasis	continuous/granular	continuous
rCBV, mm/s	0.13±0.04*	0.16±0.03*	0.23±0.06	0.27±0.04
pCBV, mm/s	0.19±0.06*	0.24±0.06*	0.40±0.04*	0.52±0.09
tpCBV, s	39.0±11.0*	28.0±9.5*	11.8±3.9*	7.3±1.5

* $p < 0.01$, vs. the NC group.

Table 4. Dynamic capillaroscopy of ring finger nailfold capillary loops in blackfoot disease (BFD), arteriosclerosis (ATS) and normal control

	BFD (n = 14)	ATS (n = 14)	Control (n = 14)
Status of blood flow	granular	continuous	continuous
rCBV, mm/s	$0.18 \pm 0.04^{b,c}$	0.26 ± 0.11	0.36 ± 0.10
pCBV, mm/s	$0.24 \pm 0.05^{b,c}$	0.49 ± 0.22^{b}	0.68 ± 0.23
tpCBV, s	$33.0 \pm 11.7^{b,c}$	15.1 ± 9.6^{a}	8.5 ± 1.5

[a] $p < 0.05$, vs. control; [b] $p < 0.01$, vs. control; [c] $p < 0.01$, vs. ATS.

ry blood flow. In a normal capillary, blood flow shows periodic fluctuations with a frequency of 6–10 cycles/min. The cause of this intermittency is called vasomotion, which is controlled by the precapillary sphincters [8]. It has been indicated that both myogenic and metabolic factors are involved in the regulation of cutaneous capillary blood flow following arterial occlusion. The hyperemic response after 1 min occlusion is largely of myogenic origin; metabolic factors are probably responsible for the prolonged hyperemia seen with longer durations of occlusion [17]. The most important factor found thus far affecting the precapillary sphincter is the concentration of oxygen in the tissue [8]. A decrease of oxygen tension in the local tissue results in an increased frequency of contraction of the precapillary sphincter, thereby allowing the blood to carry an increased quantity of oxygen to the tissue. The data presented herein demonstrate that both rCBV and vasodilator response in blackfoot disease are markedly impaired. Although rCBV is intact in arteriosclerotic patients, its hyperemia response deteriorates. One of our most important findings was that given the same status of thermoregulatory flow and eyeground arteriosclerotic classification, the regulatory mechanism for cutaneous nutrient microcirculation was more significantly affected in blackfoot disease than in the arteriosclerotic group. This demonstrates the sensitivity of dynamic capillaroscopy. This difference in the regulatory reactivity between blackfoot disease and arteriosclerosis may explain the poorer outcome of blackfoot disease in the lower extremities [20]. A possible explanation for the poorer regulation of cutaneous capillary blood flow in blackfoot disease is the degeneration of the capillary endothelial cells [24]. Pohl [14] reported that endothelial cells act as part of the vascular oxygen-sensing system. Hypoxia induces a relaxant factor which stimulates the precapillary sphincter and induces vasodilation. The role of this mechanism in blackfoot disease needs to be investigated further.

Our results suggest that dynamic capillaroscopy is a sensitive method for evaluating the cutaneous nutritive status in various circulatory disorders. It also provides a new approach to the early detection of circulatory disturbances resulting from different mechanisms such as arteriosclerosis, Buerger's disease, diabetes mellitus, collagen vascular diseases, and perhaps even for some types of heart disease.

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