Morphometric analysis of skin microvasculature in the diabetic foot

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ABSTRACT

Introduction: This study was designed to evaluate the histopathological features of skin microvasculature in patients with a diabetic foot, specifically the number of blood vessels, number of endothelial cells and endothelial thickness.

Methods: This study involved 41 diabetic foot patients admitted to Hospital Universiti Sains Malaysia for surgical management of foot problems. Skin biopsies were taken for histological evaluation following surgical procedures, such as wound debridement or local foot amputation. The skin microvasculature features examined were the number of blood vessels, the endothelial thickness of the vessels and the cross-sectional endothelial cell count. The findings were compared with the similar parameters of non-diabetic patients (control) and analysed.

Results: The mean blood vessel count (BVC), endothelial cell thickness (ECT) and endothelial cell count (ECC) for the diabetic group were 12.56 +/- 2.77, 4.81 +/- 1.5 µm and 7.07 +/- 1.88, respectively. The mean BVC, ECT and ECC for the non-diabetic control group were 5.25 +/- 1.98, 1.9 +/- 0.55 µm and 4.11 +/- 1.17, respectively. The mean BVC, ECT and ECC for the diabetic group were significantly higher than those for the non-diabetic control group.

Conclusion: The increased number of blood vessels to the skin and their endothelial cell number and thickness may be the contributing factors for problems related to the diabetic foot, such as tendency for skin ulceration, infection and poor wound-healing in these patients. These may also contribute to secondary changes of diabetic foot lesions, indicating failure of adequate vascularisation of the foot.

Keywords: diabetes mellitus, diabetic foot, endothelial cells, microvasculature, skin

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INTRODUCTION

The skin functions to protect the underlying structures and also acts as the first-line barrier against infection. The integrity of the skin is mainly maintained by its vasculature. Adequate skin blood flow is crucial for nutrients, oxygen and cellular supply to the skin. Defects in the microvasculature of the skin predispose the patients to ulceration, infection and poor tissue healing. Patients with poorly-controlled diabetes mellitus very commonly present with foot lesions. Foot ulcer is usually the primary lesion. The pathogenesis of foot ulcer in patients with diabetes mellitus has been studied extensively. Among the common explanations for the development of the foot ulcer is abnormal blood flow to the area affected.[1-6] Most of the investigations pertaining to foot blood perfusion were performed using non-invasive techniques, which give indirect evidence of vascular anomalies. Histopathological features of the skin vasculature of patients with diabetes mellitus have rarely been reported. Studies of microscopical features of the blood vessels may give further information and contribute to a better understanding of diabetic foot problems.

METHODS

This cross-sectional study was conducted at Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan from August 2003 through August 2005. There were two groups of patients in this study. Group I comprised patients with type 2 diabetes mellitus, and were admitted for foot problems which required surgical procedures and whose diabetic status was confirmed based on hospital records. Group II comprised non-diabetic patients, who were admitted for various other surgical procedures which included thyroid, breast, inguinal and lower limb. Verbal and written informed consent was obtained prior to the study procedures. Both male and female patients were included in this study.

A skin biopsy was obtained surgically from each subject. The biopsies were taken at the surgical margin of the primary lesions (e.g., skin from the neck for thyroid procedures, skin from the chest/breast for breast procedures, and skin from the inguinal region for hernia procedures). Each skin biopsy measured approximately 2 cm × 1 cm, divided into two halves, and were then immediately immersed into two separate containers containing 10% formalin. They were processed overnight

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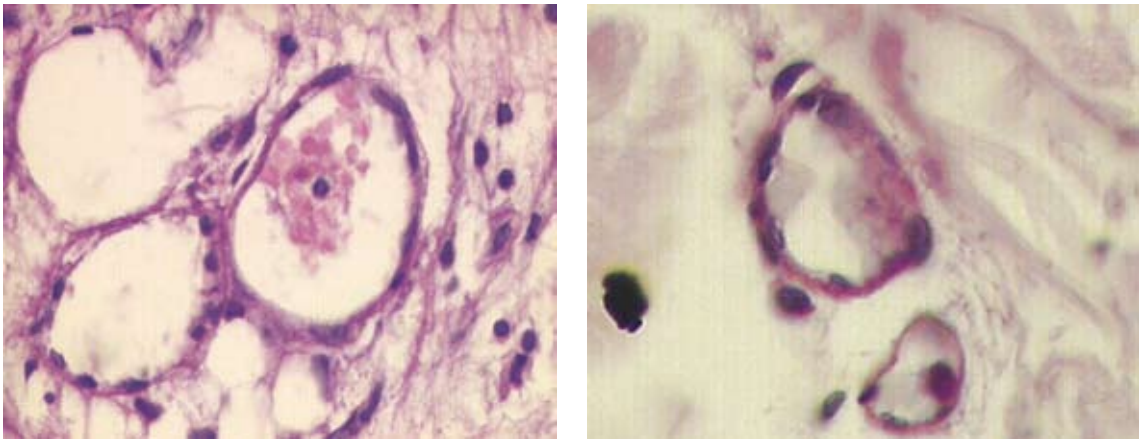

Fig. 1 Photomicrographs show (a) microvasculature of the skin in a patient with diabetes mellitus (Group I) (PAS × 40); (b) microvasculature of the skin in a non-diabetic patient (Group II) (PAS × 40).

with a tissue processor and then sectioned using a microtome (Leica, RM2145, Germany) The skin samples were subsequently processed using a vacuum filtration tissue processor (Tissue-Tek VIP, E150 Sakura, Japan). All the prepared tissue sections were then stained with haematoxylin and eosin to assess the vascular morphology.

The tissue sections were subjected to standard avidin-biotin complex (ABC) immunohistochemical method for FVIII-related antigen. The FVIII-related antigen would stain blood vessels. The blood vessel count (BVC) in the dermis were performed under high power field (× 400) microscope magnification and the mean of five readings were recorded. The endothelial cell count (ECC) in each vessel was also performed and the mean was calculated. The tissue sections were subjected to periodic acid-Schiff (PAS) stain and five blood vessels in the dermis were identified for each sample. The endothelial cell thickness (ECT) of all endothelia present in the vessel was measured in micrometre (μm) using an image analyzer (Leica, QwinLite V 2.3, England) and the mean thickness was calculated.

The Statistical Package for Social Sciences version 12.0.1 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. The distribution of all numerical variables was checked for normality and presented by mean (± standard deviation). To analyse the difference between group means, Student's *t*-test for the two groups (two independent means) was used for variables with normal distribution. The level of significance (α) was set at 0.05 and p-value < 0.05 was accepted as being significant.

RESULTS

There were 41 patients available in the diabetic group (Group I) and 36 patients in the non-diabetic control group (Group II). There were 39 males and 38 females with patients' age ranging from 30 to 82 years. Since there was no significant difference between mean BVC, ECT and ECC of male and female patients, their data was analysed together in this study (Table I). The mean BVC, ECT and ECC for the diabetic group were 12.56 ± 2.77, 4.81 ± 1.5 μm and 7.07 ± 1.88, respectively. The mean BVC, ECT and ECC for the non-diabetic control group were 5.25 ± 1.98, 1.9 ± 0.55 μm and 4.11 ± 1.17, respectively. The mean BVC, ECT and ECC for the diabetic group were significantly higher than those for the non-diabetic control group ($p < 0.034$, $p < 0.001$, $p < 0.005$, respectively) (Table II).

DISCUSSION

The causes of foot ulcer in patients with diabetes mellitus are multifactorial. Impaired perfusion, foot deformities, poor vision, greater body mass, sensory and autonomic neuropathy are known risk factors for foot ulceration.[5] Many authors have reported about the possible causes of blood vessel abnormalities in various conditions, which include poor oxygenation or poor blood flow to the extremities. However, actual histopathological features of microvascularity to the skin of a diabetic foot are rarely reported. It has been shown in many reports that the majority of patients with diabetes mellitus have impaired foot circulation, including large vessel abnormalities such as atherosclerosis, calcification and thickening of the tunica media.[7,8] Increased intima-media thickness was associated with high blood glucose, [9] and hyperperfusion was associated with increased blood vessel formation.[8,10] A similar mechanism probably causes endothelial cell thickness, as observed in our study. Therefore, narrowing of the blood vessel calibre is not only due to thickening of the basal membrane, but also contributed by endothelial cells thickening. Based on our observation, the endothelial cells become three times (4.81 ± 1.5 μm versus 1.9 ± 0.55 μm) thicker than normal cell. Circumferentially, this will produce significant narrowing of the blood vessels. Endothelial cell thickening may also be related to the

Table I. The blood vessel count (BVC), endothelial cell count (ECC) and endothelial cell thickness (ECT) of Group I and Group II.

Group I (n = 41)				Group II (n = 36)			
Case	BVC	ECC	ECT (μm)	Case	BVC	ECC	ECT (μm)
D1	16.20	6.20	5.45	C1	7.60	3.00	.96
D2	11.20	11.80	7.15	C2	5.60	2.80	1.76
D3	18.80	7.20	5.90	C3	4.00	4.20	1.20
D4	18.20	10.80	6.80	C4	4.20	7.80	1.87
D5	14.40	5.60	6.07	C5	4.20	3.20	1.80
D6	15.20	6.60	3.09	C6	5.20	4.00	1.84
D7	12.40	6.60	4.99	C7	4.20	4.00	2.10
D8	12.20	6.20	2.84	C8	4.20	2.10	2.30
D9	16.40	9.20	6.20	C9	4.40	4.80	1.58
D10	20.00	7.00	3.88	C10	4.00	2.00	2.30
D11	12.20	7.90	4.25	C11	4.60	4.00	1.28
D12	12.00	8.00	5.10	C12	4.00	6.80	2.54
D13	16.20	10.10	8.90	C13	5.20	3.00	1.53
D14	14.00	5.58	4.72	C14	4.20	4.20	1.70
D15	13.00	9.20	6.30	C15	5.00	4.80	1.74
D16	14.70	7.90	4.20	C16	12.60	4.00	1.20
D17	13.00	8.40	6.10	C17	3.80	4.60	1.50
D18	16.60	10.00	6.95	C18	4.10	2.80	1.50
D19	12.40	6.80	3.51	C19	6.00	4.00	1.60
D20	10.20	9.80	7.72	C20	8.00	5.60	1.84
D21	9.60	7.00	5.07	C21	10.00	4.00	1.51
D22	10.60	8.40	5.30	C22	4.40	5.80	2.73
D23	15.20	9.80	7.70	C23	4.00	3.80	1.40
D24	11.00	4.20	3.18	C24	3.80	4.80	1.68
D25	12.00	8.10	6.30	C25	6.00	6.00	4.20
D26	10.10	6.80	4.22	C26	7.00	3.80	2.43
D27	13.20	9.60	5.20	C27	4.80	4.00	1.58
D28	11.20	5.60	3.05	C28	5.20	5.20	1.40
D29	12.60	6.00	2.50	C29	5.00	3.80	1.33
D30	10.00	8.80	4.49	C30	4.20	4.40	1.84
D31	11.80	6.60	4.01	C31	4.00	3.00	2.40
D32	12.80	4.60	6.30	C32	4.40	4.80	1.77
D33	11.00	6.80	4.25	C33	6.00	5.20	2.60
D34	11.28	8.80	3.65	C34	7.20	5.30	2.80
D35	13.30	7.60	6.25	C35	5.30	4.30	2.25
D36	12.50	8.60	5.60	C36	4.20	2.90	1.31
D37	11.00	7.00	6.24				
D38	9.20	10.00	4.00				
D39	10.10	3.90	7.12				
D40	11.50	5.31	4.70				
D41	11.00	5.42	8.22				

endothelial dysfunction associated with endothelium-dependent vasodilation impairment in patients with diabetes mellitus.[2]

In this study, the exact incidence of peripheral vascular disease in both studied groups was not known, as preoperative vascular evaluation, e.g. Doppler imaging and angiography, was not performed. The implication of peripheral vascular disease in the microvasculature of the diabetic foot therefore could not be determined. Blunted responsiveness of cutaneous blood flow with local heating, and following exercise, in patients with diabetes mellitus has been shown.[11] They also had altered skin blood flow response to a very much lower pressure applied locally[12] and failure of increased blood flow to the leg during exercise, as compared to normal subjects.[4] Increase in the number of cutaneous blood vessels observed in our patients might be the secondary response to the failure of vasodilatations observed in patients with diabetes mellitus. When the body fails to respond to an increased requirement of blood supply to the peripheral tissues for long periods, due to failure of vasodilatation, it compensates for this insufficiency by promoting neovascularisation. With the increased number of blood vessels seen in our series, sufficient evidence of neovascularisation around the ulcers in diabetic feet was observed.

Cutaneous vasodilatation in the normal foot is relatively lower in comparison to other parts of the body,

Table II. The comparison of morphometric features of the skin microvasculature between patients in Group I and Group II.

	Group I Mean ± SD (n = 41)	Group II Mean ± SD (n = 36)	Mean difference (95% CI)	t-test	p-value
BVC	12.56 ± 2.77	5.25 ± 1.98	-7.33 (-8.32,0.34)	-14.71	0.034
ECC	7.07 ± 1.88	4.11 ± 1.17	-2.96 (-3.59,-2.33)	-9.32	0.005
ECT (μm)	4.81 ± 1.5	1.9 ± 0.55	-2.92 (-3.38, -2.46)	-12.57	< 0.001

BVC: blood vessel count; ECC: endothelial cell count; ECT: endothelial cell thickness

e.g. the forearm.[1] Apart from being the weight-bearing part of the body, the foot is also subjected to constant pressure from the ground and footwear. The inability to sense even slight pressure can be detrimental to the skin in patients with diabetes mellitus.[5] Therefore, an increase in vascularity (12.56 ± 2.77 vs. 5.25 ± 1.98) may improve perfusion of the foot in these patients. Overperfused nutritive capillary circulation in the feet of patients with diabetic neuropathy has been demonstrated by Netten et al,[3] and our findings support their observations.

Abnormalities of the arterial flow pattern precede the skin ulceration even in the absence of lower limb ischaemia. A monophasic pattern was noted to replace the normal triphasic pattern of arterial blood flow at the level of the dorsalis pedis, posterior tibial arteries and distal arteries.[6] This might indicate that even though neovascularisation occurs in patients with a diabetic foot, this mechanism is not effective enough to compensate for hypoperfusion due to failure of vasodilatation. Increase in the number of vessels distally might also explain the arterial flow changes reported. Clinically, the degree of vascular insufficiency seemed so critical that even successful bypass grafting surgery fails to improve ischaemia completely.[13] Treatment using hyperbaric oxygen may play a role in the management of diabetic foot ulcer. Significant improvement in ulcer healing was observed by improving oxygenation.[14] The presence of circulating endothelial cells may reflect ongoing vascular injury in diabetic type 2 patients. McClung et al reported a higher number of circulating endothelial cells in these patients. A presence of a higher number of endothelial cells in the skin blood vessels of patients with diabetes mellitus, as observed in our series, may support their findings. This may indicate the high turnover of the cells due to ongoing vascular injury in this group caused by high blood glucose.[15]

Diabetic foot was associated with an increased number of blood vessels to the affected skin. However, the larger number of blood vessels was not necessarily associated with improved blood supply to the foot lesions. This was possibly because the newly-formed blood vessels were abnormal, as evidenced by the increased numbers of endothelial cells and endothelial thickness shown in this study, and also increased basal membrane thickness as reported elsewhere. These unfavourable changes in the diabetic foot may possibly contribute to the failure of vasodilatation in response to various stimuli, and therefore can possibly predispose the patients to worse diabetic foot complications.

The major limitation of our study was that all the parameters measured from the skin biopsy of diabetic feet (Group I) were not compared to similar skin sites in non-diabetics (Group II). This might result in inaccurate findings as the number and other features of the blood vessels in the skin of the foot and other parts of the body are different. Skin biopsy from the foot of non-diabetics is difficult to obtain as they rarely present with foot problems requiring surgical interventions. For this study, the non-diabetic patients were assured that the skin biopsy was performed during the primary procedure, and therefore no additional anesthesia was necessary. They were also informed that the closure of the wound would not be problematic and no new scar would be created. There was no immediate postoperative complication reported from the procedure and during follow-up.

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