HISTOMORPHOMETRIC ANALYSIS OF THE ENDOTHELIAL CELL IN SKIN MICROVASCULATURE AMONG DIABETIC FOOT ULCER PATIENTS

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Changes affecting endothelial cells in skin microcirculation of diabetic foot patients are important in the development of foot ulceration and subsequent failure to heal existing ulcers. We studied 30 subjects, involving 10 controls, 10 with neuropathic ulcer and 10 with neuroischaemic ulcer. The specimens were taken using skin punch biopsy and were routinely processed for light microscopy. The mean endothelial cell count (ECC) and endothelial cell thickness (ECT) were performed by calculating and measuring all the endothelial cells of 3 selected arterioles in each slide of every patient. There is significant decrement in ECC among the diseased group as compared to the controls, $F(2,27)=5.31$, $p=0.01$. The ECT of the diseased group were significantly higher than the controls, $F(2,27)=52.42$, $p<0.01$. The Tukey post-hoc comparisons indicate that the ECC and ECT of neuropathic group (M=5.63, 95% CI [4.87, 6.40], M=6.20, 95% CI [5.51, 6.90]) and neuroischemic group (M=5.70, 95% CI [4.98, 6.42], M=6.77, 95% CI [6.41, 7.12]) were not significant as $p>0.05$. In conclusion, the decrement of ECC and the increment of ECT among diabetic foot patients could be due to the alteration in the endothelial cell function and structure as the results of the metabolic disease.

Keywords : diabetic foot, microcirculation, endothelial cell count, endothelial cell thickness

INTRODUCTION

Diabetes mellitus (DM) becoming a major threat to the worldwide public health as it is reaching pandemic proportion and currently is considered among ten leading mortality causes globally [1, 2]. Of all the adverse consequences that could arise from this disease, foot disorder remains one of the most feared complication as it leads to significant morbidity and prolonged hospitalization, causing immense social, emotional and financial impact towards patients [3, 4]. Unlike diabetic macroangiopathy, the role of microvascular abnormalities in the pathogenesis of diabetic foot ulcer (DFU) has not yet been established. There are also many studies that emphasized the functional impairment of endothelial cells (ECs) in DFU [5-8], but seldom being described according to their actual anatomical structure. The primary aim of this study was to determine the histomorphology of skin microvascular ECs of the lower limb and their changes in response towards diabetic state.

MATERIALS AND METHODS

The study was conducted from 1st January 2011 until 30th June 2011 in Hospital Tengku Ampuan Afzan, Kuantan, involving 10 control subjects and 20 diabetic foot patients with their age ranging from 33 to 75 years. All these patients are known to have type II DM for duration of 1 to 25 years.

The subjects were categorized into control group, neuropathic ulcer (NPU) and neuroischemic ulcer (NIU) group according to the assessment of peripheral pulses, ankle-brachial index and criteria listed in Neuropathic Symptoms Score and Modified Neuropathic Disability Score. The demographic data obtained are as follows: age, gender, races and duration of illness. All patients were treated with antibiotics preoperatively and their glucose and electrolyte levels were optimized. Verbal and written informed consents were obtained prior to the procedures from those patients who had undergone minor operation for lesion up to ankle joint (e.g: wound debridement) or minor amputation (e.g: Ray amputation) for the first time. For the control group, skin samples were taken from the dorsomedial aspect of the foot, just proximal to the metatarsophalangeal joint region. All samples are taken using skin biopsy punch (Accu-Sharp Punch 5.0mm). Patients were given general anaesthesia during the operations, while the control subjects were given topical anaesthesia (Emla cream 5%) to avoid any changes that could occur to the microvascular due to its pharmacological effects [9].

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Each sample measured 0.5cm X 0.5cm X 0.5cm and kept in different containers containing neutral buffered formalin. Fixed tissues left for 24-48 hours at room temperature before being processed overnight using automated tissue processor (Leica TP 1020). Next, the samples were put into paraffin blocks and cut into smaller sections (5 to 7μm) by using microtome (Leica RM 2245). Then, the sections stained with hematoxylin-eosin (H&E).

For the assessment of dermal arterioles, the endothelial cell count (ECC) and endothelial cell thickness (ECT) performed under high power field (X 40) microscope magnification (Olympus BX 51) and the mean of three readings were recorded. The values were measured in micrometer (μm) and the images were captured and analyzed using 5MP Dino-Eye digital microscope AM-7023.

All calculations performed by PASW Statistics version 18. The numerical variables were checked for their normality and presented by mean (standard deviation). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to test the mean differences between these three groups. Independent student t-test was applied between the diseased groups; while qualitative variables were calculated by using Chi-square test. The level of significance was set at p-value<0.05.

RESULTS AND DISCUSSION

There were 30 subjects altogether in this study, involving 17 males and 13 females. Among them were 25 Malays, 4 Chinese and an Indian with their age ranging from 33 to 75 years. From these 20 individuals who were having DFU, 15 of them were diagnosed of having DM for more than 10 years. No significant differences of age, gender and races noted among these 3 categories, enabling them to be comparable to each other. There was also no significant difference in term of age, gender, races and duration of illness with regards to their ECC and ECT; therefore these data were analyzed collectively.

As shown in Table 1, there were significant decrement in ECC among NPU and NIU groups as compared to controls, $F(2,27)=5.31$, $p=0.01$. Meanwhile, the ECT of both diseased groups were significantly higher than the controls, $F(2,27)=52.42$, $p<0.01$. The Tukey post-hoc comparisons indicate that the ECC and ECT of neuropathic group (M=5.63, 95% CI [4.87, 6.40], M=6.20, 95% CI [5.51, 6.90]) and neuroischemic group (M=5.70, 95% CI [4.98, 6.42], M=6.77, 95% CI [6.41, 7.12]) were not significant as $p>0.05$.

Table 1. Demographic characteristic and endothelial cell assessment in subjects with neuropathic ulcers, neuroischemic ulcers and control individuals

		Control Mean (SD) n = 10	Neuropathic ulcer Mean (SD) n = 10	Neuroischemic ulcer Mean (SD) n = 10	*p*
Age		54 (6.7)	56 (13.2)	61 (6.7)	0.10
Gender	Male	8	4	5	0.17
(n)	Female	2	6	5	
Race *(n)*	Malay	10	7	8	0.20
	Chinese	-	3	1	
	Indian	-	-	1	
Duration of illness		-	10.3 (4.2)	11.5 (7.3)	0.66
Endothelial cell count (ECC)		7.13 (1.37)	5.63 (1.07)	5.70 (1.01)	$< 0.05^a$ $> 0.05^b$
Endothelial cell thickness (ECT)		3.78 (0.52)	6.20 (0.96)	6.77 (0.49)	$< 0.05^a$ $> 0.05^b$

Data are n or mean (SD)
[a]Control group vs neuropathic and neuroischemic groups
[b]Neuropathic group vs neuroischemic group

Basically, this study has revealed to us that the numbers of ECs among NPU and NIU patients are lesser. Instead of multiplying their numbers, the ECs become reduced, even though in response towards injury. The size of ECs are also enlarge as evidence by their thickness (see Fig. 1). Fadini et al. and Hamed et al. have stated that the involvement of peripheral vascular disease in DM revealed profound effect to the microvascular ECs [10, 11]. But since most of the samples that are included in this study were among NPU and NIU patients who had prolonged exposure towards hyperglycaemia, the morphological changes between both groups may not differ significantly. It is because the autoregulation impairment of the microcirculation becoming more apparent with increasing duration of illness [12].

ECs are needed to regulate the vascular homeostasis, maintain the blood flow and nutrient delivery, and also preserve the balance between proangiogenic and antithrombotic properties.

However, the exposure towards hyperglycaemia has affected extracellular and intracellular activities of the ECs via interrelations between different biochemical pathways [13, 14]. The formation of oxidants further disrupts various signalling cascades that alter the gene expression and protein function, which subsequently visualized by the morphological changes of the ECs.

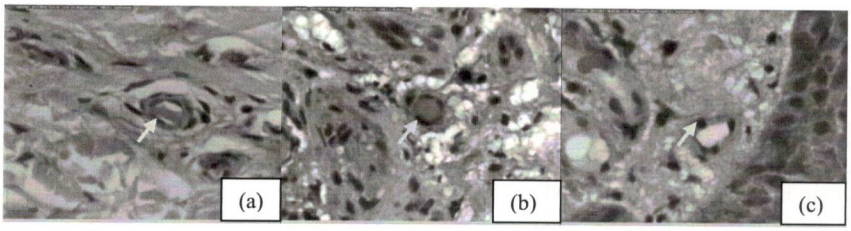

Fig. 1. Pictomicrographs show (a) the elliptical shape of the ECs of the control individual (H&E X 40) as compared to (b) larger size of ECs noted from the arterioles of neuropathic ulcer patient (H&E X 40). But the ECs from the neuroischemic patient (c) are most prominent (H&E X 40).

Few studies done by analysing nerve microvasculature showed that hyperplasia of ECs is a response towards deprivation of its normal functions in maintaining vascular homeostasis and hypoxic-induced state [15, 16]. However, these hypotheses were in contrary to Yasuda et al., who proposed that the occurrence of ECs hyperplasia that was seen in other types of tissue could be different than the nature of skin microvascular ECs in DM patients [17]. The ECs hyperplasia also may not be a conclusive feature in DM patients as normal individuals may also have it [18]. Therefore, other studies have proposed that the enlargement of ECs sizes may occur in DM patients [18, 19] and contribute towards decrement in luminal area, which further disrupt the normal hemoerheology [20, 21]. Nonetheless, the pathological explanation for the microvascular ECs hyperplasia and hypertrophy are still not yet well understood as pathophysiological aspect in both changes could be differ [19].

Apart from that, the ischemia of the vessel wall and its degenerative changes contribute towards desquamation of microvascular ECs. Long standing exposure towards hyperglycaemia can cause production of cytokines and growth factors, formation of signalling complexes, new gene promotion within the ECs, expression of cell adhesion molecules and induction of diapedesis, which may contribute towards ECs damage; thus resulting in induction of apoptosis and delayed cell proliferation [22-26]. As a consequence, reduction in number of ECs together with inactivation of angiogenic properties contributes towards wound healing impairment in DFU patients [5, 26]. Delayed wound healing was deliberately associated with reduction in circulating endothelial progenitor cells that are responsible to differentiate into mature ECs.

CONCLUSION

The skin microvascular ECs adapt functionally and structurally in response towards stress and injury, such as in diabetic state. These morphological changes are best explained by interrelation of various metabolic pathways that occur either intra or extracellularly. Failure of ECs proliferation and induction of apoptosis may cause reduction in ECC among DFU patients. While the enlargement of ECs size occurs in close relation with mediators activities within the cells during inflammatory response. It is best to include those who are asymptomatic DM or impaired glucose tolerance test individuals in this study so that we can assess the early morphological changes of skin microvasculature.

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