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CLINICO-PATHOLOGICAL ASSESSMENT OF VASCULAR LESIONS IN COMPLICATED DIABETIC FOOT

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ABSTRACT

Background: The pathological lesions of microcirculation in diabetic foot have not been previously fully studied. Identification of such lesions and their association with clinical findings in complicated diabetic foot will allow the expansion of the knowledge related to the pathological background of this condition. The aim of this study is to perform a quantitative report on microvascular changes of complicated diabetic foot and correlate these changes with clinical findings in such patients. **Patients and Methods**: This is a prospective study on all cases admitted to the surgical department, King Fahd Hospital of University with diabetic foot problems who required foot amputation between January 2014 and September 2015. Preoperative diagnosis was based on history and comprehensive neuro-vascular physical examination ± Computed angiogram (CTA). Skin and tissue biopsies were taken from the amputated foot and subjected to histopathological and immunohistochemical study. The findings were compared with similar parameters of non-diabetic patients (controls). **Results:** During the study period, 26 patients (22 diabetic and 4 controls) were included. Of the diabetic patients, 16 (73%) had peripheral arterial disease (PAD). There was significant difference between diabetic and control groups in small blood vessel basement membrane thickness (22.46±10.28 microns in diabetic vs. 10.51±3.15 microns in control, p=<0.001) and microvascular area density (41.61±19.54/mm^2 in diabetic vs. 13.27±2.44/mm^2 in control p=<0.001). However there was no significant difference in small vessel (microvascular) changes between diabetics with and without PAD. Arteriolar hyalinosis and mononuclear inflammatory cell infiltrate was seen in all diabetic cases. **Conclusion:** Ischemia of the diabetic foot is secondary to pre-existing diabetic microangiopathy which is often aggravated by superimposed multi-segment arterial disease.

KEYWORDS: Diabetic Microangiopathy, peripheral vascular disease, diabetic foot. Presented in part at the 65th ESCVS Congress, Belgrade, Serbia, April 2016.

INTRODUCTION

The actual histo-pathological features of microvascularity of 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.[1,2] Recent studies have proven that microangiopathy on the foot level cannot be viewed as an obliterating microangiopathy[3] (as it is commonly recorded in diabetic retinopathy and nephropathy). Ischemia, secondary to the multi-segmental arterial disease overlap with the pre-existent structural microvascular changes and functional microangiopathy.[4] The alterations of the microcirculation can also explain the delayed healing noticed in patients suffering from diabetes mellitus,[5,6] and, even more, the high incidence of diabetic foot infections.[7] Identification of vascular lesions in complicated diabetic foot will allow the expansion of the knowledge related to the pathological background of this condition. The aim of this study is to perform a quantitative report on microvascular changes and correlate these changes with clinical findings of complicated diabetic foot.

PATIENTS AND METHODS

This is a prospective study on all cases admitted to the department of Surgery, King Fahd Hospital of University with diabetic foot problems who required foot amputation between January 2014 and September 2015. The study was approved by local ethical committee.

Written informed consent was taken from each patient to participate and publish any data of the study. All patients were subjected to a comprehensive physical examination especially vascular assessment of foot pulses. Neurological assessment included 10 g monofilament testing and vibration perception using tuning fork. Measuring the ankle-brachial index (ABI) was obtained from each extremity using a Multi Doppler II Advanced Bi-directional Doppler (Huntleigh, UK). The higher of arm and ankle pressures (dorsalis pedis or posterior tibial) was used to calculate ABI. Patients were considered to have PAD if the ABI was < 0.9. If ABI was difficult to measure due to calcified and uncompressible vessels or there was tissue loss extending to the ankle, CTA was performed. Diabetes Mellitus (DM) was defined as self-reported physician diagnosis, use of diabetes medication, fasting glucose of 126 mg/dL in two separate occasions, or 2 hours post 75 g oral glucose tolerance test of 200 mg/dL in two separate occasions or random blood glucose 200mg/dL in the presence of classical symptoms of hyperglycemia. Preoperative diagnosis was based on history, comprehensive neuro-vascular physical examination, ±CTA. Skin and tissue biopsies were taken from the amputated foot and subjected to histopathological and immunohistochemical study. The findings were compared with similar parameters of non-diabetic patients (controls) who required foot amputation.

Histopathology and Immunohistochemistry: Each specimen was formalin fixed, paraffin embedded and sectioned at a thickness of 4 microns. Sections were then deparaffinized in xylene, hydrated in descending grades of alcohol and stained with H and E, Masson's trichrome (to highlight fibrosis), and immunohistochemically for CD31 (to highlight vascular endothelium). Immunohistochemical staining was performed in a Ventana Benchmark automated immunostainer according to the manufacturer's instructions (Ventana Medical Systems Inc., Strasbourg) using the labeled streptavidin-biotin (LSAB) method with 3,3'-diaminobenzidine (DAB) as chromagen. For each specimen, histpathologic alterations were reported for: epidermal and basement membrane thickness in microns. Basement membrane thickness was performed by measuring the mean of thickness of 10 blood vessels in each case. Small vessels area density was assessed by measuring the mean of most vascular area per mm2 in 10 fields. Micro-measurements and counting were performed in a Ventana Iscan Coreo scanner / image analyzer. Presence of arteriolar hyalinosis and arteriolar obstruction was reported. Intimal thickening, thrombosis or calcifications in large vessels were checked. Interstitial tissue fibrosis and presence of inflammatory cells and nerves hypertrophy were also reported.

All stained sections were separately examined by two experienced pathologists (MS and TS) who were unaware of patient characteristics and clinical details.

Statistical analysis: Data for groups were summarized either as the mean ± standard deviation (SD) or percentage of the risk factors. Differences between the groups were tested initially for statistical significance using t-test, chi-square test, Fisher's exact test as appropriate. Significance was set at $p < .05$ for all comparisons. Statistical analyses were performed using SPSS 15 software (Chicago, Il, USA).

RESULTS

During the study period, 26 patients (22 diabetic and 4 controls) were included. CTA was performed in 18 patients. Of the diabetic patients, 15 (68%) were male and 16 (73%) had PAD (Table 1). Indication of amputation in diabetic patients was extensive gangrene of the foot for mangle extremity± non-reconstructable PAD. There was significant difference between the diabetic and control groups in epidermal thickness (114.97±155.43 microns in diabetic vs. 70.35±13.57 microns in control, p=<0.001), small blood vessel basement membrane thickness (22.46±10.28 microns in diabetic vs. 10.51±3.15 microns in control, p=<0.001) and microvascular area density (41.61±19.54 vessels /mm^2 in diabetic vs. 13.27±2.44/ mm^2 in control, p=<0.001) (Table 2, Fig. 1–3). Arteriolar hyalinosis was seen in all diabetic cases Fig. 4 However, there was no arteriolar obstruction. Thrombosis and intimal thickening were seen in large vessels in patients with PAD. However there was no significant difference in small vessel (microvascular) changes between diabetic with and without PAD (Table 3). In the interstitial tissue there was mononuclear inflammatory cell infiltrate (Fig. 5) and variable fibrosis (Fig. 6) was seen in all diabetic cases studied. Nerve hypertrophy was seen in 3 cases.

Table 1: Diabetic Patients characteristic.

Characteristic	No. 22
Age Mean (years±SD)	52.23±12.61
Male (%)	15 (68%)
Duration of DM Mean (years ±SD)	17.37±6.51
Retinopathy (%)	10(45%)
Nephropathy (%)	4(18%)
Neuropathy (%)	22(100%)
Peripheral arterial disease (%)	16 (73%)
Smoking (%)	14(64%)
Dyslipidemia (%)	21(95%)
Hypertension (%)	13(55%)
Poor glycemic control	22(100%)

Table 2: Difference between Diabetic and control groups.

	Control group (No=4)	Diabetic group (No=22)	P value
Age Mean (±SD)	42.41±9.85	52.23±12.61	0.137
Male (%)	3 (75%)	15 (68%)	0.785
Epidermal thickness (micron)	70.35±13.57	114.97±155.43	<0.001
Basement Membrane Thickening (micron)	10.51±3.15	22.46±10.28	<0.001
Microvessel Area Density / mm^2	13.27±2.44	41.61±19.54	<0.001

Table 3: Difference between Diabetic microvascular changes with and without PAD.

	Diabetic with PAD(No=16)	Diabetic without PAD(No=8)	P value
Age Mean (±SD)	63.64±8.72	54.43±13.61	0.68
Male (%)	11 (69%)	4 (50%)	0.41
Epidermal thickness (micron)	110.52±60.43	139.29±114.78	0.48
Basement membrane thickening (micron)	18.39±8.25	25.52±9.38	0.39
Microvessel Area Density/ mm^2	40.28±18.34	41.97±19.69	0.84

Legends

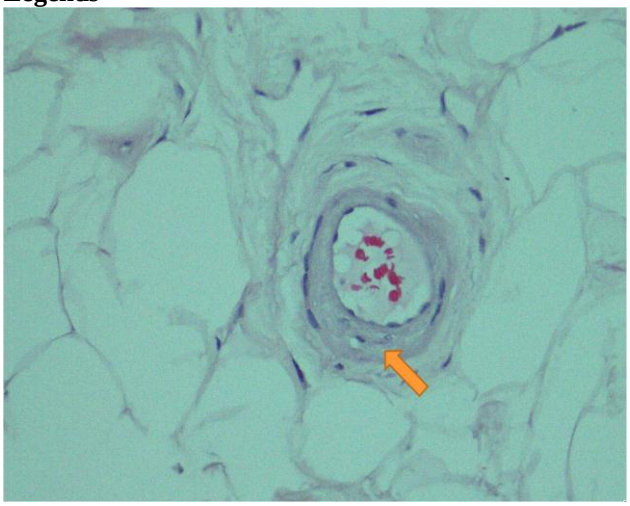

Fig 1: Diabetic case: Basement membrane thickening. Small vessel showing thickened intimal basement membrane in subcutis (arrow). H and E x 400.

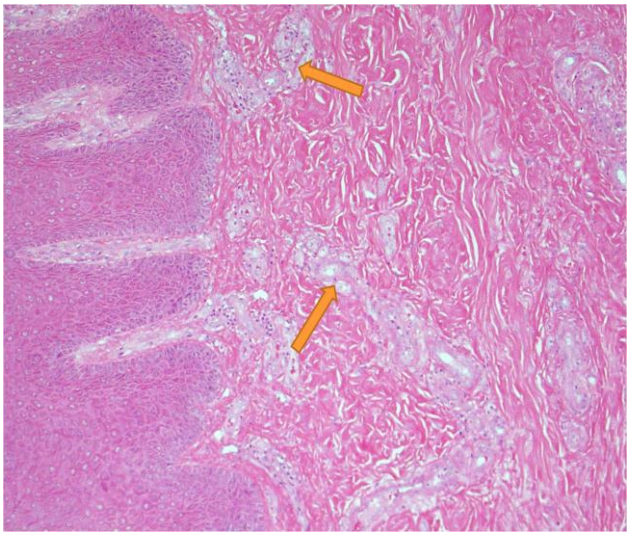

Fig 2: Diabetic case: Neovascularization. Arrows point at newly formed vessels in dermis. H and E x 200.

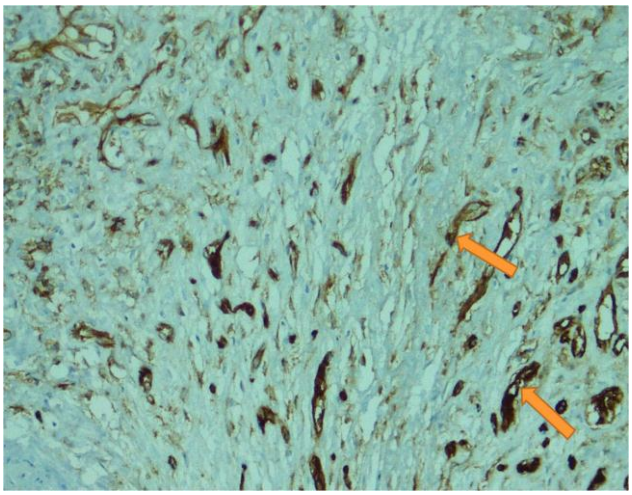

Fig 3: Diabetic case: Neovascularization. Arrows point at endothelium of newly formed vessels highlighted by positivite immunostaining for CD31. Immunhistochemistry x 200.

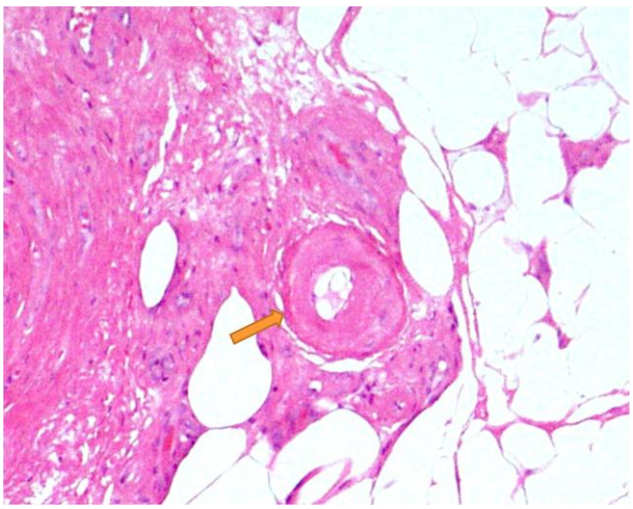

Fig 4: Diabetic case: Hyalinosis. Section showing a hyalinosed small vessel in deep dermis/subcutis (arrow). H and E x 400.

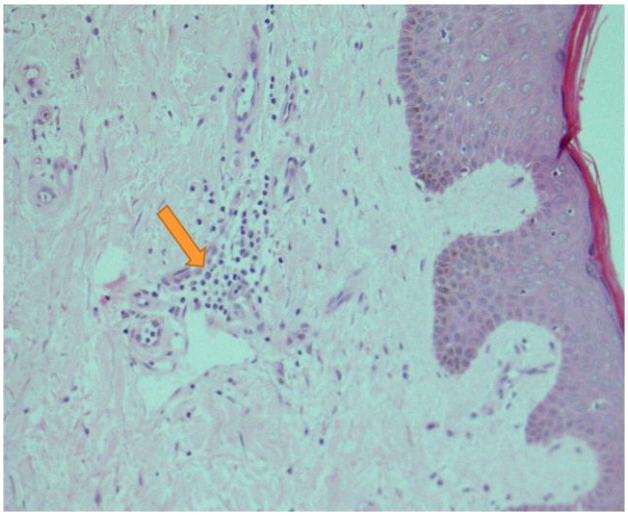

Fig 5: Diabetic case: Section of skin showing mild focal dermal mononuclear inflammatory cell infiltration. H and E x 100.

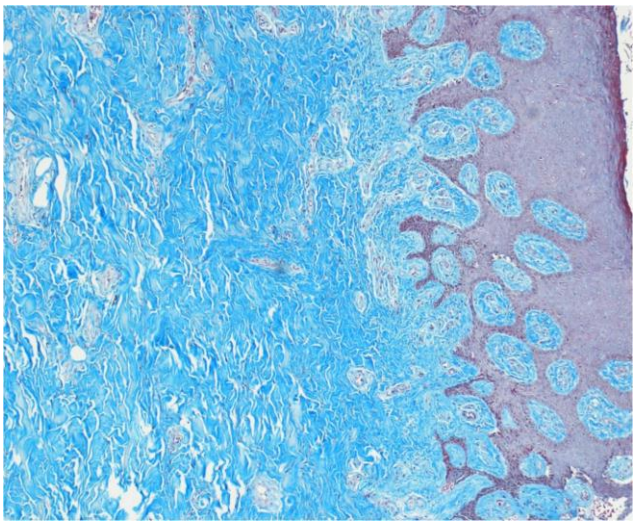

Fig.6: Diabetic case: Trichrome stain showing dermal fibrosis. X 100.

DISCUSSION

Diabetic foot is one of the most common complications of DM[8] It was found that the incidence of major amputations (below and above knee) is 27.2%[9]-36.7%[10] in diabetics with foot complications. Understanding the precise microscopic mechanisms that lead to amputation in diabetic foot may help designing new therapeutic strategies to treat such cases. The pathogenesis of diabetic microangiopathy is complex and is not completely understood. Long standing hyperglycemia triggers many pathways which end by endothelial damage.[11] In the present study all patients had poor glycemic control.

DM is a major risk factor for atherosclerosis and its extent.[12,13] Sixteen (73%) of our patients had clinical, radiological and pathological evidence of large vessel disease compared to 87% in a previous study.[14] In the present study and others,[3] microangiopathy was present in all patients who had atherosclerosis and more than half of cases, ischemia secondary to in large vessel arteriosclerosis overlap with the microagiopathy. There was no significant difference of pathological degree of microangiopathy between cases who had atherosclerosis and those without. Presence of other risk factors of atherosclerosis increases the complications of both microvascular[11] and peripheral arterial disease.[13] Most of the diabetic groups in the present study are dyslipidemic (95%), smoker (64%) and hypertensive (55%). Duration of DM is another risk factor of microvascular complications.[15] In all diabetic group patients duration was more than 10 years with mean 17.37±6.51 years.

The present study was the first quantitative report on microvascular changes and correlates these changes with clinical findings of complicated diabetic foot. Thickening of basement membrane and excessive proliferation of sub-endothelial layer have been shown in our study and others.[3,16] These changes can cause dysfunction of vascular endothelial cells which will contribute to the reduction in vasodilatation and impaired wound healing in diabetic patients.[17] Contrary to previous study[16] there was no arteriolar obstruction in our study and as well as in other studies.[18] The present study has few limitations. It is a case control observation study. There is also big difference between the case (n=22) and the control (n=4) groups. The highly significant difference between the 2 groups may compensate for the lower power of the study.

Arteriolar hyalinosis is another finding in our as well as in other studies.[19] The increase of arteriolar resistance and also the increase of circulation through the arteriovenous shunts will decrease the blood quantity reaching the capillary level.[16] Presence of mononuclear inflammatory cell infiltrate is another finding in the present study and others[16,20] which is one of the main factors associated with failure of ulcers to heal.[21]

CONCLUSION

Ischemia of the diabetic foot is secondary to pre-existing diabetic microangiopathy which is often be aggravated by superimposed multi-segment arterial disease.

ABBREVIATIONS

ABI: ankle-brachial index, CTA: Computed angiogram, PAD: peripheral arterial disease. DM: Diabetes Mellitus, LSAB: labeled streptavidin-biotin, DAB: 3,3'-diaminobenzidine SD: Standard deviation

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AVAILABILITY OF DATA AND MATERIALS

At present, the data are not available to be shared

AUTHORS' CONTRIBUTIONS

ME contributed to this study's conception, design and analysis, and drafted manuscript. AE contributed data acquisition, and interpretation of data. MS contributed pathology examination, the data acquisition and interpretation of data. TE contributed pathology examination and interpretation of data. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interest.

CONSENT FOR PUBLICATION: Not applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the King Fahd Hospital of University. All subjects provided written informed consent prior to participation in the study

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