The Structural Changes of the Skin Surrounding Ulcers in Diabetic Foot

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Abstract

Background: Diabetic foot is a serious complication of diabetes which aggravates the patient's condition and abnormal regulation of the hemodynamics in the small vessels may contribute to the development of microangiopathy.

Objective: To investigate the histological changes in the skin tissue covering the area surrounding the ulceration of the diabetic foot.

Patients & Methods: In the present study 45 patients were collected from Al-Jumhuri Teaching Hospital in Mosul-Iraq during the period from December 2015 to December 2016.The patients were subdivided into 3 groups (15 for each group).Group I were the control group (non-diabetic but have traumatic ulceration); group II were insulin-dependent diabetics (Diabetes mellitus type-I) & group III were non-insulin dependent diabetes (Diabetes mellitus type-II). Specimens of skin were obtained from the area surrounding the diabetic ulcer.The specimens were fixed in 10% neutral buffered formalin for 24 hours then histological sections were prepared and stained by Hematoxylin and Eosin, and Orcein-VanGieson stains for examination by light microscope. All the work was done in the Department of Anatomy, Mosul College of Medicine.

Results: The skin sections showed the presence of epidermal hyperkeratosis with regular acanthosis & chronic inflammatory cells infiltration in the dermis; fibrous tissue deposition in tunica media of the small arteries and arterioles; obvious degeneration of Schwann cells in the peripheral nerves & finally degeneration of regional sweat glands.

Conclusions: The diabetic foot ulcer causes morphological & structural changes in the skin tissue surrounding it.More significant incidence of diabetic foot ulceration in female, smoker, hypertensive, and those having disturbance in renal functions and the severity of changes appeared to be more in non-insulin dependent diabetic patients.

***Keywords*:** Diabetic foot, Skin, Microangiography, Neuropathy.

Introduction:

Diabetic foot ulceration is frequently associated with peripheral neuropathy, which is a known cause of morbidity &mortality[1]. The incidence is rising due to ageing process and increased risk factors for atherosclerosis such as smoking & obesity, which is usually associated with diabetes[2]. The diabetic foot lesion involves a wide range of structural changes affecting the nerves (autonomic & peripheral neuropathy), blood vessels (macro & microangio-graphy), joints & bone lesions of foot and skin [3]. The exact mechanism underlying diabetic ulceration is not well known yet, although many mechanisms has been proposed including genetic factors[4]. However peripheral neuropathy forms the major cause combined with arterial insufficiency[5]. The first pathological change is vasoconstriction associated with vascular abnormalities, such as thickening of the capillaries basement membrane &hyperplasia of endothelial lining cells with subsequent hypoxia and as the disease progress, neuronal dysfunction occurs [1]. The microvascular changes occurs early in diabetes, parallel with neuronal ischaemia is a characteristic feature in diabetic neuropathy[6]. Diabetic neuropathy is the major problem in diabetics, which can be prevented by controlling blood glucose level & maintenance of normoglycaemia [7]. The term diabetic foot involves multiple changes at the level of small and large blood vessels, nerves, bones &soft tissues besides abnormalities of the microcirculation, which results in capillary insufficiency, all these leads to alterations in the foot biomechanisms, which leads to tissue destructions and severe infections which might need amputations[8].

The microangiopathy in diabetes can affect different organs at different degrees, like diabetic nephropathy or retinopathy[9]. Recently it is well established that there is a close association between the abnormalities in microcirculation & diabetic neuropathy[10], proving the fact that microvascular changes are closely linked to the diminished nerve conduction and muscular activity[11]. The abnormal neural function contributes to the development of microangiopathy in diabetic foot ulcer[12], and a great development in the field of management of diabetic foot has been made by increasing range of antibiotic therapy & by exploring invasive and non-invasive angiographic techniques [13]. The present study aims to investigate the structural &vascular changes accompanied diabetic foot ulceration and to evaluate the severity and pattern of the condition in relation to the type of diabetes.

Patients and Methods:

In this study 45 patients were collected from Al-Jumhuri Teaching Hospital in Mosul during the period from December 2015 to December 2016 and subdivided into 3 groups(15 for each).Group I is the control group, (they have no diabeties but they have an traumatic ulcetation in their foot caused by car accidents or fall from hight), group II suffering from type I diabetes(insulin-dependent) and, group III suffering from type 2 diabetes (non-insulin dependent). Skin fragments were taken from the area surrounding the ulcers.Detailed history, thorough physical examination and some investigations has been done.The history includes age, sex, occupation,

duration of diabetes, drugs taken and family history to exclude any genetic role. Tissue preparations was performed in Department of Anatomy, College of Medicine, University of Mosul.

The specimens (pieces of about 1 cm in thickness) were put in a fixative solution (10% neutral buffered formalin) for 24 hours, then dehydrated in ascending concentrations of ethanol (70% for overnight ,90% as 2 changes for one hour each and then in 2 changes of 100% alcohol for 2 hours each).

The specimens were then put in 3 changes of xylene for 1 hour each).Complete removal of the clearing agent was done by immersing the specimens into 3 successive paraffin baths in oven for 1 hour each and fanially, paraffin blocks were prepared by embedding the tissue specimens using paraffin wax at melting point of 55-60 C and these blocks were ready for sectioning using Reichert Rotary Microtome. Serial sections of about 4 *MM* in thickness were cut from each block, then the sections were collected and mounted on glass slides to be stained by Haematoxylin & Eosin and Orcein-VanGieson stains for microscopical examination .

Results:

Among the 30 diabetic patients of groups II & III we noticed the followings demographic variables (see table .1):

1-40%(12) cases were males and 60%(18) were females.

2-70% (21) of the cases were smokers.

3-80%(24) of the cases were hypertensives.

4-90% (27) of the cases were having renal problems (nephropathy).

From the table, it seems obvious that there is significant increase in the incidence of diabetes in female patients (P-value=0.01) compared with non significant incidence in male (P-value=0.1).The smokers(70%) and hypertensive patients (80%) have significant increase in the incidence of diabetes (P-value=0.01) compared with non significant incidence in non smoker and non hypertensive patients (P-value=0.1). Patients with renal insufficiency (90%) have significant increase in the incidence of diabetes (P-value=0.01) compared with those with no renal insufficiency(P-value=0.3).

The microscopical examination of the skin fragments from tissue around the ulcers areas showed specific changes which can be tabulated as follows:

A-In the insulin dependent patients (group II):

1. Mild hyperkeratosis in the epidermis (increase thickness of keratin layer in the dermis) with regular acanthosis due to hyperplasia of the stratum spinosum with chronic inflammatory cellular infiltration (mainly lymphocyte and eosinophils) compared to control group (see figs.1 &2).

2. Disorganization and degenerative changes of the sweat glands surrounded by prominent deposition of lymphocytes (see fig. 3).

3. Congestion of the dermal blood vessels with obvious perivascular lymphocytic infiltration arranged in concentric layers (see fig. 4).

4. Disturbance of the normal architecture with focal areas of necrosis involving destruction of the vascular structures (see fig. 5) and focal melanin pigmentation in the dermis (fig.6)**.**

5. The arterioles & small arteries showed swollen endothelial lining with excessive proliferation of the subendothelial connective tissue layer with fibrous tissue deposition in the tunica media (see fig. 7).

6. On using Orcien-Van Gieson stain, heavy collagen fibers deposition are seen around the blood vessels (see fig. 8).

B-In Non-insulin dependent diabetic patients (group III):

1. More marked hyperkeratosis compared to that of group II (insulin dependent), which appeared as a thickened keratin covered the epidermis with obvious severe acanthosis (fig. 9).

2. Few inflammatory cells infiltrated in the dermis as lymphocytes and plasma cells (see fig. 10).

3. Dilated acini of the sweat glands with degenerative changes of their lining epithelium and dilatation of their ducts (fig.11).

4. The peripheral nerves showed an obvious vacuolar degeneration of Schwan cells, which eventually results in disappearance of some nerve fibers(see fig. 12)

Table 1: Statistical significance of the demographic variables in patients with diabetes mellitus

Variables		No. (N=30)	%	P-values
Sex	Female	18	60%	0.01(S)
	Male	12	40%	0.1(NS)
Smoking	Yes	21	70%	0.01(S)
	No	9	30%	0.2(NS)
Hypertension	Yes	24	80%	0.01(S)
	No	6	20%	0.2(NS)
Renal insufficiency	Yes	27	90%	0.01(S)
	No	3	10%	0.3(NS)

S=Significant (P≤0.05); NS=Non-significant (P>0.05)

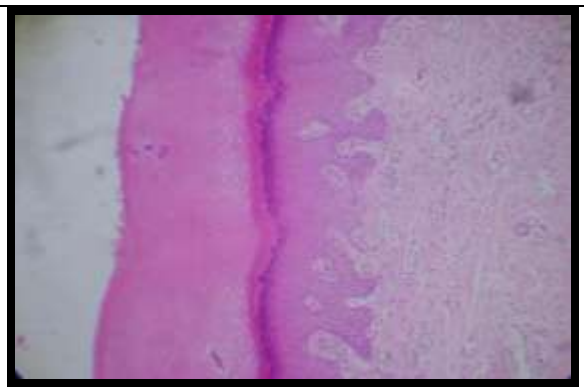

Figure 1: Micrograph of skin tissue from control showing normal skin consisting of epidermis(white arrow) and dermis (black arrow) (H&E X100).

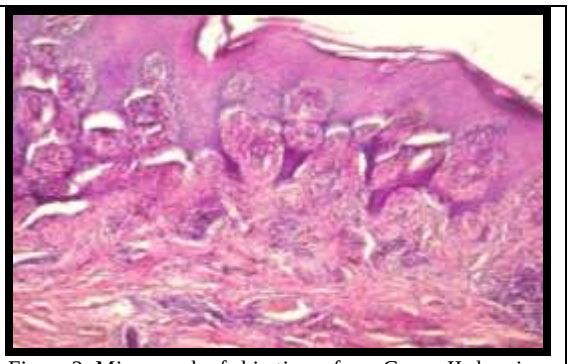

Figure 2: Micrograph of skin tissue from Group II showing mild hyperkeratosis in the epidermis (white arrows) with regular acanthosis (black arrows) and chronic inflammatory cells infiltration (arrow heads) (H&E X150).

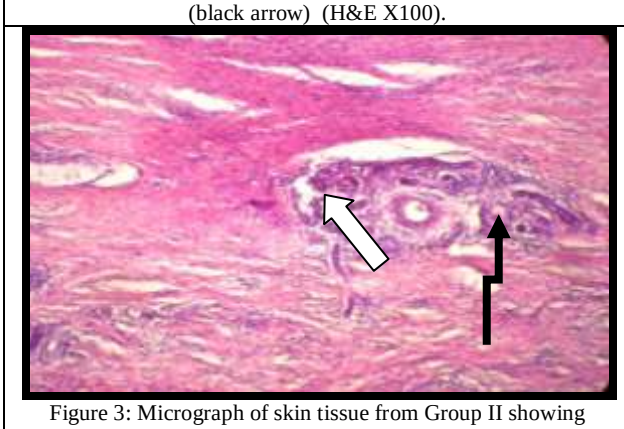

Figure 3: Micrograph of skin tissue from Group II showing degenerative changes of the sweat gland (black arrow) surrounded lymphocytes(white arrow) (H&E X400).

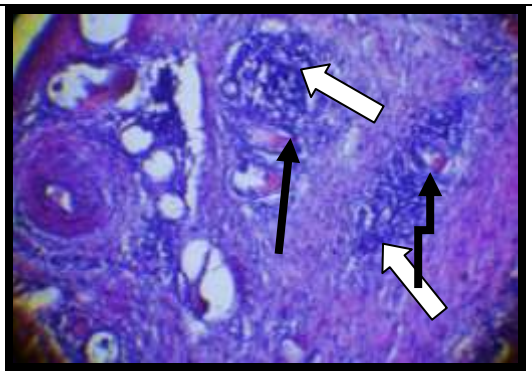

Figure 4: Micrograph of skin tissue from Group II showing congestion of the blood vessels in the dermis (black arrow) with perivascular lymphocytic infiltration (white arrows) (H&E X100).

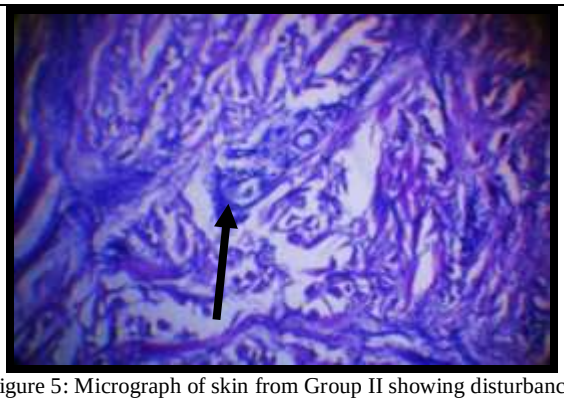

Figure 5: Micrograph of skin from Group II showing disturbance of skin architecture with focal areas of necrosis (black arrow) (H&E X100).

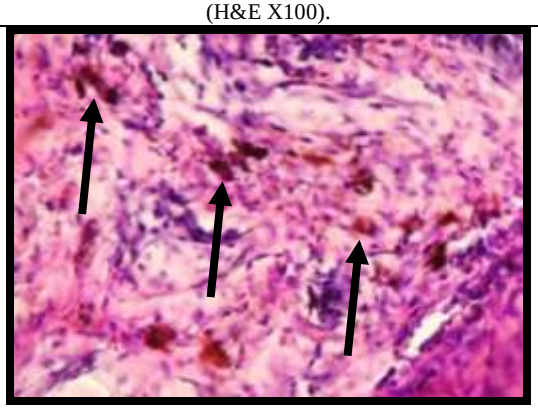

Figure 6: Micrograph of skin from Group II showing focal melanin pigmentation in the dermis (black arrows) (H&E X150).

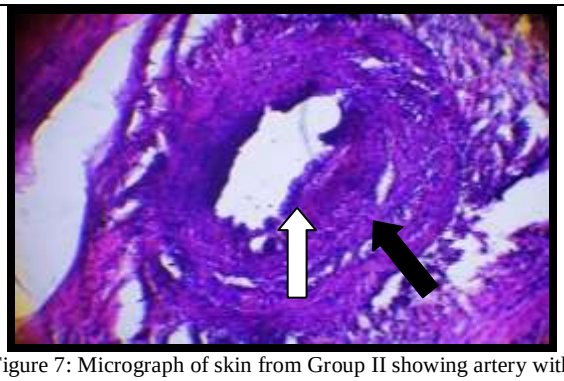

Figure 7: Micrograph of skin from Group II showing artery with excessive proliferation of the subendothelial connective tissue layer (white arrow) and fibrous tissue deposition in tunica media (black arrow) (H&E X150).

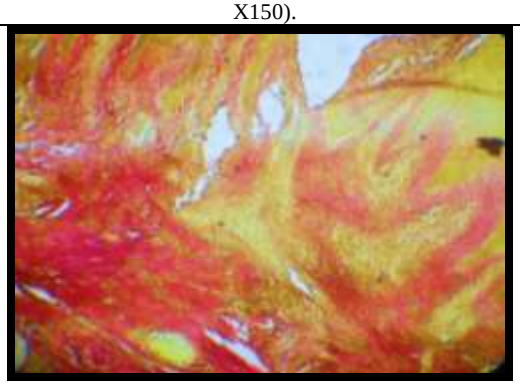

Figure 8: Micrograph of skin from Group II showing hyperacanthosis of epidermis (white arrows) with heavy collagen fiber deposition around the blood vessels (black arrows) (Orcien-Van Gieson X400).

Figure 9: Micrograph of skin from Group III showing more hyperkeratosis as thickened keratin covering the epidermis (black arrows) with sever acanthosis (white arrows) (H&E X100)

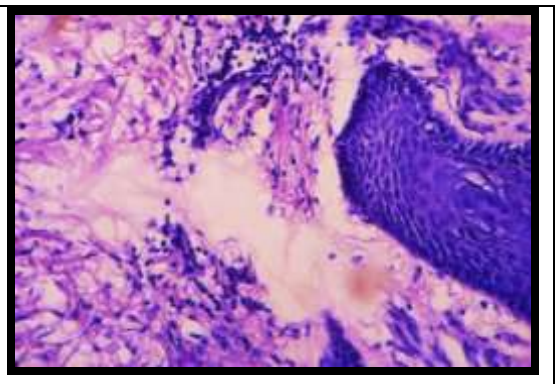

Figure 10: Micrograph of skin from Group III showing few inflammatory cells in the dermis (black arrow) (H&E X150)

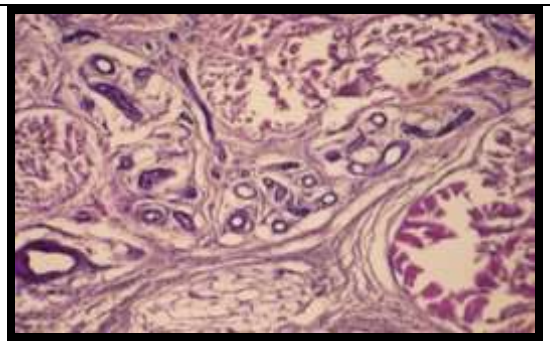

Figure 11: Micrograph of skin from Group III showing dilated acini of the sweat glands with degenerative changes of their lining epithelium (black arrows) and dilatation of their ducts(white arrow) (H&E X150

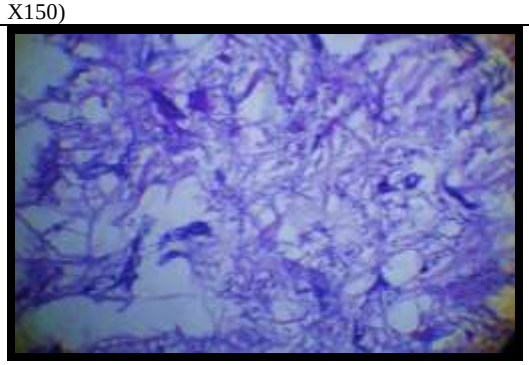

Figure 12: Micrograph of skin tissue from Group III showing vacuolar degeneration of Schwann cells of the peripheral nerve (black arrows) (H&E X400).

Discussion:

The duration of diabetes, hypertension, smoking & elevated cholesterol are important risk factors for the development of diabetic foot ulcer [(14)].

In the present study, hyperkeratosis with regular acanthosis in the epidermis and chronic inflammatory cells infiltration might be due to release of pro-inflammatory cytokines like prostaglandins, leukotrienes and interleukins which causes an inflammatory response. This finding is in agreement with what has been reported before[(15)]. Furthermore, vascular congestion in the dermis could be attributed to the release of vasodilator substances, then the stagnant blood flow in the dilated vessels will cause tissue hypoxia, which is followed by degenerative changes in the sweat glands & focal areas of necrosis in the foot. This finding is nearly similar to what has been noted by Nicolosi& Botek in 2015 [(16)]. In the exhausted areas more fibroblasts are seen which leads to deposition of huge amount of collagen fibers[(17)]. In addition alveolar macrophages may release fibroblast chemotactic factors leading to more fibroblast proliferation and fibrous tissue deposition in tunica media of the vessels of the region [(18)].

The focal necrotic areas could be provoked by mitochondrial damage due to oxidative stress, as mitochondrial swelling may lead to rupture of the outer mitochondrial membrane & then release of cytochrome C that will activate the proapoptotic Bax protein, which triggers cellular apoptosis followed by necrosis [(19)].The abnormalities in the vascular structure & function are caused by loss of sympathetic tone, specially at the level of capillaries, small and medium sized arterioles leading to local ischaemia with a decrease in the blood flow& nutrition to the tissues[(20)]. The alterations of the microcirculation can also explain the delay healing of diabetic foot ulcer [(21)].The frequency and severity of wound infection may be related to high glucose levels & occlusive microvascular disease [(22)]. and, during the progression of diabetic foot ulceration there are some morphological changes in the peripheral nerves [(23)].

Conclusions:

1. The diabetic foot ulcer causes morphological & structural changes in the skin tissue surrounding it due to alteration in the structure and function of regional blood vessels and nerves.
2. More significant incidence of diabetic foot ulceration in female diabetic patients compared with male and in diabetic patients who are smoker, hypertensive, and having renal impairments.
3. The severity of morphological changes in the blood vessels, peripheral nerves in the epidermis and dermis appeared to be more in non-insulin dependent diabetic patients compared with those with insulin dependent diabetes.

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