

The Histological Changes of the Skin Lesion in Diabetic Foot

Dr. Muna Zuhair Alhamdany¹

¹ University of Mosul

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Abstract

To investigate the histological changes in the skin tissue covering the area surrounding the ulceration of the diabetic foot. Patients Methods: The study was performed on 30 patients who were classified into 3 groups 10 patient in each. Group I is the control group have no diabetes, group II with diabetes mellitus type I, and Group III patients with diabetes mellitus type II. All the diabetic patients showed various degrees of skin lesions in the foot. Specimens of skin tissue were obtained from the area surrounding the diabetic ulcer from Al-Jumhuri Teaching Hospital and the histological analysis was performed in the

Index terms— diabetic foot, microangiopathy, neuropathy

1 I. Introduction

Diabetic foot is the most common complication of diabetes. The ulceration is frequently associated with peripheral neuropathy which is a well-known cause of morbidity and even mortality in the diabetic patients (1). The incidence is rising as a result ageing and increased risk factors for atherosclerosis such as smoking and obesity commonly associated with diabetes (2). The diabetic foot lesions involves a wide range of structural changes affecting the nerves in the form of autonomic and motor neuropathy, blood vessels as diabetic macro and microangiopathy, joint and bone lesions of the sole, and skin and nail lesions (3). The exact mechanism underlying the pathogenesis of diabetic ulceration is not well known, many mechanisms have been proposed even there may be a genetic influence which increase the susceptibility to such complications (4). However, peripheral neuropathy is the major cause combined with arterial insufficiency caused by atherosclerotic occlusion of the tibioperoneal arteries (5). The first pathological change in the development of diabetic foot ulcer is vasoconstriction associated with vascular abnormalities, such as thickening of the basement membrane of the capillaries and hyperplasia of their endothelial cells lining with subsequent diminished oxygen tension and hypoxia, as the disease progresses, neuronal dysfunction occurs (1). Microvascular changes occurs early in diabetes, parallel to the progression of neuronal ischemia which is a characteristic feature in diabetic neuropathy thus both(D D D D)

C vascular and neural abnormalities determine the severity of structural, functional, and clinical dysfunction (6).

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Following the debridement of the foot ulceration and removal of the skin tissue fragments from inside and around the ulcer, the specimens were put in a fixative solution (10% neutral formalin) for 24 hour then each specimen was cut into 1 cm thick slices and dehydrated in graded alcohol solutions (70% alcohol for overnight, two changes in 90% alcohol one hour for each and two changes in 100% alcohol for two hours) then the specimens were immersed in xylene using three changes with one-hour interval for each. Complete removal of the clearing solution was made by immersing the tissue specimens into three successive paraffin bathes in oven, one hour for each. Finally paraffin blocks were prepared by embedding the tissue specimens using paraffin wax (melting point is 55-60Co) and these paraffin blocks were now ready for sections using Reichert Rotary Microtome, serial paraffin sections of 4 micrometers in thickness were cut from each block, the sections were collected and mounted on glass slides then the sections were stained with Haematoxylin and Eosin for histological analysis.

2 III. Results and Observations

This study was performed on 30 persons, 20 of them were diabetic and 10 of them were not, 8 (40%) male and 12 (60 %) female, 14(70%) smoker and 6(30%) were not, 16 (80%) hypertensive and 4(20%) were not, and ??(90%) suffering from kidney disease and ?(10%) were not.

3 C

The Histological Changes of the Skin Lesion in Diabetic Foot Diabetic neuropathy is the major problem in diabetes mellitus, which may be prevented by the control blood glucose level and maintenance of normoglycaemia (7) . The term "diabetic foot" involves multiple changes on the level of small and large blood vessels, nerves, bone and soft tissues besides abnormalities of the microcirculation which results in capillary insufficiency, all lead to alterations in the foot biomechanics which promotes tissue destructions and severe infections even sometimes, resulting in amputations (8) . The microangiopathy in diabetes can affect different organs to a different degree, like diabetic nephropathy or retinopathy) (9). Recently it is well established that there is a close connection between the abnormalities of the microcirculation and the diabetic neuropathy (10) proving the fact that microvascular changes are closely linked to the diminished nervous conduction and the potential of muscular action (11) . The abnormal neural function contributes to the development of microangiopathy in diabetic foot ulcer manifested as thickening of the basal membrane of the capillaries and proliferation of the endothelial cells lining of both arteries and arterioles, resulting in ischemic changes and ulceration (12) . A great improvement in the field of management of diabetic foot has been made by increasing range of the antibiotic therapy and by exploring invasive and noninvasive angiographic techniques (13) .

Our present study aims to investigate the histological and vascular changes accompanied to the diabetic foot ulceration and to evaluate the severity and pattern of progress of the condition in relation to the type of diabetes and the control of blood glucose level.

4 II. Patients, Materials and Methods

In this study, 30 patients were classified into 3 groups 10 patients in each. Group I is the control group have no history of diabetes, group II suffering from diabetes mellitus type I, and Group III patients with diabetes type II. The specimens of skin fragments were collected from the area surrounding the diabetic foot ulcer from diabetic patients who were admitted for surgical management. Detailed history, thorough physical examination and biochemical testing including serum glucose, renal function and liver function tests and x-ray of the affected foot were carried out, the history includes their age, sex, occupation, duration of diabetes, drug history and family history to exclude genetic predisposition of the condition. All the diabetic patients were affected by peripheral neuropathy and showed various degrees of skin lesions in the foot. Specimens of skin tissue were obtained from Al-Jumhuri Teaching Hospital and the histological analysis was performed in the Department of Anatomy, College of The table showed that female affected with diabetes more than male, and statistical significant increase in the incidence of diabetes in smokers (70%) and in those suffering from hypertension (80%) and renal insufficiency (90%) thus $P < 0.05$.

The histopathological examination of skin fragments from tissue around revealed specific microscopic changes which reflect the pathogenic background of the lesions of diabetic foot.

IV. Histological Findings a) In the control group ? Skin tissue from the control group showed normal epidermis as stratified squamous epithelium and dermis which a connective tissue layer (Figure 1). ? Mild hyperkeratosis in the epidermis (increase thickness of the keratin layer of the epidermis) with regular acanthosis due to hyperplasia of the stratum spinosum in addition to dense chronic inflammatory cells infiltration mainly lymphocytes and eosinophils and at some time associated with polymorphonuclear neutrophils (Figure 2). ? Disorganization and degenerative changes of the sweat gland which are surrounded by prominent deposition of lymphocytes (Figure 3). ? Congestion of the blood vessels in the dermis with obvious perivascular lymphocytic infiltration arranged in concentric layers "muffs" around them, sometimes penetrating to the media layer (Figure 4). ? Disturbance of the histological architecture with focal areas of necrosis involving the destruction of the vascular structures (Figure ??). Focal melanin pigmentation in the dermis (Figure ??). ? The large arterioles and arteries of muscular type showed swollen endothelial cells lining, excessive proliferation of the subendothelial connective tissue layer and fibrous tissue deposition at the level of media (Figure ??).

5 c) In Non-insulin dependent diabetes (NIDD)

? Marked hyperkeratosis more than insulin dependent diabetes appeared as thickened keratin covering the epidermis with obvious severe acanthosis (Figure 9). ? Mild inflammatory cells deposition including lymphocytes and plasma cells (Figure 10). ? Dilated acini of the sweat glands with degenerative changes of their lining epithelium and dilatation of their ducts (Figure 11). ? The peripheral nerves showed an obvious vacuolar degeneration of Schwann cells that eventually have resulted in the disappearance of the nerve fibers (Figure 12).

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7 V. Discussion

The duration of diabetes, hypertension, smoking, and raised serum cholesterol are important risk factors for the development of diabetic foot ulcers in patients with diabetes. However, various pathogenic mechanisms of vascular and haemodynamic dysfunction have been proposed (14) . Platelet dysfunction, immunological mechanisms, presence of adhesion molecules have been described in relation to diabetic foot (1) .

Aguiar et.al, 2007 (15) stated that microcirculation is involved in the pathogenesis of diabetic foot which is sometime termed as "small vessel disease". Endothelial dysfunction precedes the appearance of the microvascular lesions and it is proven by vasoconstriction, marked increase in the microvascular blood flow and vascular permeability and alterations of anti-thrombotic properties of the endothelium (16) .

In this study, hyperkeratosis with regular acanthosis in the epidermis and dense chronic inflammatory cells infiltration might be due to release of proinflammatory cytokines like prostaglandins, leukotrienes, and interleukins which cause inflammatory response. This finding previously reported by (17) . Furthermore, vascular congestion in the dermis could be due to the release of vasodilator substances then the stagnant blood in the dilated vessels will cause tissue hypoxia followed by degenerative changes of the sweat gland and focal areas of necrosis. The finding agrees with that observed by (18) . deposition of collagen fibers occurs due to chronic inflammatory reaction, thus more fibroblasts might reach the area leading to more collagen fibers deposition (19) . Moreover, it has been suggested that alveolar macrophages may release fibroblast chemotactic factors leading to more fibroblast proliferation and fibrous tissue deposition at the level of media (20) .

The focal necrotic areas could be provoked by mitochondrial changes mediated by oxidative stress. Ibrahim, (2013) (21) stated that oxidative mitochondrial swelling may lead to rupture of the outer mitochondrial membrane and release of cytochrome C to activate the proapoptotic Bax protein which triggers cellular apoptosis followed by necrosis depending on the level of ATP.

Abnormalities in the vascular structure and function are caused by loss of the sympathetic tone, especially on the level of capillaries and small and medium arterioles leading to local ischemia, increase of the arteriolar resistance and consequently a decrease of the blood flow and nutritive circulation of the tissues (22) . The alterations of the microcirculation can also explain the late healing diabetic foot ulcer and even a raised suspicion of infections (23) . The frequency and severity of wound infection may be related to high glucose levels and the contribution of occlusive microvascular disease (24) . During the progression of the diabetic foot ulceration, the nervous involvement is sustained by the morphological changes on the level of the peripheral nerve (25) .

Our present study makes a difference by identifying the vascular and nervous changes on the level of cutaneous areas, along with the presence of the inflammatory infiltrate on the dermis and structural modifications of the sweat glands.

8 VI. Conclusions

The identification of vascular and nervous morphological structures in the complicated diabetic foot allows the extension of the knowledge related to the pathological background of this condition. With the progression of diabetes mellitus, the vascular lesions, which appeared on the microcirculation level are aggravating consequently involving arterioles and arteries of muscular type and are being accompanied by nervous lesions shown through morphological changes of the peripheral nerves and these changes were accompanied with lesions involving the epidermis, dermis, and muscles.

9 VII. Recommendations

The microvascular changes in relation to the severity and progress of the diabetic foot ulceration and the role played by the vascular mediators such as serotonin, 5-hydroxytryptamine in diabetes require further studies and recordings using both light and electron microscopy. Angiographic studies of medium and small arteries to investigate occlusive changes in diabetic foot ulceration were recommended. Epidemiological studies to evaluate the influence of diabetes and its outcome on the quality of life particularly in patients with chronic ischemia.



Figure 1: Table 1 :

	Variables	Number (N=30)	Percentage %(N=30)	P-values
Sex	Female	12	60%	0.01(S)
	Male	8	40%	0.1(NS)
Smoking	Yes	14	70%	0.02(S)
	No	6	30%	0.2(NS)
Hypertension	Yes	16	80%	0.02(S)
	No	4	20%	0.2(NS)
Renal insufficiency	Yes	18	90%	0.03(S)
	No	2	10%	0.3(NS)

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Figure 2: Figure 1 :C

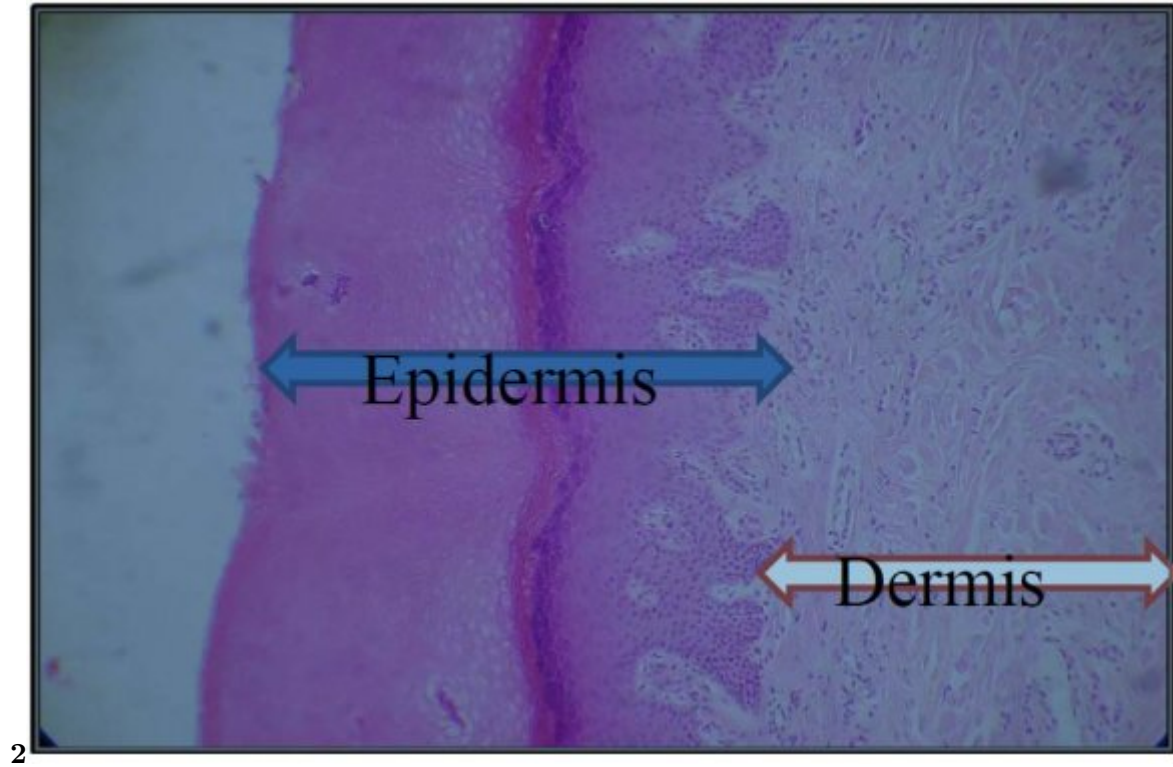


Figure 3: Figure 2 :

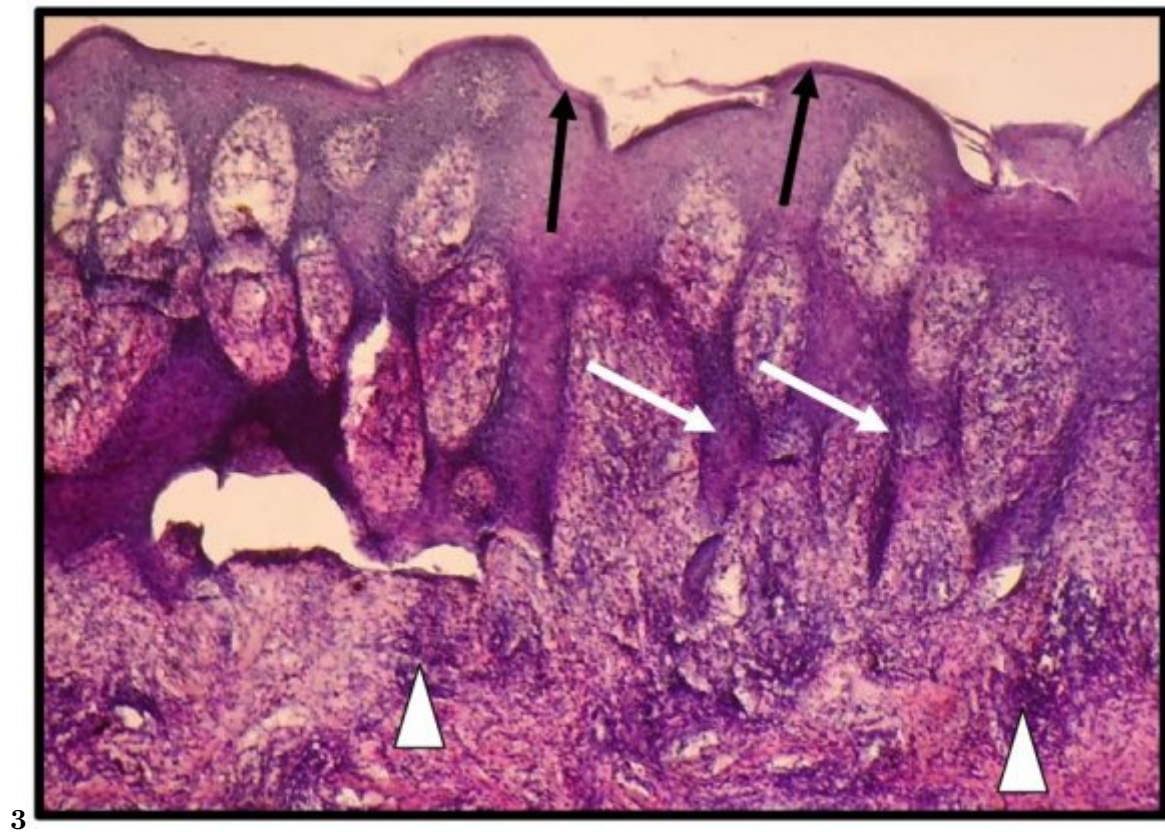
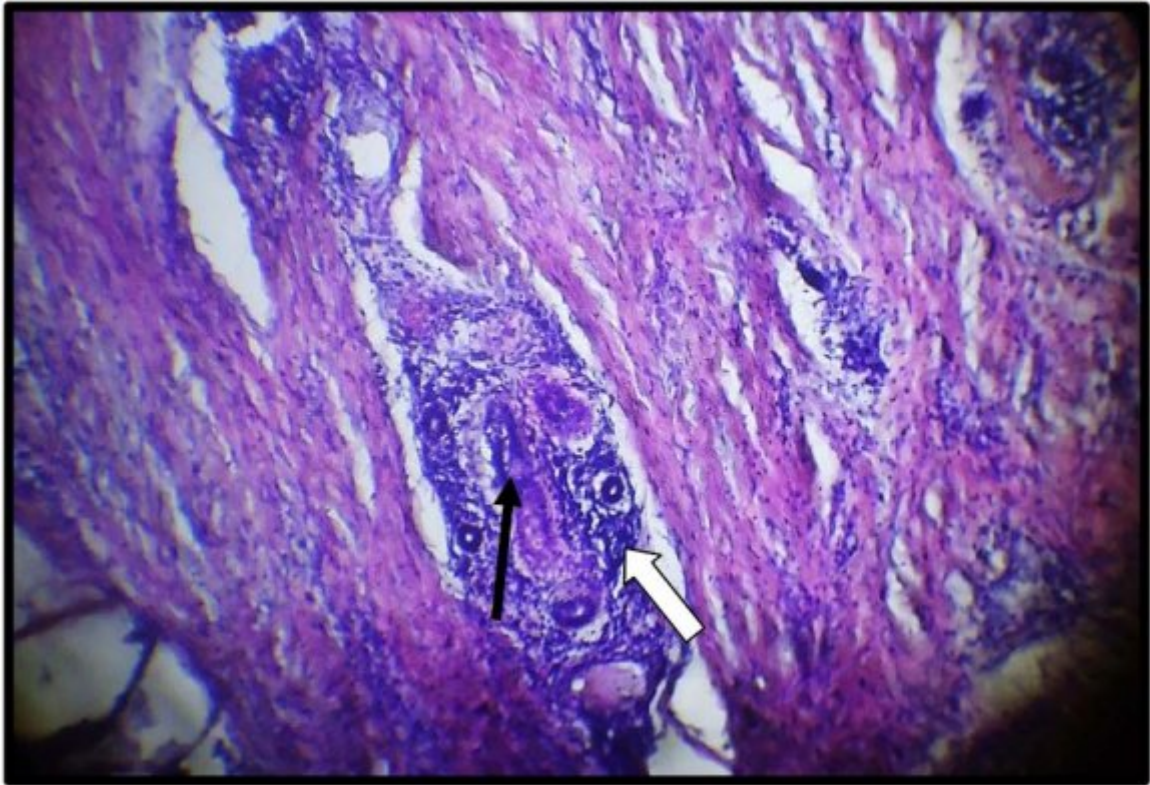


Figure 4: Figure 3 :



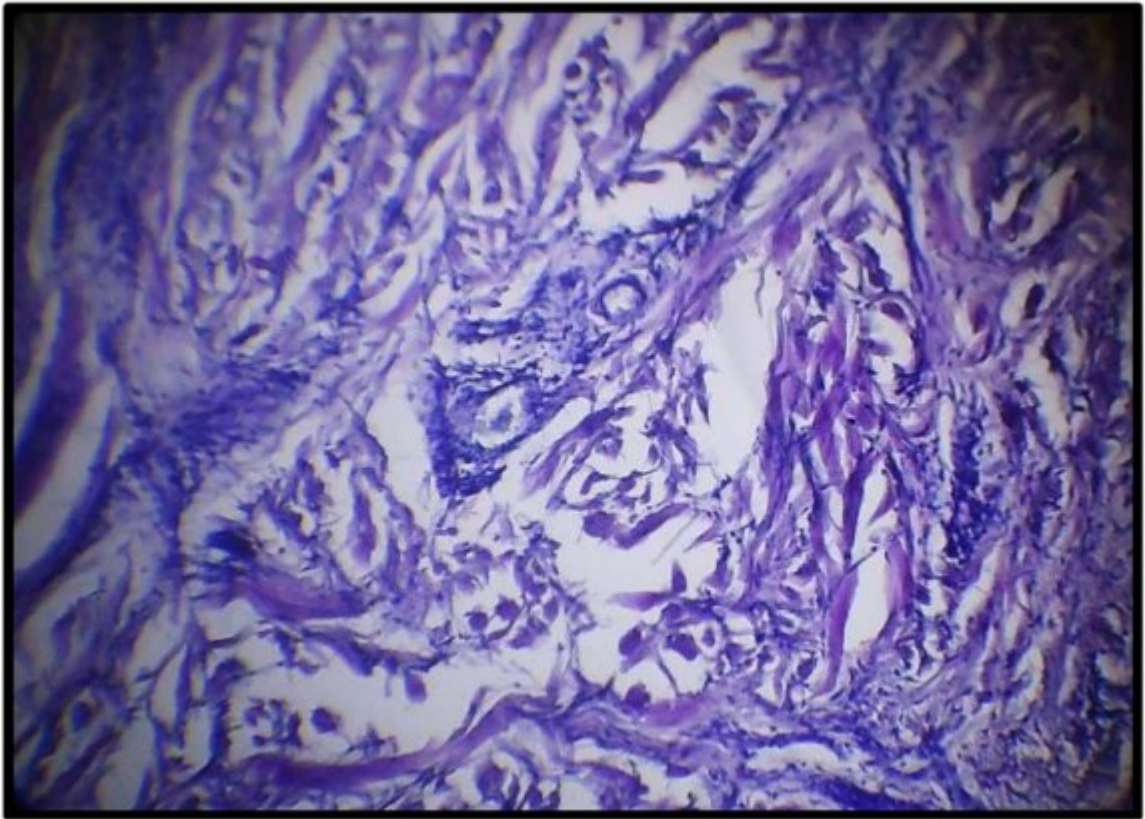
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Figure 5: Figure 4 :



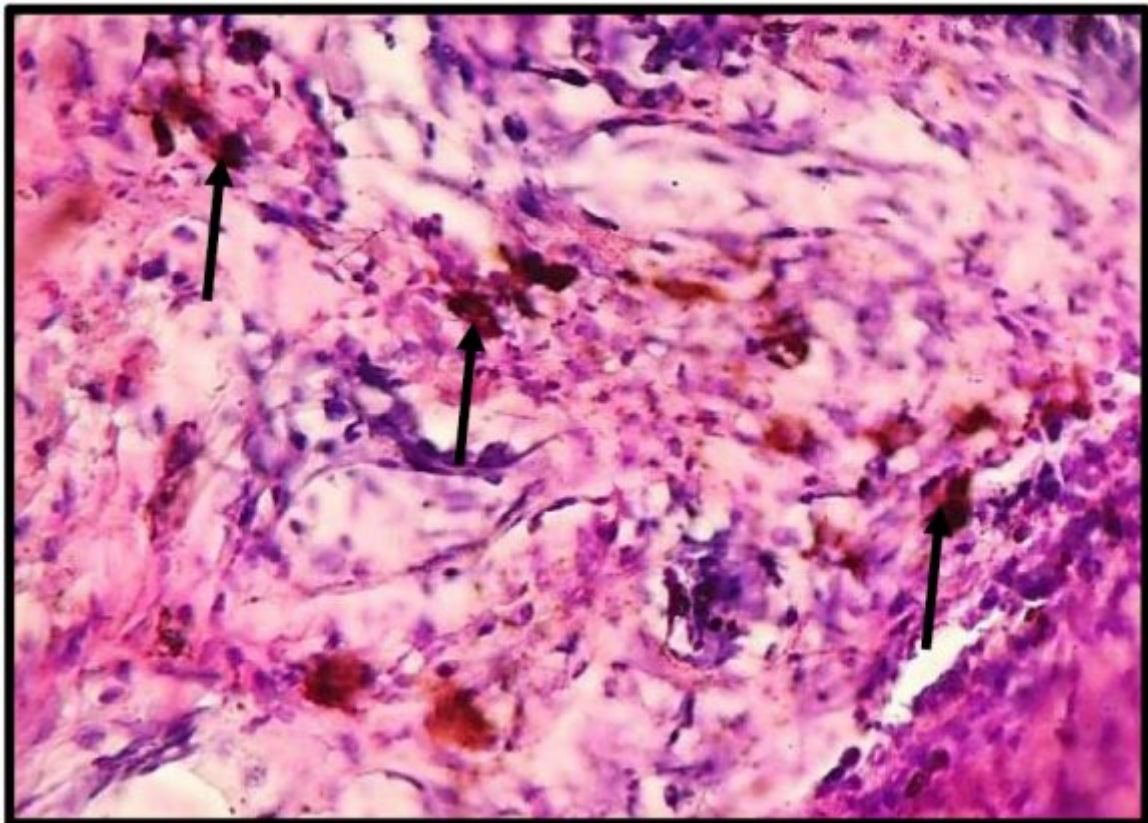
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Figure 6: Figure 5 :Figure 6 :



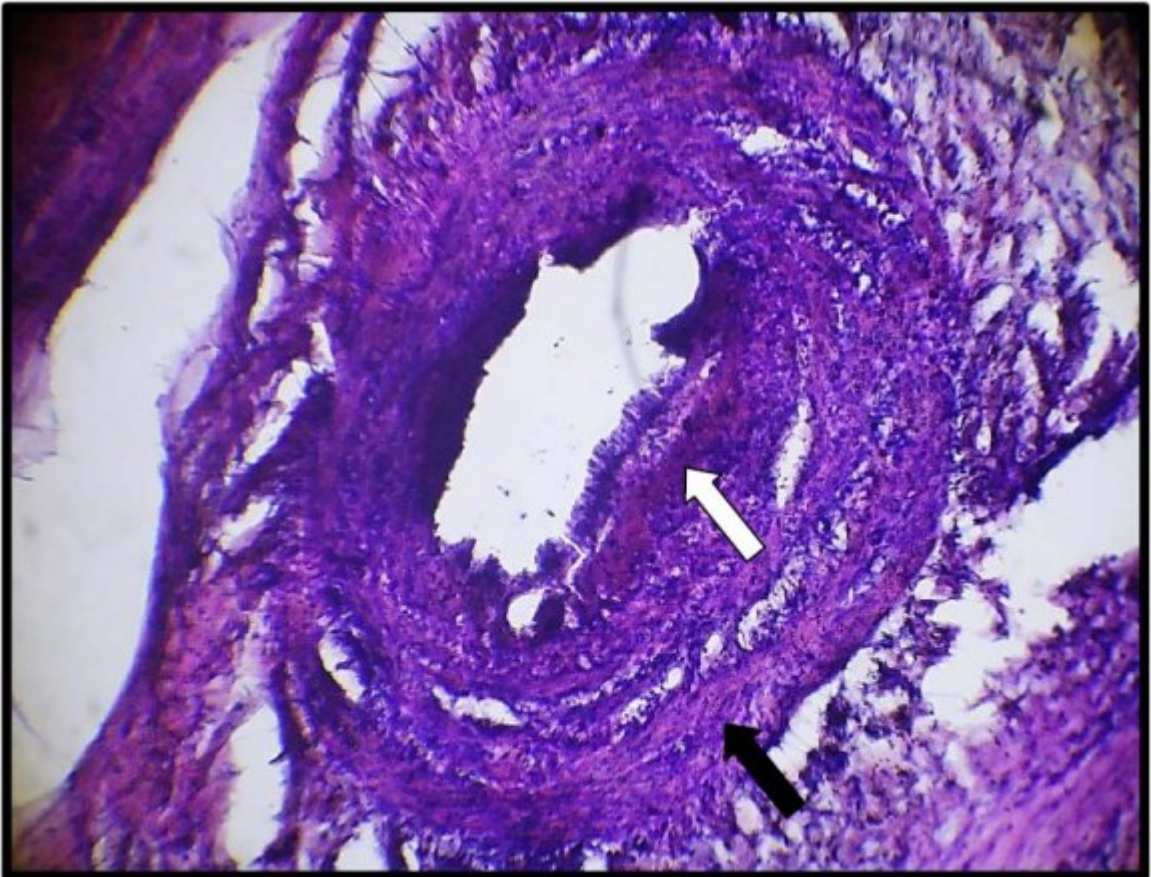
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Figure 7: Figure 7 :Figure 8 :



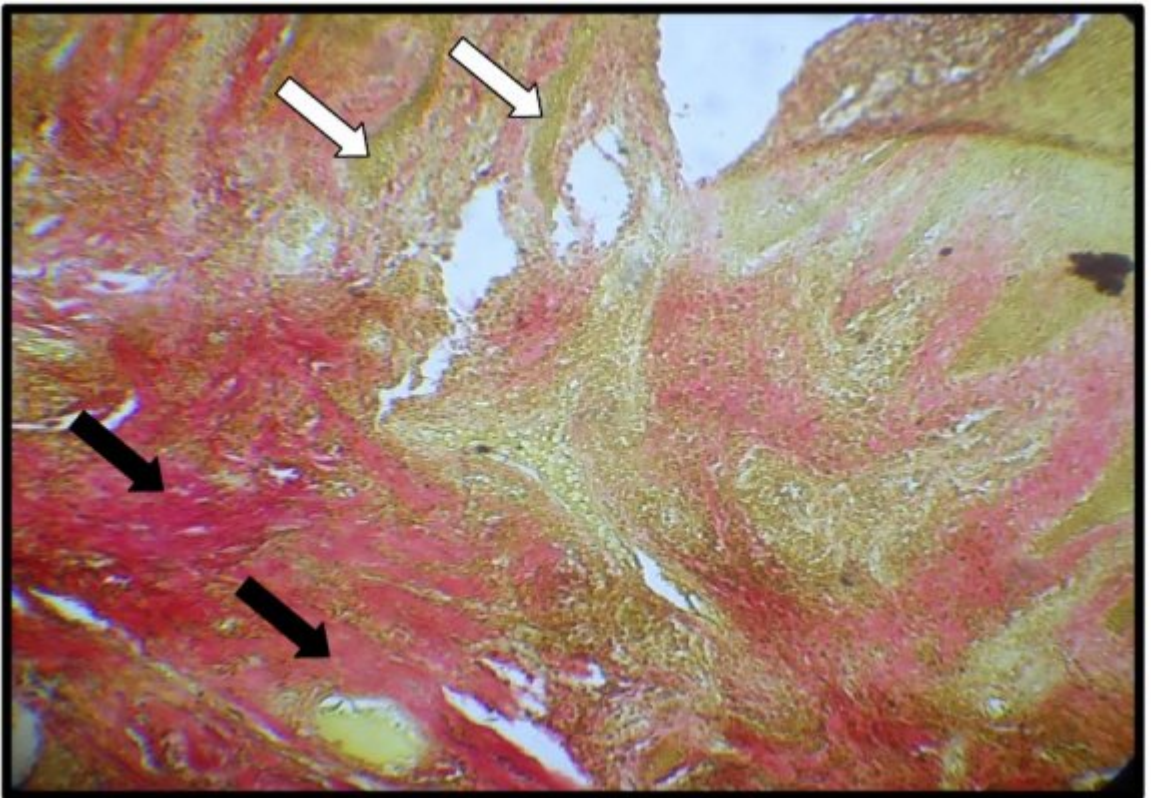
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Figure 8: Figure 9 :



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Figure 9: Figure 10 :



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Figure 10: Figure 11 :

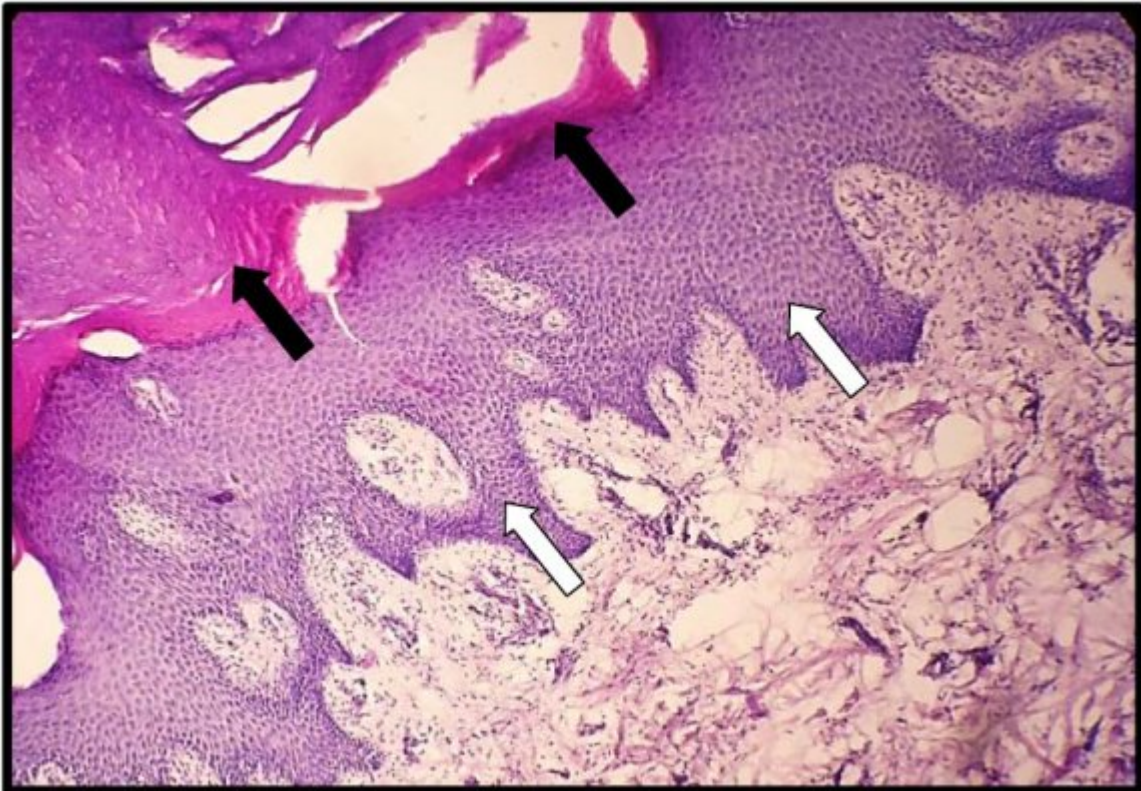
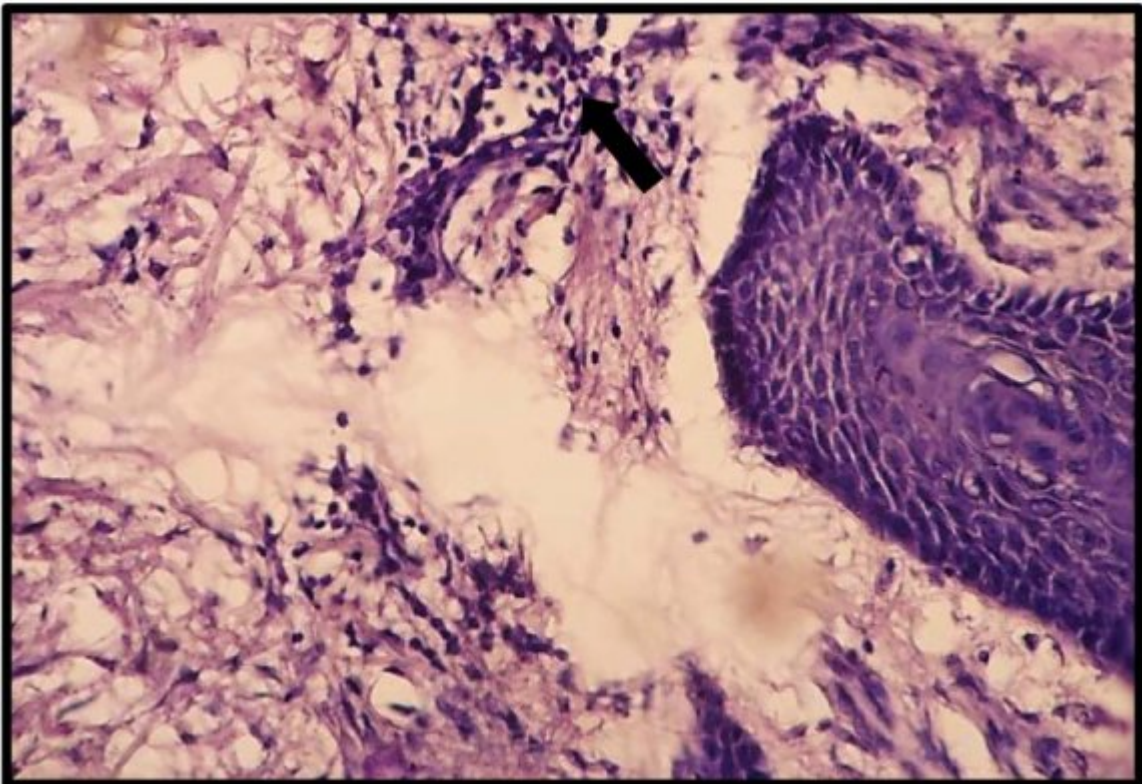


Figure 11: C



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Figure 12: Figure 12 :

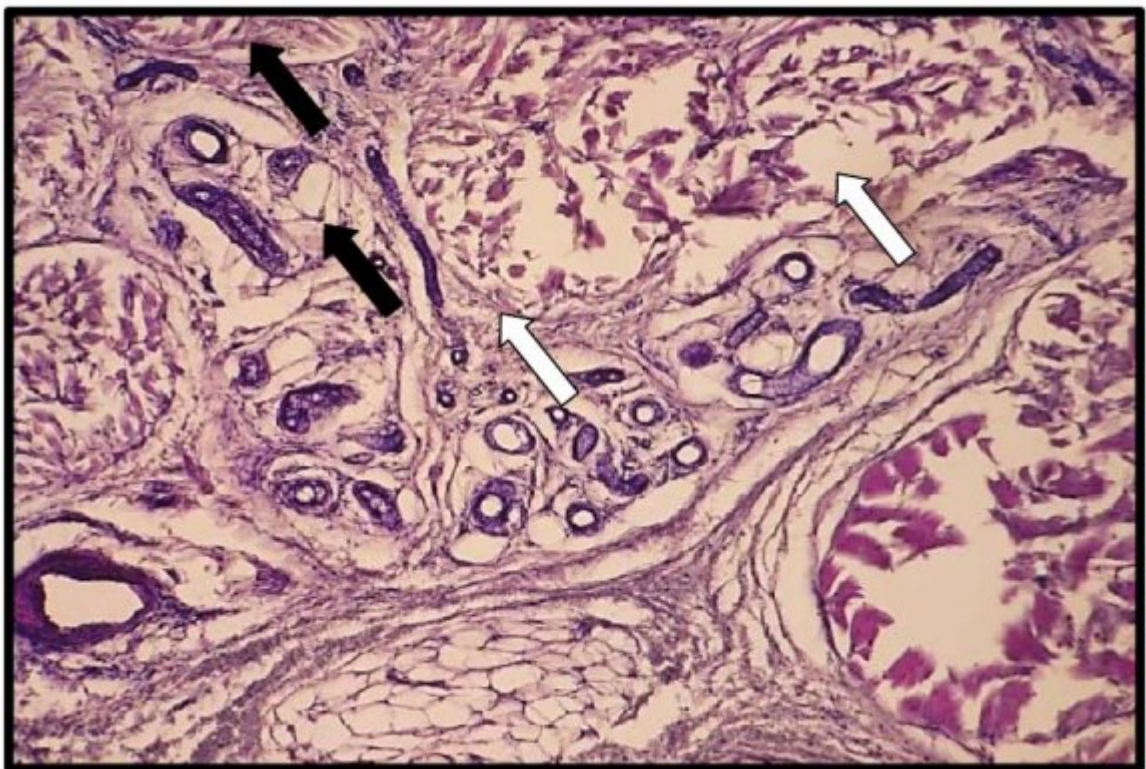


Figure 13: C

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