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Study of histopathological changes and clinical profile in diabetic limb amputations in a tertiary care hospital North India

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Abstract

Background: The prevalence of diabetes mellitus has increased by more than 60% from 1990-2001, and it is estimated that the number will increase by 165% from 2001-2050. **Aim of the study**: To study clinical profile and histomorphological changes in vessels, nerves, muscles, skeleton and soft tissues in diabetic limb amputations. **Material and methods**: A retrospective study was done from January 2011 to June 2012 in the Department of Pathology in collaboration with Department of General Surgery and Microbiology, in an urban tertiary care institution, on 25 patients undergoing diabetic limb amputations. **Results**: The present study showed that the identified risk factors for lower limb amputations are male sex, peripheral neuropathy, peripheral vascular disease and lack of awareness and practice of diabetic care protocols. 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.

Keywords: Diabetes Mellitus, histomorphological

Introduction

Despite advances in our understanding and treatment of diabetes mellitus, diabetic foot disease still remains a terrifying problem. It is the most common cause of non-traumatic lower limb amputation. In the western world diabetes causes hyperglycemia either due to lack of insulin production, insulin resistance or both.[1] Many manifestations like peripheral arterial disease (PAD), neuropathy, nephropathy and immunopathy are among the major comorbidities of the disease.

The prevalence of diabetes mellitus has increased by more than 60% from 1990-2001, and it is estimated that the number will increase by 165% from 2001-2050.[2]The diabetics are at 80% increased risk of cellulitis, 4 fold increased risk of osteomyelitis and 2 fold risk of both sepsis and death caused by infections when compared to non-diabetics.[3, 4]Patients with diabetes mellitus have impaired leucocyte function.[5] Neutrophils and macrophages show inadequate migration and reduced chemotaxis at the site of wound due to metabolic abnormalities in diabetes mellitus.[6]

A common complication among patients suffering from diabetes mellitus is ulceration. Foot ulcer incidence rate is between 2% and 10% among patients with diabetes mellitus. Fifteen percent of diabetes mellitus patients experience foot ulcer at some time over the course of their disease. Ulcers form as a result of minor trauma to the toes often already compromised by peripheral neuropathy and/or vascular insufficiency. Frequently the ulcers enlarge, reducing the likelihood of healing. Soft tissue and bone infection often accompanies ulcerations.[7]

Diabetes mellitus is associated with macrovascular and microvascular disease. The pathogenetic changes are determined on the level of vessels, nerves, bone skeleton and soft tissues. Histologically atherosclerotic lesions of extremities have arterial obstruction, fissures, ulceration, hemorrhage and thrombosis. Mononuclear cell infiltration in or around epineurial vessel wall, perivasculitis and necrotizing arteritis has been observed. Calcification of medial layer of Mônckeberg's sclerosis is more characteristic in diabetics.[8]

Osteomyelitis of the underlying bone has been seen in 20-96% amputations. Histological findings in acute osteomyelitis showdestruction of the bone and infiltration by polymorphonuclear granulocytes in the cortex and bone marrow. Thrombosis of medullary or periosteal small blood vessels is seen. In chronic osteomyelitis, there is destruction of bone and infiltration of lymphocytes, histiocytes and plasma cells. In acute exacerbation of chronic osteomyelitis, infiltration of polymorphonuclear granulocytes is present in the background of chronic osteomyelitis.[9]

Wound healing failure is frequently the result of a delay in treatment. Early intervention of these lesions promotes rapid healing of the lesion and prevents limb amputation. Vascular evaluation and intervention are critical in the presence of vascular insufficiency. Broad spectrum antibiotics and meticulous local wound care achieve remission in mild to moderately severe infections.

The present study was planned to evaluate the morphological changes accompanying diabetic limb amputation due to scarcity of descriptive information on these changes especially the clinical profile and histomorphological changes in vessels, nerves, muscles, skeleton and soft tissues.

Materials and Methods

The present study was conducted in the Department of Pathology in collaboration with Departments of General Surgery, and Microbiology, Government Medical College and Hospital, Chandigarh on 25 patients undergoing diabetic limb amputations. Detailed history, clinical examination, relevant investigations and details of any medical/surgical intervention, were recorded. Only confirmed cases of diabetes mellitus and amputation specimens with bony tissue were included. Cases of non-diabetic gangrenous amputations were not included in the study.

Amputated specimens were received in the department of pathology in 10% buffered formalin. Sections were taken from ulcerated/necrotic areas from the amputated specimen. Sections from soft tissue, neurovascular bundle and from the underlying bone were also taken.These were processed and 3-5µ sections were cut and stained with Hematoxylin and eosin.

Special stains like Periodic acid Schiff (PAS), Van-Gieson, Gram's and Grocott were done wherever required. Clinical assessment of sensory symptoms like burning or shooting pain, electrical or sharp sensations, numbness etc. and clinical examination using simple hand held devices was done. For evaluation of peripheral neuropathy 10 g monofilament pressure testing, vibration sensation testing with a 128 Hz tuning fork and ankle reflex testing with a tendon hammer were done.

Observations

The present study was conducted from January 2011 to June 2012 in the Department of Pathology in collaboration with Department of General Surgery and Microbiology, Government Medical College and Hospital, Chandigarh on 25 patients undergoing diabetic limb amputations. Pertinent clinical details of the patient were recorded.

The age of the 25 cases included in the study ranged from 30 to 90 years (median 59 years) with a mean of 58.6±10.91years. The maximum numbers of cases included were in sixth decade of life.Among the 25 cases included in the study, 16 were males and 9 were females. The male to female ratio was 1.7:1. On clinical examination 23 cases (92%) showed absent pulses in lower limbs (dorsalis pedis and posterior tibial arteries). Fourteen (56%) cases showed loss of sensations. Below knee amputations were more common, 20 cases (80%) than above knee amputations 2 cases (8%) (Table 1).

Table 1: Type of amputations (n=25)

Type of amputation	N	%
Above knee amputation	2	8
Below knee amputation	20	80
Tarsometatarsal	3	12

Classification of ulcer according to Wagner system:Twenty one cases (84%) had grade 2 and 3 ulcers. Two (8%) cases had grade 4 ulcers. One case (4%) had grade 1 ulcer and one case (4%) had grade 5 ulcer (Table 2).

Table 2: Classification of ulcer according to Wagner system (n=25)

Grade	N	%
0	0	0
1	1	4
2	8	32
3	13	52
4	2	8
5	1	4

Sections were taken from the ulcer and the normal skin adjoining the ulcer to see the dermoepidermal changes. Sections from deeper soft tissue including muscle and connective tissue were taken to see the peripheral nerves, vessels and soft tissue changes. Sections from neurovascular bundles were taken to see changes in large and medium sized muscular arteries and nervous elements. Sections were taken from bone underlying the ulcers to see changes in bone histomorphology.

Epidermis showed ulceration and the surface was covered with necrotic slough and inflammatory exudate in all the cases (Figure 1). Dermis showed presence of inflammatory cell infiltrate, mainly formed of acute inflammatory cells in 13 cases (52%) and mixed inflammatory cells in 12 cases (48%). Dermis showed destruction and disorganization of sweat glands (Figure 2) and connective tissue necrosis in all the 25 cases (100%) (Table 3). Bacterial colonies were seen in both epidermis and soft tissues in all the cases.

Table 3: Soft tissue changes (n=25)

Soft tissue changes	N	%
Ulceration of epidermis	25	100
Acute inflammatory cells	13	52
Mixed inflammatory cells	12	48
Sweat gland destruction/disorganization	25	100
Connective tissue necrosis	25	100
Bacterial colonies	25	100

Small arterioles and capillaries showed changes of vasculitis and destruction in all cases (Figure 3). Meta-arterioles, small and medium arterioles showed presence of the chronic inflammatory infiltrate arranged in concentric layers (“muffs”) around them, sometime penetrating the media level and deposition of eosinophilic hyaline material in intima and media in all cases. Thrombosed vessels showing thrombus adherent to the arterial wall and occluding most of the lumen were seen in 100% of cases. Arterioles showed obstruction, due to excessive proliferation of the subendothelial connective tissue and sometimes, by a fibrous change, was seen in 23 cases (92%) (Figure4). Calcium deposit in large and medium sized muscular arteries in tunica media level (Monckeberg sclerosis) was seen in 17 cases (68%) (Figure 5) (Table 4).

Table 4: Vascular changes (n=25)

Vascular changes	N	%
Destruction of vascular structures and vasculitis	25	100
Hyaline thickening of vessels	25	100
Thrombosed vessels	25	100
Atherosclerosis	23	92
Monckeberg sclerosis	17	68

Nerve changes were seen in sections from soft tissues and neurovascular bundles lying below the ulcer. Twenty cases (80%) showed microvasculitis (Figure 6). Eighteen cases (72%) showed both epineural and perineural vasculitis. One case (4%) showed inflammation in perineurium only, one case (4%) showed inflammation in epineurium only and 5 cases (20%) did not show any change in nerves.(Table 5).

Table 5: Nerve changes (n=25)

Nerve changes	N	%
Micro-vasculitis	20	80
Epineural/perineurium vasculitis	18	72
Perineurium only	1	4
Epineurium only	1	4
Absent	5	20

Muscle and soft tissue showed myositis in all 25 cases (100%). Focal areas of necrotic muscle fibers were seen in 23 cases (92%). These fibers were swollen and eosinophilic, lacked striations and nuclei (myonecrosis). Focal areas of muscle atrophy were seen in all the cases. In addition to the above findings, the present study showed the presence of singly scattered myogenic giant cells (Figure 7) in 8 cases (32%) (Table 6).

Table 6: Muscle and soft tissue changes (n=25)

Muscle and soft tissue changes	N	%
Myositis	25	100
Myonecrosis	23	92
Muscle atrophy	25	100
Myogenic giant cells present	8	32

Sections from bone underlying ulcer showed destruction and infiltration of cortex and the bone marrow by polymorphonuclear granulocytes in 2 cases (8%), lymphocytes and plasma cells in 3 cases (12%) and by polymorphonuclear granulocytes imposing upon lymphoplasmacytic cells in 12 cases (48%)(Figure 8). Bone necrosis was seen in 7 cases (28%) (Table 7).

Table 7: Bone changes (n=25)

Bone changes	N	%
Acute osteomyelitis	2	8
Chronic osteomyelitis	3	12
Acute exacerbation of chronic osteomyelitis	12	48
Bone necrosis	7	28

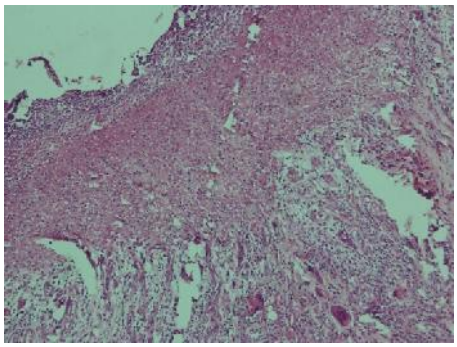

Figure 1: Photomicrograph showing ulceration of epidermis (H&E, x10)

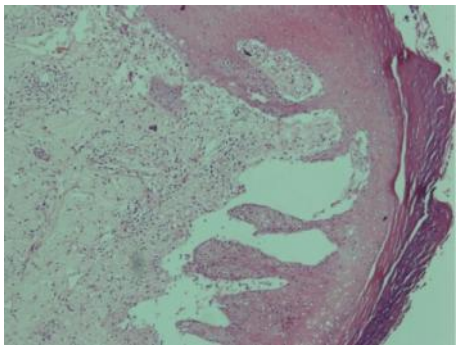

Figure 2: Photomicrograph showing destruction of sweat glands (H&E, x10)

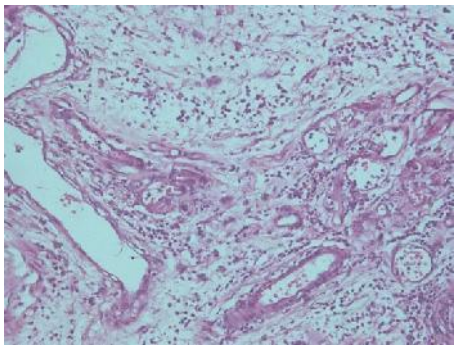

Figure 3: Photomicrograph showing vasculitis of small arterioles and capillaries (H&E, x40)

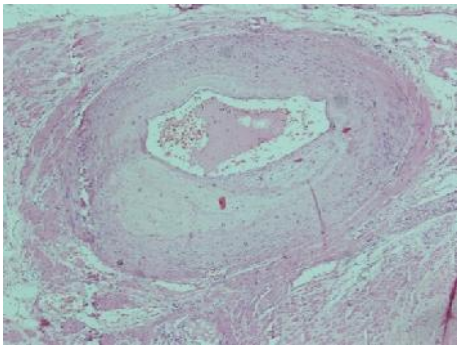

Figure 4: Photomicrograph showing atherosclerosis of a large arteriole (H&E, x10)

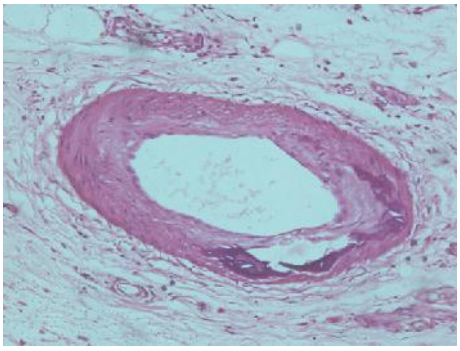

Figure 5: Photomicrograph showing Monckeberg sclerosis (H&E, x40)

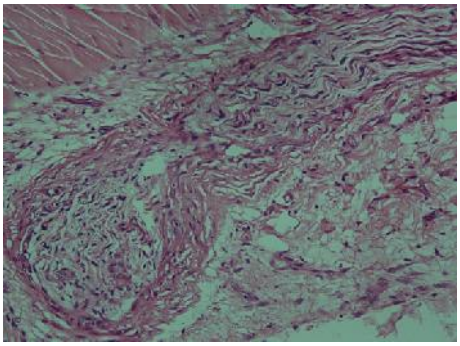

Figure 6: Photomicrograph showing microvasculitis (H&E, x40)

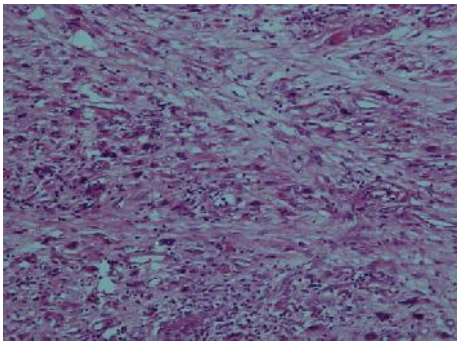

Figure 7: Photomicrograph showing myogenic giant cells (H&E, x20)

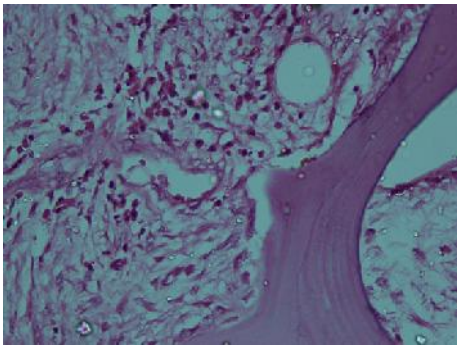

Figure 8: Photomicrograph showing destruction and infiltration of bone by lymphocytes and plasma cells (H&E, x40)

Discussion

Diabetes mellitus is a metabolic disorder in which there is abnormality in the metabolism of glucose due to qualitative and quantitative deficiency of insulin. The number of patients with diabetes mellitus is increasing by epidemic proportions and the disease leads to end-organ damage due to years of hyperglycemia, which in turn results in a major burden on health care providers. Lower extremity disease, including peripheral neuropathy, foot ulceration, peripheral arterial disease, or lower extremity amputation, is twice as common in diabetic persons compared with non-diabetic persons. In the present study, we included 25 consecutive patients with confirmed diabetes mellitus undergoing above or below knee amputation having bone in it. Cases of non-diabetic gangrenous amputations were not included in the study.

Clinical profile

In the present study, the age range of cases with diabetes mellitus was 30 to 90 years (median 59 years) with a mean of58.6±10.91 years. Maximum numbers of cases were in the fifth and sixth decade of life. Out of 25 cases, 16 were males and 9 were females with male: female ratio of 1.7:1.

In a study by Ngim et al in 36 cases, the age range was 23 to 74 years with a mean of 54 years. Out of 36 cases, 25 were males and 11 were females with male: female ratio of 2.3:1.[10] In a study by Sharma et al in 43 cases, the age range was 37 to 96 years with a mean age of 61±1.7 years. Out of 43 cases 24 were males and 19 were females with male: female ratio of 1.3:1.[11] (Table 8).

Table 8: Comparison of patient's age in different studies

Study by	Male: female ratio	Age range
Ngim et al, 2012	2.3:1	23 to 74 years
Sharma et al, 2006	1.3:1	37 to 96 years
Present study	1.7:1	30 to 90 years

In the present study, more males presented with diabetic foot lesions, which is consistent with the other studies. In a study by Ngim et al 58% cases presented with foot gangrene. Very few studies have similar results. Most other studies have identified foot ulcer as the most common presentation of diabetic foot syndrome. The high incidence of foot gangrene may be due to a number of factors including seeking alternative medical care where irritant topical agents were applied to the affected foot, self-medication, ignorance and poverty, with consequent delay in presentation to hospital. In study by Sharma et al grade 2 ulcers were seen in 53.4% cases and grade 3 ulcers in 20.9% cases. This study showed Wagner grade 3 ulcers in 52% cases and grade 2 ulcers in 32% cases, showing that the deep foot ulcers were the most common presentation in diabetes mellitus patients. (Table 9)

Table 9: Wagner grades, number of cases in each grade and their percentage

Wagner grades	Study by Sharma et al (n=43)		Present study (n=25)	
	N	%	N	%
0	0	0	0	0
1	5	11.6	1	4
2	23	53.4	8	32
3	9	20.9	13	52
4	5	11.6	2	8
5	1	2.9	1	4

The present study correlates with the study done by Ngim et al showing that the below knee amputations were much more common than the above knee amputations (Table 10). Other studies in developed countries showed, most amputations were minor amputations around the foot.

Table 10: Comparison between types of amputation

Type of amputation	Study by Ngim et al(n=19)		Present study(n=25)	
	N	%	N	%
Below knee	10	53	20	80
Ray amputation	7	37	3	12
Above knee	2	10	2	8

Histomorphological changes:

Skin: In a study by Popescu et al on a group of 25 diabetes mellitus patients showed ulceration of epidermis in all cases.[12] Dermis showed lymphomononuclear and neutrophilic cell infiltrate along with absence or degeneration of sweat glands. The present study showed ulceration of epidermis in 100% cases. Dermis showed acute inflammatory cells in 13 cases (52%) and mixed inflammatory cells in 12 cases (48%). Sweat gland destruction/disorganization, connective tissue necrosis and bacterial colonies were seen in 100% cases.

Vessels: In a study by Popescu et al small arterioles and capillaries showed flat smooth inflated endothelial cells. Large arterioles and arteries of muscular type showed presence of fibrous tissue in tunica media and calcium deposit in intima (mediocalcinosis). The present study showed changes of vasculitis and destruction of small arterioles and capillaries in all cases. Meta-arterioles, small and medium arterioles showed presence of chronic inflammatory infiltrate arranged in concentric layers ("muffs") around them. Hyaline thickening was seen in tunica intima and tunica media in all cases. Large arterioles and arteries of muscular type showed changes of atherosclerosis in 23 cases (92%). Seventeen cases (68%) showed calcium deposition in tunica media (Mönckeberg's sclerosis). Thrombosed vessels were seen in 100% cases. A study by Soor et al on 58 cases showed atherosclerosis and medial calcification histologically. They concluded that, atherosclerosis and medial calcification are significant underlying lesions in diabetic patients undergoing lower limb amputation. Medial calcification can cause significant stiffening of the arterial wall and a reduction in its ability to respond to vasodilator stimuli.[8]

Nerve changes: In a study by Younger, nerve tissue biopsies were taken from 107 cases with confirmed diabetic neuropathy.[13] Sections were examined under microscope to see mononuclear inflammatory cell infiltration in or around the epineurial vessel walls. Microvasculitis was seen in 3 cases (3%) and perivasculitis in 26 cases (23%). In the present study, the nerve changes were seen in the peripheral nerves, present in soft tissues and in neurovascular bundles, present below the ulcer. Twenty cases (80%) showed microvasculitis and one case (4%) showed inflammation in perineurium. In addition, 18 cases (72%) showed both epineural and perineural vasculitis and one case (4%) showed inflammation in epineurium only. The neuropathological findings are summarized in Table 11.

Table 11: Neuropathological findings

Cellular response	Study by Younger (n=107)		Present study(n=25)	
	N	%	N	%
Perivasculitis	26	23	1	4
Microvasculitis	3	3	20	80

Muscle and soft tissue: A study by Popescu et al showed presence of myositis in all cases.[12] The present study showed myositis and muscle atrophy in 100% cases. Myonecrosis was seen in 23 cases (92%). In comparison to the other related studies, the present study showed the presence of myogenic giant cells in 8 cases (32%).

Bone: A study by Sanchez et al in a series of 185 diabetic cases showed 94 cases (50.8%) of acute osteomyelitis, 43 cases (23.2%) of chronic osteomyelitis and 45 cases (24.3%) of acute exacerbation of chronic osteomyelitis.[14] The present study showed 13 cases (52%) of osteomyelitis, out of which 2 cases (8%) were showing acute osteomyelitis, 3 cases (12%) showed chronic osteomyelitis and 8 cases (32%) showed acute exacerbation of chronic osteomyelitis. (Table 12). Bone necrosis was seen in 7 cases (28%). The present study showed more cases of chronic osteomyelitis than acute osteomyelitis because the mean duration between the occurrence of foot lesions and presentation to hospital was 7 weeks.

Table 12: Types of osteomyelitis

Type of osteomyelitis (OM)	Study by Sanchez et al (n=185)		Present study (n=25)	
	N	%	N	%
Acute OM	94	50.8	2	8
Chronic OM	43	23.2	3	12
Acute exacerbation of chronic osteomyelitis	45	24.3	8	32

Conclusion

The present study showed that the identified risk factors for lower limb amputations are male sex, peripheral neuropathy, peripheral vascular disease and lack of awareness and practice of diabetic care protocols. 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. Within the context of the evolution of diabetes mellitus, the vascular lesions, which appeared on the microcirculation level, are aggravating; they are consequently involving arterioles and arteries of muscular type and are being accompanied by nervous lesions shown through morphological changes of the peripheral nerves. The overall morphological contest of the complicated diabetic foot (compulsorily accompanied by complications) involves lesions of the epidermis, dermis, and muscles.

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Conflict of interest: None declared

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