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ORIGINAL ARTICLE

Microbiologic and histopathological findings of osteomyelitis in diabetic patients

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Abstract Osteomyelitis can present as a severe complication in diabetic patients. The present study was structured to evaluate the histopathological characteristics of bone infection in the feet of diabetic patients and to associate any clinical variables seen with each type of bone infection. Eighty-four cases of osteomyelitis were evaluated microbiologically and histopathologically after samples were collected under sterile conditions in an operating room. The data generated were analysed using SPSS software. Polymorphonuclear neutrophil (PMN) infiltrates were associated with acute osteomyelitis (AO) (22 cases (26.2%)) and chronic acute osteomyelitis (CAO) (10 cases (11.9%)), whereas a strong association was observed between mononuclear infiltrates and chronic osteomyelitis (CO) (33 cases (39.3%)) and fibrosis (19 cases (22.6%)). Ulcer longevity on hospital admission varied for each condition: AO (10.9 + 22 weeks), CO (29.1 + 20.6 weeks), CAO (31.5 + 22.2 weeks) and fibrosis (30.5 + 23 weeks) ($P < 0.05$) (values expressed as mean + SD). The same was true for the presence of pathogenic bacteria: AO (16/22, 72.7%), CO (27/33, 81.8%), CAO (7/10, 70%) and fibrosis (16/19, 84.2%). There was significant correlation between histopathological features and presence of bacteria and longevity of the ulcer on presentation ($P < 0.05$). Osteomyelitis in the feet of diabetic patients should be classified as four specific but interlinked groups: acute, chronic, acute on chronic and fibrosis.

Keywords Diabetic foot ulcer · Microbiological features · Risk factors

Introduction

Diabetic foot ulcers (DFU) are chronic wounds, which often have major long-term effects on the quality of life (National Institute for Health and Clinical Excellence 2011; Abetz et al. 2002). It has been reported that approximately 25% of diabetics will experience some type of DFU during their life (Singh et al. 2005). Failure to diagnose early and provide appropriate treatment may result in serious outcomes. The consequence can be a rapid DFU infection with the potential to cause serious complications such as osteomyelitis. In general, osteomyelitis can complicate 10–20% of mild or moderate infections and 50–60% of severe infections in the feet of patients hospitalized with diabetes (Jeffcoate and Lipsky 2004). Osteomyelitis is defined as a disease generally caused by a microbial pathogen and, to identify the aetiological agent, bone samples are needed for histologic and microbiological analyses (Butalia et al. 2008). Several studies have provided data on the histopathologic features of bone specimens collected from infected foot in diabetic patients as well as the presence of many diagnostic criteria that are routinely used by pathologists (Aragon-Sanchez et al. 2008; Cierny et al. 2003).

Based on our previous experience with diabetic patients who were subjected to amputation, fibrosis was the most frequent diagnosis. Nonetheless, osteomyelitis has always been suspected before carrying out any operation and thus a microbiological confirmation of bone infection should be available.

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Considering this experience, we decided to assess histopathologic and microbiological changes of osteomyelitis as part of the surgical protocol. To our knowledge, no association between the cell cluster and the histopathologic types has previously been reported. Therefore, we attempted to gather a group of patients in whom bone infection was confirmed by histopathologic analysis and the cell lines were observed in the samples obtained following surgical biopsy, to assess the characteristics of histopathologic changes and to understand whether there is an association between the histopathology and clinical variables found in different patients.

Methods

Study protocol A cross-sectional descriptive study was conducted on 105 patients who suffered from osteomyelitis and were hospitalized at Velayat Training Hospitals affiliated to the Qazvin University of Medical Sciences (Qazvin, Iran) and Tamin Ejtemaei Hospital in Takestan (located in Qazvin province) from May 2012 to April 2016.

Patients with peripheral arterial disease, as indicated by an ankle brachial pressure index (ABPI) <0.8 verified by Doppler ultrasound, and signs of arterial disease such as the presence of ulcers with a "punched out" appearance, base of wound poorly perfused, pale, dry, cold legs/feet (in a warm environment), shiny, taut skin, dependent rubor, pale or blue feet and gangrenous toes were excluded from the study. According to all clinical signs, the results of probe-to-bone test and radiologic findings, only 84 patients with diabetes-associated osteomyelitis were evaluated.

Surgical treatment and sampling techniques Bone surgery was performed on patients by removing the entire portion of affected bone, i.e. if osteomyelitis was present on the head of a metatarsal bone, then the whole head was removed. After bone removal, a piece of affected bone and the surrounding soft tissue were sampled under sterile conditions and transferred to microbiology laboratory while the rest was immersed in 10% buffered formalin and sent to the pathology lab. The resected tissue was cut, the sample decalcified, processed and stained with haematoxylin-eosin and finally studied by an independent pathologist who was blind to the clinical data of the patients.

According to previous diagnostic criteria (Aragon-Sanchez et al. 2008; Cierny et al. 2003), acute osteomyelitis (AO) was diagnosed with a multifocal infiltrate consisting of polymorphonuclear neutrophils (PMNs), lymphocytes and plasma cells at the bone marrow level, with clear polymorphonuclear predominance in which there was a variable proportion of osteo- and soft tissue necrosis concentrations. Chronic osteomyelitis (CO) was diagnosed in patients with a multifocal infiltrate consisting almost entirely of macrophages, lymphocytes and plasma cells, with mononuclear predominance at the bone marrow level, where some concentrations of reshaped bone with a variable bone neogenesis, osteoid formation and variable fibrosis were observed. Chronic AO (CAO) was diagnosed when there was a PMN infiltrate over a background of CO. The fibrosis stage was diagnosed when there was bone marrow fibrosis with few or no lymphocytic infiltrates present at the bone marrow level.

Ethical approval The study was approved by the Ethics Committee of Tamin Ejtemaei Hospital in Takestan before the study was initiated, and all protocols used in the present work were conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Statistical analysis Data were analysed using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA). The Student's *t* test, chi-square, or Fisher's exact test were also used, where appropriate, for comparing the clinical and laboratory data between the AO, CO, CAO and fibrosis groups. Continuous data are presented as mean ± standard deviation. A P value <0.05 was considered significant.

Results

Of 84 patients with osteomyelitis who took part in this study, 56 (67%) were male and 28 (33%) female. The mean age was 60.6 ± 5.4 years (ranged between 28 and 81 years). (Demographic data of patients is summarized in Table 1.)

Among 84 patients with osteomyelitis, 67 patients (79.7%) showed radiologic signs. The probe-to-bone test result was positive in 81 patients (96.4%), and 49 patients (58.3%) demonstrated clinical signs of infection.

In addition, 68 pathogenic microorganisms were isolated from patients with osteomyelitis. The most common isolated microorganisms were gram-negative and gram-positive bacteria observed in 34 (50%) and 32 (47%) isolates, respectively. In two cases (3%), yeasts were found to be the causative agents of infection. (The microorganisms isolated from DFUs are presented in Table 2.)

According to the results of this study, osteomyelitis was classified into four histopathologic types on the basis of the cell clusters and histopathologic changes.

Considering the findings of the present work, PMN leukocyte infiltrate was associated with AO (22 cases (26.19%)) and CAO (10 cases (11.9%)), whereas a strong association was observed between the mononuclear infiltrate and CO (33 cases (39.28%)) and fibrosis stage (19 cases (22.61%)).

AO is characterized by the presence of PMNs, variable extent of necrosis, signs of bone remodelling and formation of osteoid (Fig. 1). In addition, periostitis and variable fibrosis were observed in some cases at the bone marrow level.

Table 1 Demographic and clinical data of patients with osteomyelitis (n = 84)

Gender: male/female (mo. of patients)	56/28
The mean ± SD evolution time from the onset of diabetes	18.6 ± 9.4 years
No. of patients under treatment with oral hypoglycaemic agents	31
No. of patients under insulin therapy	53
No. of patients with HbA1C > 7%	76
The mean ± SD duration of the ulcer at the time of admission	28.5 ± 22 weeks

CAO is characterized by a multifocal infiltrate at the bone marrow level consisting of lymphocytes, plasma cells and PMN leukocytes. Bone necrosis was also observed, with slight bone remodelling and osteoid formation as well as bone marrow fibrosis (Fig. 2).

CO is characterized by the presence of lymphocytes and plasma cells, some bone necrosis and bone remodelling of varying extent, osteoid formation and varying degrees of bone marrow fibrosis (Figs. 3 and 4).

Fibrosis is characterized by the presence of little lymphocytic type inflammatory infiltrate (which is accompanied by a few plasma cells in some cases) at the bone marrow level. There was no necrosis, bone remodelling or osteoid formation; however, there was bone marrow fibrosis (Fig. 5). No cell clusters were observed in the advanced stages since they were replaced by significant bone marrow fibrosis with variable fibrosis at the periosteum level. (The inflammatory infiltrates and histopathologic finding in each types of osteomyelitis are shown in Tables 3 and 4, respectively.)

The duration of ulcers from hospital admission was different for each classification: AO (10.9 + 22 weeks), CO (29.1 + 20.6 weeks), CAO (31.5 + 22.2 weeks) and fibrosis (30.5 + 23 weeks) ($P < 0.05$). The presence of pathogenic bacteria also varied for each classification: AO (16/22, 72.7%), CO (27/33, 81.8%), CAO(7/10, 70%) and fibrosis(16/19, 84.2%). There was significant correlation ($P < 0.05$) between the histopathologic type and the isolation of bacteria from bone culture and duration of the ulcer from hospital admission. The association between the diagnostic criteria and the histopathologic types of osteomyelitis is shown in Table 5.

Table 2 Frequency of pathogenic microorganisms that were isolated from DFUs

Microorganism	No. and percentage
Gram-negative bacteria	34 (50%)
Pseudomonas aeruginosa	14 (20.6%)
Escherichia coli	10 (14.7%)
Klebsiella spp.	4 (5.9%)
Bacteroides fragilis (anaerobic)	3 (4.4%)
Proteus mirabilis	3 (4.4%)
Gram-positive bacteria	32 (47%)
Staphylococcus aureus	23 (33.8%)
Multiple resistant *S. aureus* (MRSA)	5 (7.2%)
Streptococcus spp.	2 (3%)
Clostridium perfringens (anaerobic)	1(1.5%)
Streptococcus spp.	1 (1.5%)
Yeasts	2 (3%)
Candida albicans	2 (3%)
Total	68 (100%)

Discussion

Aragon-Sanchez et al. (2008) and Craig et al. (2003) found an indirect relationship between the histopathologic patterns of osteomyelitis and different stages of this lesion. They also described three most frequent histopathologic types according to the cell clusters and histopathologic changes (AO, CO and CAO). In their study, AO and CAO were the most common diagnosis which is different from the finding of the present study in which CO was the most prevalent diagnosis. These inconsistencies might be due to the demographic characteristics of patients in these studies. In their predominantly inpatient series, the authors found that many patients were with severe soft tissue infections. In the present study, we found a relationship between the duration of ulcers and the osteomyelitis characteristic.

In contrast to previous studies, we identified four clearly defined histopathologic types (AO, CO, CAO and fibrosis)

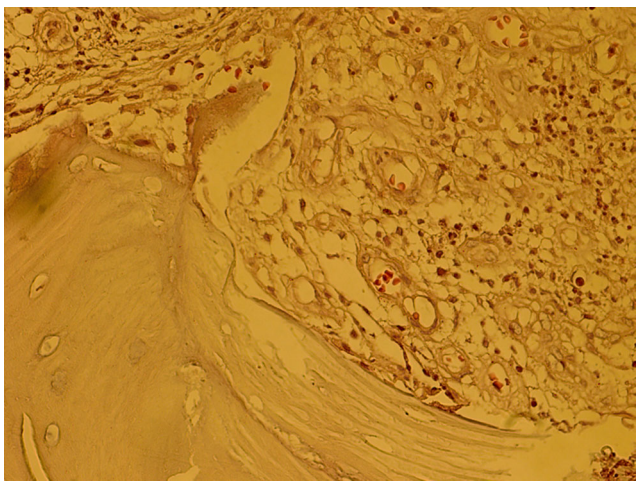


Fig. 1 Acute osteomyelitis represented by vascular congestion, PMN leukocyte infiltration and osteonecrosis. (×400, haematoxylin and eosin)

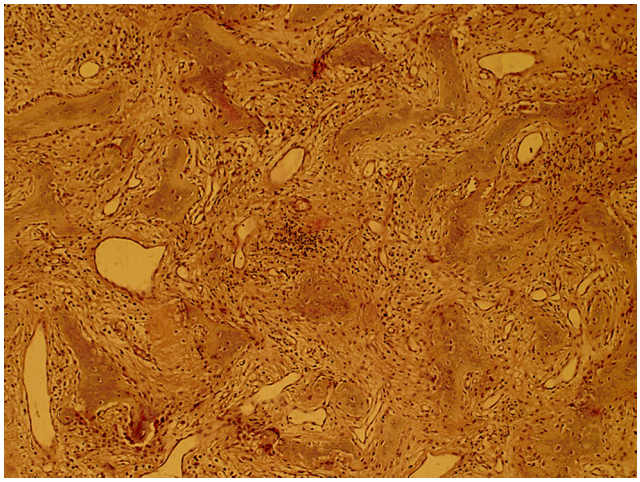


Fig. 2 Chronic acute osteomyelitis represented by multifocal infiltrates of lymphocytes, plasma cells and PMN leukocytes, bone remodelling and varying degrees of bone marrow fibrosis. (×200, haematoxylin and eosin)

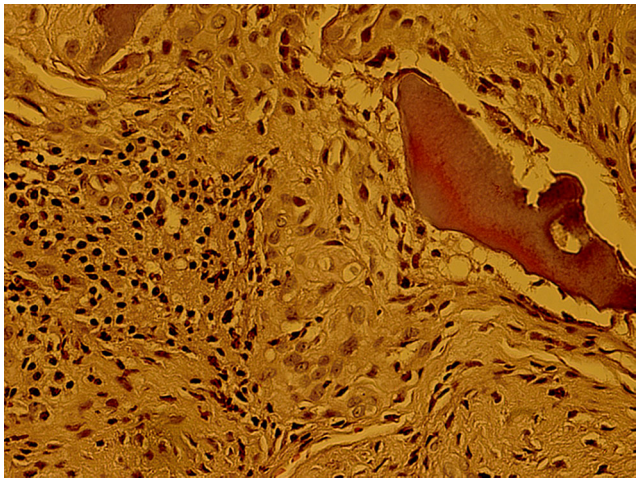


Fig. 4 Chronic osteomyelitis represented by lymphocyte and plasma cell infiltration, bone remodelling and varying degrees of bone marrow fibrosis. (×400, haematoxylin and eosin)

according to the cell clusters and histopathologic changes found in the samples. Fibrosis was observed in the lesions with positive osteomyelitis, especially in lesions with a long-term evolution and the absence of clinical signs of infection. The absence of clinical inflammatory signs could probably be related to the avascular state of the surrounding tissue and bone marrow. Therefore, it would be difficult to diagnose lesions at the levels of care that normally will serve these patients.

Vascular disease and diabetes have a complex relationship, as diabetes is a risk factor for vascular insufficiency. Hyperglycaemia allows pathologic organisms to reproduce at an increased rate so that the infection and the ischemic bed become a permanent chronic cycle (Prasad 2007; Lipsky 1990; Hannan and Attinger 2009).

We also found that fibrosis was the only histopathologic change observed in 22.61% of patients. Fibrosis is usually considered as a pathologic type in which the majority of collagen formation occurs; however, it is also regarded as the final stage of any type of osteomyelitis during which the damaged bone is subsequently replaced by fibrosis as part of the healing process (ROSAI 2004). If this is the case, then it would be logical to think that it may be unnecessary to operate on these patients.

Explanations for the clinical presentations and the outcomes of osteomyelitis could be associated with four primary factors: the number of pathogenic organisms, their virulence, local and systemic host immunity and local tissue perfusion and bone vascularity.

Diabetic patients are considered as systemically compromised hosts with variable degree of peripheral vascular disease and insufficiency. Diabetes mellitus with concomitant alterations in host defences can profoundly influence the pathophysiology and clinical presentation of osteomyelitis.

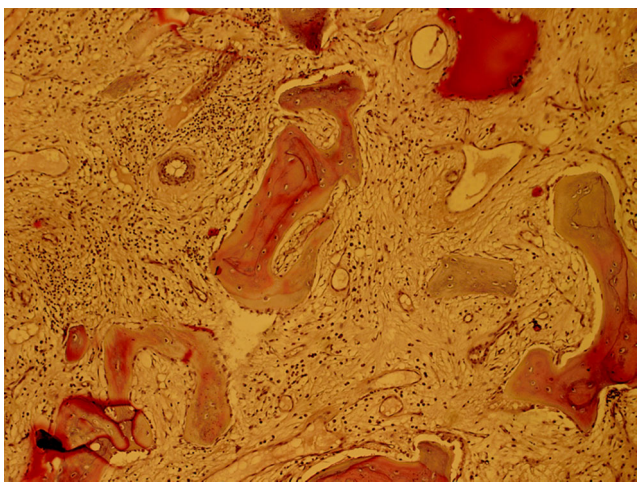


Fig. 3 Chronic osteomyelitis represented by lymphocyte and plasma cell infiltration, bone remodelling and varying degrees of bone marrow fibrosis. (×200, haematoxylin and eosin)

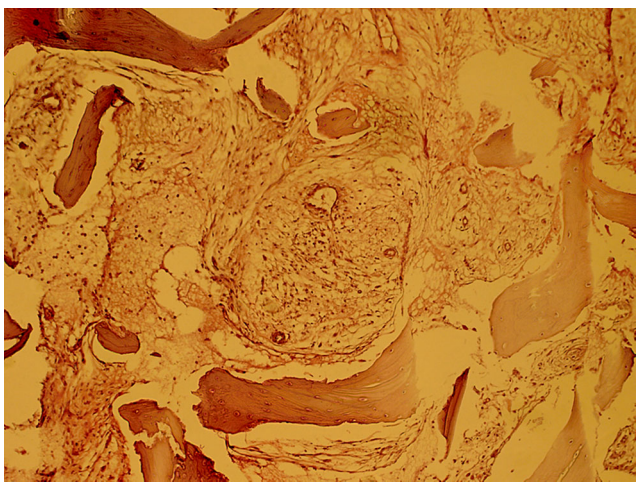


Fig. 5 Fibrosing osteomyelitis represented by few lymphocytes and plasma cell infiltration and obvious bone marrow fibrosis. (×200, haematoxylin and eosin)

Table 3 Inflammatory infiltrate found in the bone biopsy according to the types of osteomyelitis

Type of osteomyelitis	Inflammatory infiltrate present in the sample
Acute osteomyelitis	Lymphocytes, plasma cells, PMNs and histiocytes
Chronic acute osteomyelitis	Lymphocytes, plasma cells, and PMNs
Chronic osteomyelitis	Lymphocytes, plasma cells, and scarce PMNs
Fibrosis	Lymphocytes or no cell cluster

Diabetes mellitus affects the mechanisms of immunomodulation and the predisposing conditions for osteomyelitis through the following strategies: decreased level of leukocyte chemotaxis, phagocytosis activity, leukocyte lifespan, defective antibody synthesis, decreased complement levels, vasculopathy and therefore decreased vascularity of bone and surrounding soft tissue that lead to lower vascular perfusion and the lack of an effective inflammatory reaction, decreasing healing rate due to reduced tissue vascular perfusion and defective glucose metabolism.

Diabetic patients also have malnutrition that may lead to decreased wound healing process and diminution of immunological responses (Lipsky 1990; Hannan and Attinger 2009).

This implies that diabetes mellitus leads to a lower level of inflammatory responses, compared to normal individuals. Moreover, sometimes the aetiology of osteomyelitis is associated with low virulence pathogenic organisms (only after PCR-based detection methods) without any clinical symptoms and signs of infection, leading to chronic low-grade inflammation and fibrosing type histopathologic findings (Tiemann et al. 2014). As mentioned above, the exact aetiology of the fibrosing form of osteomyelitis in diabetic individuals remains unknown.

The type of fibrosis caused by osteomyelitis in diabetic patients is not compatible merely with an infection-induced osteomyelitis, and it seems that the infection phenomenon alone may not justify the occurrence of fibrosis. We believe that there is no doubt about the infectious nature of the fibrosis stage because the microbiological findings showed that the bone culture was positive in 84.2% of patients in whom fibrosis was diagnosed.

The common microorganisms isolated and characterized in this study were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and methicillin-resistant *S. aureus* (MRSA). These microorganisms were observed in the bone culture of patients with fibrosis stage. The increase in gram-negative to gram-positive bacteria ratio may be due to the susceptibility of immunocompromised patients to hospital acquired infections, either after colonization with environmental strains or following invasive surgical procedures, that cause such organisms to exert their pathogenic effects by producing endotoxins (Hunt 2003). The infections by *S. aureus* were reported to develop due to the exposure to contaminated hands of caregivers rather than to the overuse of antibiotics (Hartemann-Heurtier et al. 2004). However, patients who had received prolonged, inappropriate or broad-spectrum antibiotics or had long-term hospitalization, chronic wound or surgical procedure were most likely to have infection and bacterial colonization by organisms such as *P. aeruginosa* and MRSA. Furthermore, the presence of diverse virulence factors including lipopolysaccharide, exotoxin A, leukocidin, extracellular slime, proteases, phospholipase and several other enzymes make *P. aeruginosa* the most clinically significant bacteria among diabetic patients (Bodey et al. 1983).

We hypothesised that for all forms of osteomyelitis, there are cases where there is a restitution of bone tissue by fibrosis so that, during this process, a nidus of bacteria is often trapped inside the fibrous tissue. Since fibrous tissue does not contain blood vessels, antibiotic drugs are unable to penetrate into the site of infection. Such conditions allow the bacteria to grow, and persistent infection is often developed. We suggest that, after initiation of bone infection, a polymorphonuclear cell infiltrate would often be found. Following the acute phase, if the infection is not resolved, a chronic phase is maintained, which may eventually lead to fibrosis or become acute with the presence of polymorphonuclear cells over a background of chronic infiltrate. We think that osteomyelitis is not a

Table 4 Histopathologic findings according to the characteristics of the different types of osteomyelitis

Histopathologic finding	AO (n = 22)	CAO (n = 10)	CO (n = 33)	Fibrosis stage (n = 19)	P value
Bone necrosis	16 (72.7%)	9 (90%)	3 (9.1%)	0	<0.05*
Bone sequestrum	0	3 (30%)	1 (3%)	0	<0.05*
Bone remodelling	10 (45.4%)	8 (80%)	9 (27.2%)	1 (5.2%)	<0.05*
Osteoid formation	10 (45.4%)	7 (70%)	9 (27.2%)	4 (21%)	<0.05*
Bone marrow fibrosis	11 (50%)	8 (80%)	28 (84.8%)	17 (89.4%)	<0.05*
Cartilage affected	0	2 (20%)	0	0	<0.05*
Periostitis	13 (59.1%)	4 (40%)	5 (15.1%)	0	<0.05*
Periosteal fibrosis	0	1 (10%)	10 (30.3%)	8 (42.1%)	<0.05*

*p value less than 0.05 was considered significant

Table 5 Relationship between the diagnosis criteria and the histopathologic types of osteomyelitis

Type of osteomyelitis	Clinical sign of infection			Probe-to-bone test result			Radiologic signs of osteomyelitis		
	+	−	*P* value	+	−	*P* value	+	−	*P* value
AO ($n = 22$)	13	9	>0.05	22	0	<0.05*	19	3	<0.05*
CAO ($n = 10$)	6	4	>0.05	9	1	<0.05*	8	2	<0.05*
CO ($n = 33$)	19	14	>0.05	31	2	<0.05*	28	5	<0.05*
Fibrosis ($n = 19$)	11	8	>0.05	19	0	<0.05*	15	4	<0.05*

*p value less than 0.05 was considered significant

sequential process because we found that the duration of ulcers before the development of fibrosis is no greater than the period before the development of the chronic phase of osteomyelitis and thus fibrosis should be considered as a separate phase of osteomyelitis.

Conclusion

Histopathologic classification of osteomyelitis in the feet of diabetic patients should be classified in four groups: acute, chronic, chronic acute and fibrosis.

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