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Diabetic foot infections: effective microorganisms and factors affecting the frequency of osteomyelitis and amputation

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ABSTRACT

Objectives. Diabetic foot infections are common reason for hospitalization and are associated with high morbidity and mortality. We aimed to evaluate the clinic features and predisposed causes of osteomyelitis and amputation of patients with diabetic foot infections. *Methods.* Patients with diabetic foot infections who admitted and hospitalized at Infection Diseases and Clinical Microbiology department between January 2012 and July 2014 were included. Osteomyelitis was evaluated using magnetic resonance imaging (MRI) or bone scintigraphy. Microbiological examinations (Gram staining and culture) of the debridement materials and pus aspiration materials of the lesions were performed. *Results.* Of the seventy-three diabetic foot infected patients, 37 (50.7%) were female, and 36 (49.3%) were male. The mean age of patients was 57±9.8 years. The mean duration of diabetes and HbA1c level were 13.3±5.3 years and 8.17±1.83%; respectively. Soft tissue infection without osteomyelitis was present in only 34 out of 73 (46.5%) patients. A total of 89 pathogens were identified in 52 patients whereas any microorganism was not identified in 28.7%. Polymicrobial infections were detected in 30 (41%) patients. The most common isolated microorganism was Pseudomonas aeruginosa (36.9%), followed by Staphylococcus aureus (31.5%) and Enterococcus spp. (13.6%). Of the 37 (50.7%) patients had a history of diabetic foot infection previously, osteomyelitis progression was higher (89.2%) and statistically significant in these patients. Twelve (16.4%) patients underwent amputation. *Conclusions.* Advanced age and presence of osteomyelitis were found as risk factors for amputation. In the presence of osteomyelitis, treatment of diabetic foot infections is difficult and amputation rate is higher. For this reason, diabetic foot infections should be promptly treated before the development of osteomyelitis, and multidisciplinary approach is needed.

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Keywords: Diabetic foot infection; osteomyelitis; amputation

Introduction

Diabetic foot infection is one of the complications of diabetes; it has high mortality and morbidity and is also one of the most common causes of hospitalization in diabetes [1, 2]. Diabetic foot infections generally start as a soft tissue infection secondary to a minor trauma, may advance to osteomyelitis, and eventually become a risk factor for amputation [3, 4]. Approximately one-fourth of

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patients with diabetes mellitus (DM) have a diabetic foot infection in their lifetime and 15-20% of those cases result in amputation [3-6].

The development of diabetic foot infections can be primarily prevented by education of the patient and a good glycemic control; however, a multidisciplinary approach with various targets, such as appropriate antibiotic treatment, good glycemic control, appropriate surgical debridement, and education of the patient is needed once an infection has developed [1, 6, 7]. Some classification systems have been used to define the types, severity of infections and the outcomes of the cases. The most commonly used and easiest to use is the Meggit-Wagner classification [4-6].

The isolation of the effective microorganism, in addition to the wound classification, is needed for appropriate treatment in these infections. The most frequently isolated agents are gram positive cocci such as Staphylococcus aureus in superficial infections of mild to moderate severity in acute phases; Polymicrobial infections, in which gram negative bacilli and anaerobic bacteria are effective concomitantly, in addition to gram positive cocci in advanced stages and in the presence of severe infection [1, 3, 7, 8]. The most reliable method to detect the causative agent is obtaining a culture; however, when this is not possible, culturing the aspiration material from the pus provides more reliable results compared to swab samples [9, 10].

The aim of this study was to retrospectively evaluate various aspects of patients with diabetic foot infections who were followed-up during a two and a half year period.

Methods

Patients with diabetic foot infection that were followed-up at the Infection Diseases and Clinical Microbiology Clinic of Ankara Training and Research Hospital between January 2012 and July 2014 were included in the study retrospectively. The age and gender of the patients, duration and treatment of diabetes, use of oral antidiabetic agent (OAD) or insulin, past medical history including microvascular complications such as presence of neuropathy, nephropathy, and retinopathy, past episodes of diabetic foot infections, HbA1c (glycosylated hemoglobin) values at the time of diagnosis, and the opinion of an endocrinology consultant were recorded. The lesions of the patients were classified according to the Wagner classification (Table 1) [4]. According to this classification, patients with pressure in the foot due to shoes (callus formation) (Stage 0) were excluded from the study.

Osteomyelitis was evaluated using magnetic resonance imaging (MRI) or bone scintigraphy besides the direct roentgenograms according to the stage of the patient. The bilateral lower extremity arterial Doppler ultrasonography report of each patient was analyzed in order to detect peripheral vascular disease and the joint decision of the orthopedic, cardiovascular surgery, and plastic surgery clinics was recorded.

Microbiological examinations (Gram staining and culture) of the debridement materials and pus aspiration materials of the lesions were performed. Aerobic cultures were performed in all samples, while anaerobic cultures could not be done in most patients due to technical impossibilities. Clinical samples were routinely cultured in blood agar and EMB (Eosin methylene blue) agar plaques for both aerobic and anaerobic identification. The samples were incubated for 24 hours at 35°C under aerobic conditions to identify the aerobic microorganisms. For anaerobic cultures, the samples arriving the laboratory in a capped syringe with no air inside were cultured into anaerobic blood agar, which was prepared adding 5% sheep blood and vitamin K1 (1 •g/mL). The cultured anaerobic agars were placed into anaerobic jars and an environment without oxygen is provided with a dry system gas package (AnaeroGen-Oxoid, Basingstoke, UK). An anaerobic indicator (Oxoid, Basingstoke, UK) was used as an indicator to control the anaerobic environment. To grow the anaerobic bacteria, the agars were incubated for 48 hours at 35-37°C in an anaerobic environment. The definition of microorganisms and the antibiotic sensitivities were performed using the VITEK II automated system (Biomèrieux, France) according to the CLSI (Clinical and Laboratory Standards Institute) [11] guidelines.

Table 1. Wagner classification in diabetic foot ulcers

Stage 0: Formation of bone protrusion with healthy skin and/or callus formation (risk of ulceration)
Stage 1: Superficial ulcer with no involvement of deep tissues
Stage 2: Deep ulcer involving tendon, bone, ligament of joint
Stage 3: Deep ulcer including abscess and/or osteomyelitis
Stage 4 Gangrene involving the toes and/or metatarsal region
Stage 5: Gangrene involving the heel and/or the whole foot at an unrecoverable level that necessitates amputation

Statistical analysis

Statistical analysis of the data obtained was performed using SPSS for Windows 15.0 package program. The descriptive analysis was performed and the data was expressed as number, percentage, and mean±standard deviation. The normal distribution of continuous variables was tested using the Shapiro-Wilk test. The t-test and Mann-Whitney U-test were used to compare two independent groups in case of normal distribution and non-normal distribution, respectively. The chi-square test was used to analyze the categorical variables. The level of statistical significance was determined as $p<0.05$.

Results

Thirty-seven of the patients were females (50.7%) and 36 were males (49.3%) among the total 73 patients included in the study. The mean age of the patients was 57±9.8 years (range: 34-77 years) and mean duration of diabetes was 13.4±5.3 years. The mean HbA1C level of the patients was 8.17±1.83%.

Thirty-six patients (49.3%) were on insulin treatment, 23 (31.5%) were on insulin in addition to oral antidiabetic drugs (OAD), and 14 patients (19.1%) were on OAD alone. Diabetic foot infections of fifteen patients (20.5 %) were classified as Stage 1, 19 of (26%) were Stage 2, seven (9.6%) were Stage 3, 20 (27.4%) were Stage 4, and 12 (16.4%) were Stage 5. Clinical and demographic characteristics of the patients are summarized in Table 2. Thirty-nine patients (53.4%) of patients had osteomyelitis in addition to soft tissue infection,while 34 (46.5%) had only soft tissue infection. The mean age of the patients whose disease advanced and did not advance to osteomyelitis was 63.25±5.7 years and 49.88±8.5 years, respectively, and the difference between the two groups was statistically significant ($p<0.001$). The mean duration of diabetes and HbA1c levels in patients with osteomyelitis was 15 years and 8.9%, respectively, and these were significantly higher than those of without osteomyelitis ($p=0.042$ and $p<0.001$, respectively). Past medical history revealed prior diabetic foot infections in 37 patients (50.7%); the rate of advancement to osteomyelitis (89.2%) was statistically significantly higher in these patients than those of without infection history ($p<0.001$).Clinical and laboratory findings of the patients with osteomyelitis and comparison with the patients without osteomyelitis as shown in Table 3. The rate of all antidiabetic drug use was markedly higher in patients with osteomyelitis. Amputation not performed in any of the patients who had soft tissue infection alone; however, amputations at specific levels were performed in 12 patients ((30.8%) out of 39 patients who had concomitant osteomyelitis ($p<0.001$). All of the patients who had osteomyelitis and underwent amputation were Stage 5 according to the Wagner classification ($p<0.001$). The age and the rate of presence of osteomyelitis were higher in patients who underwent amputation. Clinical and laboratory findings of patients who did and did not undergo amputation are summarized in Table 4. Anaerobic cultures were performed in 16 patients (20.8%), although aerobic cultures were performed in all patients. However, no anaerobic agent was isolated. According to the culture results, a total of

Table 2. Clinical and Demographic characteristics of the patients

Properties	Number of patients (%)
Male/Female	36 (49.3)/ 37 (50.7)
Mean age (years, range)	57±9.81 (34-77)
Mean duration of diabetes	13.4±5.33
Mean HbA1c* (%)	8.17±1.83
OAD use**	14 (19.1)
Insulin use	36 (46.3)
OAD+insulin use**	23 (31.5)
Past history of infection	37 (50.7)
Wagner classification	
Stage 1	15 (20.5)
Stage 2	19 (26)
Stage 3	7 (9.6)
Stage 4	20 (27.4)
Stage 5	12 (%16.4)

*HbA1c=Glycosylated hemoglobin, **OAD=Oral antidiabetic drug

Table 3. Clinical and laboratory findings in patients with and without osteomyelitis

	Osteomyelitis (n=39)	No Osteomyelitis (n=34)	*p* value
Wagner stage ≤3	7 (17.1%)	34 (82.9%)	<0.001
Wagner stage >3	32 (100%)	-	<0.001
Age (mean±SD)	63.25±5.7	49.88±8.5	<0.001
Duration of diabetes (year) (median)	15	9.5	<0.001
Past history of diabetic foot infection	33(89.2%)	4 (10.8%)	
OAD* use	13 (38.2%)	1 (2.6%)	<0.001
OAD*+insulin use	18 (52.9%)	5 (12.8%)	
Insulin use	33(84.6%)	3 (8.8%)	0.044
HbA1c (%) (median)**	8.9	8.3	

*OAD=Oral antidiabetic drug, **HbA1c=Glycosylated hemoglobin

89 pathogenic agents were identified in 52 patients (71.2%), while no agent was identified in other patients (28.7%). More than one agent was isolated in 30 patients (41%). The most commonly isolated bacteria in order of frequency were Pseudomonas aeruginosa (n: 27, 36.9%), Staphylococcus aureus (n: 23, 31.5%), and enterococcus spp. (n: 10, 13.6%). Among the isolated S. aureus species, 19 (21.1%) were methicillin sensitive and four (3.9%) were methicillin resistant. Methicillin resistance rate was 17% among the S. aureus species. Isolated microorganisms are summarized in Table 5. When the antibiotherapies administered to the patients were analyzed, monotherapy was applied

Table 4. Clinical and laboratory findings in patients with and without amputation

	Amputation (n=12)	No Amputation (n=61)	*p* value
Wagner stage ≤3	-	41 (67.3%)	
Wagner stage >3	12 (100%)	20 (32.7%)	<0.001
Age (mean±SD)	64.75±6.0	55.5±9.7	<0.001
Duration of Diabetes (year) (median)	11	16	0.042
Presence of osteomyelitis	12 (100%)	27 (44.3%)	<0.001
Past history of DFI*	8 (66.6%)	29 (47.5%)	0.345
HbA1c **(%) (median)	8.65	8.6	0.644

*DFI=Diabetic foot infection, **HbA1c=Glycosylated hemoglobin

to 27 patients (36.9%), while 46 patients (63%) were received combined antibiotherapy. Piperacillin-tazobactam was used in 18 (66.6%) of the patients who received monotherapy. Other antibiotics used in monotherapy were imipenem, meropenem, ampicillin-sulbactam, and glycopeptides. The most frequently used combination among the patients who received combined antibiotherapy was vancomycin-meropenem combination (41.3%). Other antibiotics used in combined antibiotherapy were ampicillin-sulbactam+ciprofloxacin, vancomycin+piperacillin-tazobactam, and glycopeptide+ rifampin.

Discussion

The development of DFI and advancement to osteomyelitis and amputation in diabetic patients are closely related with advanced age, presence of microvascular complications (retinopathy, nephropathy, and neuropathy), duration of diabetes, and blood glucose level [12-14]. Diabetic foot infections were found to occur after 40 years of age, the frequency of the infections and amputations were found to increase with age, and they were found to occur in higher rates in men compared to women [12, 13, 15]. The mean age was also found to be high in the present study; however, the male/female ratio was found to be equal (49.3% / 50.7%). Compatible with other studies, the age of the patients was significantly higher in patients who underwent amputation compared to the ones who had no amputation ($p<0.001$) [14, 16].

Effective wound care, appropriate antimicrobial treatment, and good glycemic control are of utmost importance in the multidisciplinary approach to diabetic foot infections. Chronic hyperglycemia, which develops as a result of poor glycemic control, is known to cause impairment in neutrophil functions and to provide a basis for infections [15, 16]. High levels of HbA1c were suggested in two different studies to pave the way for the recurrence of diabetic foot infections and the development of neuropathy, and as a result, become a risk factor for amputation [17, 18]. Mean HbA1c levels in the present study at the beginning were 8.17% and high values were detected in patients with a prior history of diabetic foot infection. We suggest that providing glycemic control with conversion to insulin treatment or dose adjustment in patients with initial high levels of HbA1c will be effective in controlling infections.

In western societies, diabetic foot infections are generally seen 18 or more years after the diagnosis [14, 19]. This time period was reported to be 14.4 years and 16.6 years in the studies performed by Savas *et al*. and Demirci *et al*., respectively, in Turkey [20, 21]. The same measure was a mean 13.4 years in the current study. The reason of the earlier onset of diabetic foot infections in Turkey might be due to differences in the educational and socioeconomical level of the patients.

The first step in the diagnosis and treatment planning of diabetic foot infection is to define the extension

Table 5. Isolated microorganisms

Microorganism	Number of patients (%)
Agent not specified	21 (28.7)
Single microorganism	22 (30.1)
P. aeruginosa	7 (31.8)
MSSA*	4 (18.1)
Enterobacter cloacae	3 (13.6)
MRSA**	2 (9.1)
Coagulase negative staphylococcus	2 (9.1)
Morganella morganii	1 (4.5)
Escherichia coli	1 (4.5)
Klebsiella spp.	1 (4.5)
Proteus mirabilis	1 (4.5)
More than one microorganism	30(41.1)
P. aeruginosa	20 (66.6)
MSSA*	15 (50)
Enterococcus spp.	10 (33.3)
Coagulase negative staphylococcus	5 (16.6)
Morganella morganii	5 (16.6)
Escherichia coli	4 (13.3)
Enterobacter cloacae	3 (10)
MRSA**	2 (6.6)
Proteus mirabilis	2 (6.6)
Klebsiella spp.	1 (3.3)

*MSSA=Methicillin sensitive S. aureus, **MRSA=Methicillin resistant S. aureus

of tissue damage and the isolation of the causative agent. The commonly used Wagner classification was preferred in this present study due to its easier application compared to other classifications [22]. Most of infections in our patients were Wagner 4 stage. This may be explained by the level of our hospital (tertiary hospital). Advanced age, duration of diabetes and past history of diabetic foot infection were all found the main factors effecting development of osteomyelitis in this study. A prior history of diabetic foot infection and the presence of osteomyelitis play major roles in treatment failure and advancement of the condition necessitating amputation [6, 7, 12]. An association between the presence of osteomyelitis and frequency of amputation, similar to the current study, has been reported in many previous studies [1, 4, 12, 15]. The rate of amputation in the present study was identified as 16.4%. The high rate of amputation might be due to the fact that our hospital is a tertiary hospital and thus particularly complicated cases are generally referred to this hospital. The presence of a prior diabetic foot infection and a statistically significantly high rate of osteomyelitis and amputation, especially in this group of patients, support the findings of the previous studies performed [1, 7].

Obtaining a sample by aspiration following careful cleansing with serum saline without using any antiseptic solutions in cases when deep tissue and biopsy samples could not be obtained for various reasons has been reported to be more reliable compared to superficial swab samples in the isolation of causative agents in these infections [1, 3, 4]. In the present study, debridement materials and pus aspiration materials obtained from the lesions were evaluated. Anaerobic cultures can be performed in only 16 patients with no isolation of any causative agents, while aerobic cultures were performed in each sample. The reason for the inadequate detection of anaerobic agents may be due to the fact that they could not be performed in an adequate number of patients. Anaerobic bacteria frequently are involved in generally severe infections; however, isolation rates of anaerobic agents is low due to various factors such as the difficulties in obtaining and transport of these anaerobic culture samples and technical insufficiencies.

The most commonly seen microorganism in diabetic foot infection is S. aureus, while other gram positive microorganisms, gram negative aerobic bacilli, and anaerobic microorganisms may also be the causative agent. Although the agent is a single one in mild to moderate or acute infections (most commonly S. aureus), the most frequently seen agents in severe or chronic infections are gram negatives and anaerobes and polymicrobials, in general [1, 3, 7, 22]. A total of 89 causative pathogens were identified in 52 patients (71.2%) and no agent could be identified in the rest of the patients (28.7%). The number of causative agents identified was more than one in 30 patients (41%). The most frequently isolated bacteria were, P. aeruginosa (30%), S. aureus (25.5%), and enterococcus spp. Some studies have found P. aeruginosa as the most frequently seen causative agent, similar to the current study [1, 9]. The isolation of P. aeruginosa as the most commonly seen agent in the current study may be due to the fact that 53.4% of our patients were Wagner stage ≥3. The agents in severe infections with a high risk of amputation due to advancement of the lesion into the deep tissues and accompanying osteomyelitis are most commonly polymicrobial in contrast to low risk infections.

Empirical antibiotic treatment should be started early in these patients due to the risk of fast dissemination of infection to the deep tissues. The application of surgical debridement in addition to possible empirical antibiotic treatment decreases morbidity and mortality [1, 3, 23]. There is no gold standard antimicrobial treatment regimen in the management of diabetic foot infections [3, 23]. The severity of the infection, probability of the presence of resistant microorganisms, accompanying microvascular complications, the presence of osteomyelitis, and a prior history of infection episodes should all be taken into account. Since the most commonly encountered agents in mild to moderate superficial infections are gram positive bacteria and mainly staphylococci, targeting gram positive cocci in empirical therapy is reported to be adequate and monotherapy is generally stated to be effective in this group of patients. For the initial treatment of this type of infections, oral amoxicillin-clavulanate or parenteral beta-lactam +beta-lactamase inhibitors such as ampicillin /sulbactam, piperacillin/tazobactam, ticarcillin /clavulanic acid are among the recommended drugs. The most important advantages of beta-lactam + beta-lactamase inhibitors are the possibility of the application of monotherapy, the presence of oral forms, and the avoidance of disadvantages of multiple drug use [1, 3, 24-26]. Monotherapy was administered to 27 out of 73 patients (36.9%), while combined antibiotherapy was administered to 46 patients (63%). However, 44.4% of those patients had superficial soft tissue infection, while 54.6% had osteomyelitis. Piperacillin-tazobactam was the most common (66%) used agent as monotherapy. An analysis of isolated bacteria revealed that gram negative bacteria such as P. aeruginosa and gram positive bacteria such as S. aureus ranked first. This finding is compatible with the opinion that suggests the use of a beta-lactam+beta-lactamase inhibitor such as piperacillin-tazobactam in empirical treatment which has gram negative, antipseudomonal, and anti-anaerobe efficacy, in addition to gram positive efficacy.

Anti-anaerobic agents should be included in the treatment regimen in the presence of predisposing factors for amputation due to the high rate of involvement of anaerobic bacteria in diabetic foot infections. Therefore, initially, monotherapy or combined antibiotherapy including wide spectrum antibiotics should be selected that will cover gram positive, gram negative, and anaerobic bacteria in empirical treatment. Parenteral monotherapy with beta-lactam+beta-lactamase inhibitors such as ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin-clavulanic acid, carbapenems, ciprofloxacin+clindamycin combination, or third/fourth generation cefalosporines +clindamycin/metronidazole combinations can be preferred in this group of patients [1, 3, 24-26]. Combined antibiotherapy was applied in 46 patients (63%) in this study. A deep soft tissue infection was present in 47.8% of our patients, while 52.2% had osteomyelitis. Eleven (91.7%) out of 12 patients who underwent amputation had received combined antibiotherapy. The most commonly used combination was vancomycin+meropenem (41.3%). Other antibiotics that were used in combined antibiotherapy were combinations of ampicillin-sulbactam+ciprofloxacine, vancomycin+piperacillin -tazobactam and glycopeptide+rifampin combinations. In other studies published in the literature, factors such as prior history of diabetic foot infection, presence of osteomyelitis, poor glycemic control, advanced age, accompanying microvascular complications, and Wagner stage ?3 were reported to negatively affect the treatment success and to be the predisposing factors for amputation; therefore, wide spectrum antibiotics have been recommended in this group of infections [1, 4, 12, 15]. The reason for the frequent use of carbapenem group of antibiotics and glycopeptides in the present study is the presence of recurrent cases, advanced age, and presence of life-threatening and severe infections. The frequency of amputation and presence of osteomyelitis was statistically significant. The reason for this was thought to be the failure of treatment due to the difficulties in penetration of the antibiotics to the bony tissues in osteomyelitis or delay in the diagnosis of osteomyelitis.

The multidisciplinary treatment approach involves surgical debridement, control of blood glucose levels, and some procedures such as bone resection in the presence of osteomyelitis in addition to appropriate antimicrobial treatment.

Conclusions

Diabetic foot infection is a major health problem, since it causes loss of labor, psychosocial damage, and economic loss due to high treatment costs. Appropriate foot cares and good glycemic control decrease the rate of development of infections and amputations by 50% [22]. Therefore, the primary approach to diabetic foot infection should be education of the patient regarding the complications of diabetes. In addition, a multidisciplinary approach and follow-up at all stages is necessary.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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