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Full Length Research Paper

Infections and aerobic bacterial pathogens in diabetic foot

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The aim of the study was to investigate the causative pathogens, profile of antimicrobial susceptibility of them and the extent of tissue lesions in diabetic foot infections. This is a prospective study in which infected diabetic foot presenting with Wagner grade 1 to 5 ulcers were investigated. 78 consecutive diabetic patients who were seen in the orthopaedic clinic were cultured during ordinary visits. Bacteriological diagnosis and antimicrobial susceptibility profiles were carried out and analyzed using standard procedures. Diabetic polyneuropathy was found to be a common finding (74.4%). 15 (19.2%) cultures revealed polymicrobial involvement. The most frequent organisms isolated were *Enterobacteriaceae* (36.5%), *Pseudomonas aeruginosa* (18.9%), *Enterococcus* spp. (14.9%), *and Staphylococcus aureus* (10.8%). While imipenem, meropenem, amikacin, piperacillin/tazobactam were found out to be the most effective agents against Gram-negative organisms, vancomycin, teicoplanin, chloramphenicol were the most effective agents against Gram-positives. The aetiologies of most of the ulcers were neuropathic and 81.6% of them were deep. Our study also revealed that Gram-negative bacteria were the most common pathogens in infected diabetic feet. The diabetic foot ulcer is the most important cause of non-traumatic foot amputations so it is important to know the causative pathogens of these ulcers, profile of antimicrobial susceptibility of them for their treatment.

Key words: Diabetes mellitus, foot ulcers, infection, pathogen.

INTRODUCTION

Diabetes Mellitus (DM) is a serious public health problem worldwide (Wild et al. 2004). This problem was redoubled with rising prevalence of DM ineluctable rise in foot ulcers. Contributory factors include peripheral neuropathy, vascular disease, foot deformities, local trauma and pressure. The situation is the most important cause of non-traumatic foot amputations (Wild et al., 2004; Vamos et al., 2010; Fosse et al., 2009). In Turkey prevalence of DM was 7.2% (Satman et al., 2002). Due to the increase in geriatric population, number of patients with DM is rising per year in Turkey. Therefore the number of diabetic foot and infections are also increasing.

Infection is a frequent complication of diabetic foot ulcers, and the presence of infection greatly enhances the risk of amputation (Fosse et al., 2009). Infections in diabetic foot are usually polymicrobial due to aerobic bacteria and fungi. Severe infections usually yield polymicrobial isolates, whereas mild infections are frequently monomicrobial. In cases of a severe diabetic foot infection, three to five organisms may be cultured (Lipsky, 2004).

The Wagner classification (Wagner, 1981) classifies the severity and depth of tissue injury into five grades. In the superficial grades (Wagner 1 and 2), aerobic bacteria (*Staphylococcus* spp., *Streptococcus* spp., and *Enterobacteriaceae*) are predominant pathogens while anaerobic bacteria add up in Wagner grade 3 to 5 ulcers (Pathare et al., 1998). Effective antimicrobial therapy for these infections should help to reduce morbidity.

The aim of this present study is to investigate the causative pathogens and the relation between them,

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profile of antimicrobial susceptibility and the depth of tissue injury in patients with DM.

MATERIALS AND METHODS

Participants

This is a prospective study in which 78 consecutive diabetic patients, who were admitted to the outpatient department of an orthopaedic clinic at a university hospital in a period of 18 months, were included.

The patients were included if they received no antibiotics (first episode) or if systemic antibiotic treatment was stopped at least 30 days before the time of onset of the current episode (recurrent wound).

The patients were classified at the time of their first assessment. Each patient was included only once in this study. Age, sex, clinical history, duration of DM, nephropathy, urinary incontinence, retinopathy, obesity, associated diseases (e.g., hypertension, ischemic heart disease, cerebrovascular accident), duration of foot ulcers, neuropathic or ischemic character, localization, any history of amputation were recorded, glycosylated haemoglobin was measured, and all wounds were graded according to the Wagner Classification System when they are admitted to the hospital. Grade 1 was defined as cellulitis or a superficial wound, grade 2 as subcutaneous infection, fasciitis, or tendonitis without osteomyelitis, grade 3 as osteomyelitis (Osteomyelitis was diagnosed on suggestive changes in the radiographs and bone scans), grade 4 as a localized gangrene, and grade 5 as widespread gangrene (Wagner 1981). The patients in grade 0 were not included into the study.

Microbiology and antibiotic susceptibility tests

Culture materials from all the wounds were obtained, either by washing the wound with sterile physiological saline and then making a puncture-aspiration from the base of the wound or by applying a sterile cotton swab to the wound (Shankar et al. 2005). Specimens were sent to the laboratory and processed for aerobic bacteria. To minimize bias, laboratory technicians were kept blind to the clinical data. Anaerobic cultures were disregarded because of the lack of technical and logistical support.

Bacteriological diagnosis was carried out and analyzed using standard medical microbiology laboratory procedures (Doern et al., 2003). Susceptibility testing of microorganisms was performed by the disk diffusion method and evaluated according to recommended National Committee for Clinical Laboratory Standards (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2010).

Statistical methods

Quantitative variables were expressed as means ±SD while qualitative variables were expressed as percentages. Comparison of mean values was performed using the Student's t test for continuous and chi-squared test for categorical variables. A p value≤0.05 was considered statistically significant. Analysis was performed with Statistical Package for Social Sciences (SPSS) version 13.0.

RESULTS

Demographic characteristics

78 patients were included in the study. 44 (56.4%) of them were male. Clinical features of the patients are shown in Table 1. The mean age of the patients was 59.72 ± 10.17 (min34 max79). Fifty eight patients (74.4%) had diabetic neuropathy, 25 patients (32.1%) had diabetic retinopathy, 22 patients (28.2%) had nephropathy and 11 (14.1%) had urinary incontinence. Their mean glycosylated haemoglobin was 9.7±3.5%. The aetiologies of ulcers were neuropathic in 42 (53.8%) patients, ischemic in 36 (46.2%) patients. The localization of ulcers was commonly on the distal phalanges (34.5%), with 28.2% on the sole, 20% on the heel and 17% on the interphalangeal area. 81.6% of these ulcers were deep and 71.4% of them were neuropathic in the patients with bacterial growth (Table 2). While 69.6% of the patients with bacterial growth had loss of protective sensation; all of them had deformity of foot; 75.7% of them had pain (Table 2).

The statistically significant relation was found between the bacterial growth and the deep ulcers, deformity of foot and size of cellulite (Table 2).

According to Wagner classification, the ulcers were found to be in grade 4, 3, 2, 5 and grade 1 in 26 (33.3%), 16 (20.5%), 14 (17.9%), 13 (16.7%), and 9 (11.5%) patients, respectively.

Microbiology

In 21 (26.9%) patients cultures were negative; while in 15 (19.2%) cultures revealed polymicrobial involvement and the most frequent organism isolated were *Enterobacteriaceae* (36.5%). Others were *Pseudomonas aeruginosa* (18.9%), *Enterococcus* spp. (14.9%), *Staphylococcus aureus* (10.8%), *Streptococcus* spp. (6.8%), Coagulase negative *staphylococci* (5.4%), *Candida* spp. (4%) and *Acinetobacter* spp. (2.7%). A total of 74 organisms were isolated. Thirty three of these organisms were isolated in polymicrobial cultures. *Enterobacteriaceae* (10/33), *Enterococcus* spp. (9/33) and *P. aeruginosa* (4/33) were mostly isolated in the polymicrobial cultures in predominantly grade 4 and 5 (Table 3). The majority of positive cultures were observed in grade 4 (39.4%), 5 (26.6%) and grade 3 (22.3%) (Table 4).

Imipenem, meropenem, amikacin, piperacillin/ tazobactam were the most effective agents against Gram-negative organisms while vancomycin, teicoplanin, chloramphenicol were the most effective agents against Gram-positives. The antimicrobial resistance to Gram-negative organisms and Gram-positive organisms are shown in Tables 5 and 6 respectively.

DISCUSSION

Diabetic foot ulcers are common and serious complications of chronic DM. In parallel with increased prevalence of DM, the prevalence of foot infection are increasing,

Table 1. Clinical features of the patients.

Feature	Number of patients (%)
Age (mean ± SD years)	59.72±10.17 (min34 max79)
Sex	
Male	44 (56.4)
Female	34 (43.6)
Diabetic medication	
Insulin	31 (39.7)
Oral antidiabetic	30 (38.5)
Oral antidiabetic+insulin	14 (17.9)
Associated diseases	
Hypertension	35 (44.9)
Ischemic heart disease	18 (23.1)
Cerebrovascular disease	7 (9)
Chronic heart failure	6 (7.7)
Chronic Obstructive Lung Disease	5 (6.4)
Chronic renal failure	4 (5.1)
Hyperlipidemia	3 (3.8)
Malignancy	2 (2.6)
Pregnancy	1 (1.3)
More than one disease	25 (32)
Duration of foot infection (mean±SD days)	79.0±108.7 (min 3. max 720)
>1 month	32 (41)
<1 month	46 (59)
Duration of diabetes mellitus (mean±SD months)	11.9±7.9 month
>1 year	72 (92.3)
<1 year	6 (7.7)
Glycosylated haemoglobin (mean±SD %)	9.7±3.5 (min 5.3. max 18.9)
Body mass index (mean±SD kg/m2)	27.6±4.3 (min 16.5. max 40.8)
Current or past history of smoking	19 (24.4)
Current or past history of alcohol use	7 (9)
Diabetes comorbidities	
Neuropathy	58 (74.4)
Nephropathy	22 (28.2)
Retinopathy	25 (32.1)
Urinary incontinance	11 (14.1)
History of previous amputation	13 (16.7)
History of previous diabetic foot ulcer	31 (39.7)
Insufficient foot care	37 (47.4)
Trauma	15 (19.2)
Foreign body	3 (3.8)

worldwide and also in Turkey (Wild et al., 2004; Satman et al., 2002; Kandemir et al., 2007; Ozkara et al., 2008).

This prospective study was performed to evaluate the diabetic foot infections, the causative pathogens, the antimicrobial susceptibility profiles of them and the dept of tissue injury in these patients with diabetic foot ulcers.

Table 2. The relationship between the clinical features and the culture results.

Findings		Positive growth n (%)	No growth n (%)	P
Fever		5 (100)	-	P>0.05
Location of ulcer	Deep	40 (81.6)	9 (18.4%)	P=0.027
	Superficial	17 (58.6)	12 (41.4%)	
Type of ulcer	Ischemic	27 (75.0)	9 (25)	P>0.05
	Neuropathic	30 (71.4)	12 (28.6)	
Loss of protective sensation		39 (69.6)	17 (30.4)	P>0.05
Deformity of foot		14 (100)	-	P=0.016
Pain		28 (75.7)	9 (24.3)	P>0.05
Heat of foot	Cold	11 (84.6)	2 (11.4)	P>0.05
	Hot	46 (70.8)	19 (29.2)	
Appearance of foot	Pink	51 (72.9)	19 (27.1)	P>0.05
	Pale	6 (75)	2 (25)	
Pulse positive		44 (73.3)	16 (26.7)	P>0.05
Leakage		36 (76.6)	11 (23.4)	P>0.05
Abse		21 (77.8)	6 (22.2)	P>0.05
Ecchymosis /petechia		19 (67.9)	9 (32.1)	P>0.05
Lymphedema		20 (74.1)	7 (25.9)	P>0.05
Necrosis		33 (71.7)	13 (28.3)	P>0.05
Crepitation		5 (83.3)	1 (16.7)	P>0.05
Cellulite		25 (71.4)	10 (28.6)	P>0.05
Spread of cellulite to the leg		12 (75)	4 (25)	P>0.05
Size of cellulite	≤2 cm	4 (40)	6 (60)	P=0.016
	≥2 cm	21 (84)	4 (16)	
Distance of cellulite and ulcer	Far	8 (80)	2 (20)	P>0.05
	Close	17 (70.8)	7 (29.2)	
Septicemia		2 (100)	-	P>0.05

In the patients included in this study the duration of DM and foot infection were found to be more than a year (92.3%) and less than a month (59%) respectively. The level of their mean glycosylated haemoglobin was high. Therefore it was confirmed that the complications of DM were seen in the patients who had irregular glucose levels. Hyperglycemias and other metabolic derangements cause impaired immunological (especially neutrophil) function and wound healing and excess collagen cross-linking (Boulton, 2008).

In this study diabetic neuropathy was found in 58 patients (74.4%). The most common serious complication of diabetic peripheral neuropathy that affects the foot is neuropathic ulcers. Loss of sensation results in failure to perceive damage caused by mechanical trauma-such as, friction from bad fitting shoes, penetration of pointed objects on the floor, or excessive heat from radiators or flames (Boulton, 2008).

However, as suggested by Lipsky et al. (2004) detection of neuropathy before its complications develop, is the best way to prevent diabetic foot infections.

In our study the ulcers were mostly on the distal phalanges. Infections of the lower extremities in diabetic patients commonly occur on the plantar surface of the forefoot, in particular the toes and metatarsal heads.

In this study majority of these ulcers were deep and neuropathic in the patients with positive growth. Also the significant relation was found between the bacterial

Table 3. Organisms in polymicrobial growth according to Wagner classification.

Organisms in polymicrobial growth	W1	W2	W3	W4	W5	Total
Staphylococcus aureus+Streptococcus spp.	-	-	1	-	1	2
Staphylococcus aureus+Pseudomonas aeruginosa	-	-	-	-	1	1
Escherichia coli+Enterococcus spp.	-	-	-	1	1	2
Pseudomonas aeruginosa+Enterococcus spp.	-	-	-	1	-	1
Klebsiella spp.*+Enterococcus* spp.	-	-	1	-	-	1
Klebsiella spp.*+Enterococcus s*pp.	1	-	-	-	-	1
Escherichia coli+Candida spp.	-	-	-	1	-	1
Enterococcus spp.*+Proteus* spp.	-	-	-	1	-	1
Enterococcus spp.*+Citrobacter* spp.	-	-	-	-	1	1
Enterococcus spp.*+Acinetobacter* spp.	-	-	-	-	1	1
Streptococcus spp.*+Escherichia coli+Candida spp.*	1	-	-	-	-	1
Streptococcus spp.*+Pseudomonas aeruginosa+Enterobacter cloacae*	-	1	-	-	-	1
Pseudomonas aeruginosa+Enterococcus spp.+Proteus spp.	-	-	-	1	-	1
Total	2	1	2	5	5	15

Table 4. Isolated organisms according to Wagner classification.

Microorganisms			W1	W2	W3	W4	W5	Total
No growth			3	7	2	6	3	21
ram negative bacteria	*Enterobacteriaceae*	*Escherichia coli*	2	1	3	3	2	1
		Klebsiella spp.	2	-	2	2	-	6
		Proteus spp.	-	-	-	3	2	5
		Morganella morganii	-	-	1	-	1	2
		Citrobacter spp.	-	1	-	-	1	2
		Enterobacter cloacae	-	1	-	-	-	1
	**Nonf.*	*Pseudomonas aeruginosa*	2	-	3	7	2	14
		Acinetobacter spp.	-	-	1	-	1	2
Gram-positive bacteria	*Enterococcus* spp.		1	-	2	5	3	11
	Staphylococcus aureus		1	3	1	1	2	8
	Streptococcus spp.		1	1	2	-	1	5
	Coagulase Negative Staphylococ		-	-	1	3	-	4
Yeast	*Candida* spp.		1	1	-	1	-	3
Polymicrobial			2	1	2	5	5	15

***** Nonf.; nonfermentative.

growth and the deep ulcers, deformity of foot, size of cellulite. There was bacterial growth at the cultures of the all patients who had foot deformity. These patients had friction from bad fitting shoes. This data was evaluated as the foot ulcer can be revealed out because of the mechanical trauma caused by friction from bad fitting shoes and can be infected easily.

The careful assessment is mandatory in the presence of severe diabetic foot infection. Ulcers and surrounding tissues must be evaluated for deep soft tissue involvement, presence of foreign bodies, and necrotic tissue. Categorization helps to determine the degree of risk to the patient and the limb and thus, the urgency and the method of management (Lipsky et al., 2004). As in previous studies (Candel et al., 2003), the ulcers were found to be mostly in grade 4 and 3 in this study. Severe infections usually yield polymicrobial isolates, whereas mild infections are frequently monomicrobial (Dhanasekaran et al., 2003). In cases of severe diabetic foot infection three to five organisms may be cultured

Table 5. Antimicrobial resistance among Gram-negative bacteria.

Antimicrobials	P. aeruginosa (n=14) No.	Acinetobacter spp. (n=2) No.	Enterobactericeae (n=27) No.
Mezlocillin	1	1	NT*
Ampicillin	NT	NT	22
Amoxicillin clavulanic acid	NT	NT	21
Ampicillin/sulbactam	NT	-	NT
Piperacillin/tazobactam	-	1	-
Ceftazidime	1	1	NT
Cefepime	NT	1	2
Cefuroxim	NT	NT	10
Cefazolin	NT	NT	19
Cefotaxime	NT	NT	4
Imipenem	-	-	-
Meropenem	-	-	-
Gentamicin	4	1	6
Amikacin	-	1	-
Tobramycin	1	-	NT
Netilmicin	NT	-	NT
Ciprofloxacin	-	1	5
Levofloxacin	-	1	5
Trimetoprim/sulphametoxazole	NT	NT	9

*NT; not tested.

Table 6. Antimicrobial resistance among Gram-positive bacteria.

Antimicrobials	*S. aureus* (n=8) No.	CNS* (n=4) No.	*Streptococcus* spp. (n=5) No.	*Enterococcus* spp. (n=11) No.
Ampicilin/Sulbactam	-	2	NT**	NT
Cefazolin	-	2	NT	NT
Vancomycin	-	-	-	-
Teicoplanin	-	-	NT	NT
Gentamicin	-	1	NT	NT
Erytromycin	2	2	1	NT
Ciprofloxacin	-	1	-	1
Ofloxacin	NT	1	NT	NT
Clindamycin	-	-	2	NT
Trimetoprim/sulphametaxasole	-	2	-	NT
Chloramphenicol	-	-	-	NT
Methicillin	-	3	1	NT
Tetracycline	NT	NT	2	11
Penicillin	NT	NT	1	3
Nitrofurantoin	NT	NT	NT	1
Cefotaxime	NT	NT	-	NT

* CNS; Coagulase Negative Stapylococci. **NT; not tested.

(Anandi et al., 2004; Abdulrazaka et al., 2005; Ozkara et al., 2008). The polymicrobial infection rate was low (19.2%) in this study. Similar with our findings Dhanasekaran et al. (2003) documented that 84% of diabetic foot ulcers are frequently monomicrobial. Several studies have previously described polymicrobial aetiology in diabetic foot infections (Anandi et al., 2004; Abdulrazak et al., 2005; Ozkara et al., 2008; Frykberg, 2003). Lipsky

et al. (2004) reported that polymicrobial etiology in diabetic foot ulcers may often be due to previous treatment history. However the aim of the present study is to investigate the aerobic pathogens in diabetic foot infections, disregarding anaerobic bacteria may be a limitation. Also this might be the reason of the lower polymicrobial infection rate.

Determining the causative organisms in diabetic foot infections and their antimicrobial susceptibility pattern is necessary for the antimicrobial therapy. Chronic wounds were developed more complex infections caused by Enterococci, various *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and sometimes, other nonfermentative Gram-negative rods (Anandi et al., 2004; Abdulrazak et al., 2005; Ozkara et al., 2008). Lipsky et al. (2004) reported a prospective evaluation of diabetic patients with non-limb-threatening lower extremity infections and who were not yet treated with antibiotics, and cultures revealed aerobic Gram-positive cocci as pathogens in 89% of patients.

Though previous studies (Frykberg, 2003; Ge et al., 2002) showed Gram-positive aerobes as predominant agents in diabetic foot infections, we frequently isolated Gram-negative bacteria (55.7%) compared to Gram-positive bacteria (40.5%). Similar to our findings, Shankar et al. (2005) and Gadepalli et al. (2006) showed predominant involvement of Gram-negative isolates.

Diabetic patients with foot ulcers have several factors that may be associated with a high risk of multidrug resistant microorganisms carriage, such as inappropriate antibiotic treatment, chronic course of the wound and frequent hospital admission (Kandemir et al., 2007). The causative pathogens and their antimicrobial susceptibility profiles should be considered when arranging the treatment of diabetic foot infections. In this study the most frequent bacteria isolates were Enterobacteriaceae. The majority of them were resistant to ampicillin, amoxicillin/clavulanic acid and cefazolin. Imipenem, meropenem, amikacin, piperacillin/tazobactam were the most effective agents against whole Gram-negative organisms included *P. aeruginosa* and *Acinetobacter* spp., while vancomycin, teicoplanin, chloramphenicol were the most effective agents against Gram-positives. Among the Gram-positive bacteria Enterococcus genus was isolated mostly and all of them were resistant to tetracycline, while no resistance to vancomycin was determined. Imipenem, meropenem and vancomycin were reported to be the most effective agents against to the bacteria isolated in diabetic foot infections in several studies similar with our study (Abdulrazak et al., 2005; Raja, 2007; Gadepalli et al., 2006).

Conclusions

We found that the aetiologies of the most of the ulcers were neuropathic in our study and 81.6% of them were deep. Detection of neuropathy before its complications develop is a strategic way to prevent diabetic foot infections. Our study showed that Gram-negative bacteria were the most common pathogens in diabetic foot infections. Imipenem, meropenem, were the most effective agents against Gram-negative organisms. Vancomycin was the most effective against Gram-positive organisms. Decisive therapy should be based on both the cultures and susceptibility data and the clinical response to the empirical regimen.

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