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Peripheral arterial disease increases the risk of multidrug-resistant bacteria and amputation in diabetic foot infections

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Background/aim: The aim of this study was to investigate the microbiological profile and resistance rates of diabetic foot infections (DFIs) and to determine the effect of peripheral arterial disease (PAD) on the microbiology, clinical condition, and treatment outcomes.

Materials and methods: Characteristics, laboratory and imaging data, and the treatment modalities of patients admitted to our hospital with a diagnosis of DFI (PEDIS classification 3–4) during 2005–2016 were analyzed according to the presence of PAD.

Results: Of 112 patients who were included in this study, 86 (76.8%) had PAD. Patients with PAD were older and had higher amputation rates ($P < 0.05$). A microbiological profile of patients revealed a predominance of gram-positive bacteria (57.1%). *Staphylococcus aureus* and *Streptococcus* spp. were the most frequently encountered bacteria. Incidence of *Pseudomonas* spp. infection was higher in the PAD group ($P < 0.05$). Of all patients, 24.1% had multidrug-resistant (MDR) microorganisms in their wound cultures. Presence of MDR bacteria in patients with PAD was 4.9-fold higher than that in patients without PAD ($P < 0.05$).

Conclusion: This retrospective study indicates that PAD has a significant role, especially in elderly patients with DFIs. Patients should be promptly evaluated and treated for PAD to prevent infections with resistant microorganisms and limb loss.

Key words: Diabetic foot, peripheral arterial disease, infection

1. Introduction

Approximately one-fourth of diabetic patients experience lower extremity infections in their lifetime, and 15%–20% of these infections result in amputation (1,2). Diabetic foot infections (DFIs) are also the most common complication in diabetic patients, which lead to hospitalization (3). The major risk factors for the development of DFIs include peripheral arterial disease (PAD), neuropathy, and poor glycemic control (4). PAD, defined as an occlusion of the lower extremity arteries, has a special place among these risk factors (5). In diabetic patients with PAD, changes in the peripheral vascular bed lead to hypoxia in the tissue and also cause decreased antibiotic concentrations at the infection site. Consequently, the wound healing process is impaired, treatment becomes more difficult, and the rates of amputation and mortality increase (4,6,7).

Staphylococcus aureus and beta-hemolytic streptococci are the most frequent pathogens in DFIs. These microorganisms are particularly isolated in patients who do not have vascular pathology and antibiotic exposure. Gram-negative bacteria and anaerobes are more common in patients with long-term lesions and severe ischemia (8). *Pseudomonas* is often isolated in patients living in warm-climate regions such as Turkey (7). The prevalence of multidrug-resistant (MDR) organisms in patients with DFIs increases constantly due to inappropriate use of antibiotics. The treatment of these patients becomes challenging, hospital admission becomes prolonged, and treatment cost and mortality rates increase (2,9).

The aims of this study were to investigate the microbiological profile and the resistance rates of DFIs and to determine the effect of PAD on the microbiology, clinical condition, and treatment outcomes.

2. Materials and methods

Medical records of patients admitted to our hospital with a diagnosis of DFI during 2005–2016 were retrospectively reviewed from patient files and the hospital information management system. DFIs were classified using the PEDIS infection score system (7). Patients with grades 3

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and 4 PEDIS infection scores and those having positive culture results were included in the study. Wound cultures were collected as aerobic deep tissue specimens. Patient characteristics, laboratory and imaging data, and treatment modalities were reviewed. The presence of PAD was evaluated according to Doppler ultrasound and, in some cases, lower extremity angiography results. Patients who had stenosis with velocity changes (velocity increase, monophasic/biphasic flow, or collateral flow) in Doppler ultrasound were interpreted as having PAD (10). Patients who had stenotic segments in DSA imaging were also evaluated as having PAD. The diagnosis of osteomyelitis was established by plain radiography and magnetic resonance imaging findings. A minor lower extremity amputation was defined as any amputation distal to the ankle joint, while a major amputation was any amputation through or proximal to the ankle joint (11). The definition of MDR bacteria was made according to the criteria described by the CDC and the ECDC (12).

Statistical analysis was performed using SPSS 20. Qualitative variables were expressed as percentage, while quantitative variables were expressed as mean (±standard deviation) and median (range). Comparisons between groups were made using the chi-square test for categorical variables and the Mann–Whitney U test for numeric variables. Statistical significance was defined $P < 0.05$. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by univariate logistic regression analysis.

3. Results

A total of 112 patients who met the study criteria were included in this study. PAD was present in 76.8% of the patients. General demographics, laboratory results, treatment outcomes, and the microbiological evaluation results of the enrolled patients with (n = 86) and without PAD (n = 26) are shown in Table 1. Among the study patients, 75% of them were males and the mean age was 61.4 (±10.9) years. When analyzed according to the presence of PAD, it was observed that patients with PAD were older than patients without PAD (mean age: 63.7 ± 10.0 vs 53.9 ± 10.6 years, respectively) ($P < 0.000084$). For each unit increase in age, the PAD risk increased by 1.1-fold ($P = 0.002$, OR: 1.1, 95% CI: 1.04–1.16).

During hospitalization 53.5% of patients received only medical therapy, and in addition to this, 17.9% of them underwent surgical debridement and 28.6% underwent minor or major lower extremity amputation, as shown in Table 1. Of the eight major amputations, five were below the knee, one was above the knee, one was tibiotalar, and one was transtibial. All 21 minor amputations were phalanx amputations. When the PAD and the non-PAD groups were compared in terms of treatment options, the amputation rates were found to be higher in the PAD group ($P = 0.029$). Logistic regression analysis showed a 3.9-fold difference ($P = 0.039$, OR: 3.9, 95% CI: 1.08–14.07). There was no statistical difference between the amputation group and nonamputation group in terms of age, sex, previous amputation, osteomyelitis, previous DFI, ulcer duration, or HbA1c.

Wound culture results of all patients were also evaluated in this study, which revealed a total of 140 bacteria identified in 112 patients (Table 2). Of these bacteria, 57.1% were gram-positive and 42.8% were gram-negative. The most frequently isolated gram-positive bacteria were *S. aureus and Streptococcus* spp. Methicillin resistance was 17.2% in *S. aureus* strains and 37.5% in coagulase-negative staphylococci strains. The prevalence of MRSA among all patients was 4.5%. Penicillin resistance was detected in 18.2% of enterococcal strains. Vancomycin-resistant enterococci were not detected in any of the cultures. *Escherichia coli and Pseudomonas* spp. were the most frequently encountered organisms among the gram-negative bacteria. ESBL positivity was calculated as 21.4% in enteric gram-negative rods. No carbapenem resistance was observed, except in one *Acinetobacter* strain.

Patients with and without PAD were compared in terms of the four most frequently detected bacteria (*S. aureus*, *Streptococcus* spp., *E. coli*, *Pseudomonas* spp.), as shown in Table 1. *Pseudomonas* spp. were observed in 15 patients with PAD, whereas no *Pseudomonas* spp. were detected in the wound cultures of any patient without PAD. The difference was statistically significant ($P = 0.02$). However, there were no significant differences between the two groups for other bacteria.

MDR bacteria were detected in 27 of 112 patients (24.1%). Of these bacteria, 51.8% were MDR gram-negative bacilli, 22.2% were methicillin-resistant coagulase-negative staphylococci, 18.5% were MRSA, and 7.4% were MDR enterococci. When compared in terms of MDR microorganisms, the incidence of resistant bacteria in patients with PAD was higher than those without PAD (30.2% and 7.6%, respectively). The difference was found to be 4.9-fold when analyzed with logistic regression ($P = 0.039$, OR: 4.9, 95% CI: 1.08–22.3).

4. Discussion

In this study, we examined the frequency of PAD in hospitalized patients with PEDIS infection scores of 3–4 and its effects on the clinical features, microbiology, and treatment outcomes. We observed that DFI patients with PAD were of older age, the presence of *Pseudomonas* spp. infection and MDR bacterial infections was higher, and DFIs were more likely to end with an amputation in the PAD group.

PAD occurs in 20%–30% of all diabetic patients (7). The incidence is much higher, ranging from 36% to 70%

Table 1. Patients demographics, clinical features, and microbiological evaluation according to PAD presence.

	PAD (+) (n = 86, %)	PAD (-) (n = 26, %)	Total (n = 112, %)	P-value
Patient characteristics				
Median age (min–max)	63.0 (41–85)	53.5 (28–79)	61.4 (28–85)	0.000084
Male	63 (73.3)	21 (80.8)	84 (75.0)	0.605
Antibiotic use in 6 months	57 (66.3)	18 (69.2)	75 (67.0)	0.966
Previous DFI	60 (69.8)	14 (53.8)	74 (66.1)	0.132
Previous amputation*	27 (31.4)	5 (19.2)	32 (28.6)	0.375
PEDIS infection score Grade 3 Grade 4	 71 (82.6) 15 (17.4)	 22 (84.6) 4 (15.4)	 93 (83.0) 19 (17.0)	1.000
Osteomyelitis	74 (86.0)	23 (88.5)	97 (86.6)	1.000
Laboratory results*				
White blood cell ($\times 10^3/\mu L$)	12.3 ± 4.8	11.4 ± 6.3	12.1 ± 5.2	0.095
C-reactive protein (mg/L)	99.4 ± 99.6	88.0 ± 96.4	96.7 ± 98.6	0.417
Erythrocyte sedimentation rate (mm/h)	77.7 ± 28.1	75.1 ± 27.6	77.1 ± 27.9	0.670
HbA1c (%)	9.2 ± 2.4	9.9 ± 2.5	9.4 ± 2.4	0.297
Treatment outcomes				
Only medical therapy	45 (52.3)	15 (57.7)	60 (53.5)	0.630
Debridement	12 (14.0)	8 (30.8)	20 (17.9)	0.076
Amputation Minor Major	29 (33.7) 21 (24.4) 8 (9.3)	3 (11.5) 3 (11.5) 0	32 (28.6) 24 (21.4) 8 (7.2)	0.029 - -
Exitus	3 (3.5)	0	3 (2.6)	-
Microbiological evaluation				
Monomicrobial Polymicrobial	63 (73.2) 23 (26.8)	21(80.8) 5 (19.2)	84 (75.0) 28 (25.0)	0.438
Most frequent pathogens *S. aureus* *Streptococcus* spp. *E. coli* *Pseudomonas* spp. MDR bacteria presence	 19 (22.1) 16 (18.6) 14 (16.3) 15 (17.4) 25 (29.0)	 10 (38.5) 4 (15.4) 2 (7.7) 0 2 (7.7)	 29 (20.7) 20 (14.3) 16 (11.4) 15 (10.7) 27 (24.1)	 0.125 1.000 0.353 0.020 0.019

*Data of two patients missing.

in diabetic patients with foot wounds (6,7,13,14). In this study, the frequency of PAD was found to be 76.8%, which is higher than that reported by several studies (6,7,13,14). This can be attributed to our patient group, which included only patients with severe DFIs requiring hospitalization and did not include outpatients.

Several studies have reported that PAD occurs more in elderly patients. In a study of 1002 patients diagnosed with a new diabetic foot ulcer, PAD was found in 71% of patients aged >70 years (13). In the Eurodiale study that was performed prospectively with 1232 patients, the mean age of the PAD group was significantly higher than that of the non-PAD group (69 ± 11.2 vs. 60.5 ± 12.3 years, respectively) (6). Similarly, in our study, the mean age was significantly higher in the PAD group (63 ± 10.0 vs. 53.5 ± 10.6 years, respectively, $P < 0.05$)

In diabetic patients, the presence of PAD prevents the healing of ulcers and increases the rate of amputation and mortality (15). In the Eurodiale study, the rates of major amputations and mortality were found to be significantly higher in the PAD group (8% and 9%, respectively) than in the non-PAD group (2% and 3%, respectively) (6).

Table 2. Distribution of bacteria detected in the wound cultures of patients.

Microorganisms	No.	%
Gram-positive bacteria (total)	80	57.1
Staphylococcus aureus	29	20.7
Streptococcus spp.	20	14.3
Coagulase-negative streptococci	16	11.4
Enterococcus spp.	11	7.9
Corynebacterium spp.	3	2.1
Micrococcus luteus	1	0.7
Gram-negative bacteria (total)	60	42.9
Escherichia coli	16	11.4
Pseudomonas spp.	15	10.7
Enterobacter spp.	8	5.7
Klebsiella spp.	6	4.3
Proteus spp.	5	3.6
Morganella morganii	2	1.4
Citrobacter spp.	2	1.4
Acinetobacter spp.	2	1.4
Other gram-negative bacteria	4	2.8
Total	140	100

Another study from Turkey reported a major amputation rate of 28% and a minor amputation rate of 22% in DFI patients and the authors also underlined the high incidence of PAD (89%) in the amputation group (16). Yet another study showed that patients who did not undergo revascularization with peripheral angioplasty had worse wound-healing processes and higher amputation rates (17). Lipsky also emphasized that PAD and infection were the two most important causes of amputation in diabetic patients (18). The findings of our study are consistent with these data, revealing that amputation rates were significantly higher in the PAD group than in the non-PAD group (33.7% vs. 11.5% respectively, $P < 0.05$). We could not find any significant relationship with age, sex, osteomyelitis, previous amputation, previous DFI, ulcer duration, or HbA1c. Poor glycemic control, peripheral neuropathy, previous DFI, and ulcer depth and duration have been reported as other independent risk factors for amputation in the literature (19,20).

S. aureus, streptococci, gram-negative bacilli, and anaerobic bacteria are the most frequently detected pathogens in DFIs, and their prevalence may vary with the duration and severity of infection, antibiotic use, duration of hospitalization, presence of osteomyelitis, and geographical area (3,21,22). Studies from Europe and North America (23,24) have revealed that gram-positive bacteria were the primarily isolated pathogens in DFIs, whereas conversely, in Asian studies (25), the prevalence rate of aerobic gram-negative bacteria has been reported to be higher than that of gram-positive bacilli. This difference may be due to culture sampling methods and inappropriate antibiotic use, as well as cultural, geographical, and climatic factors (26). Several studies in our country have reported similar prevalence rates of gram-negative and -positive bacteria, with a slight predominance of gram-negative ones in DFIs (27). In our study, the prevalence rate of gram-positive microorganisms was found to be higher than that of gram-negative bacteria (57.1% vs. 42.9%, respectively), which is similar to the data from Western countries. The reason for the high prevalence rates of gram-negative bacteria isolated in DFIs in our country is thought to be associated more with the culture sampling methods rather than regional differences. Regarding the pathogenesis of DFIs, it is known that staphylococci and streptococci take place at the beginning of the infection, followed by gram-negative bacilli as the infection stage progresses (7). This becomes obvious when deep tissue cultures are obtained.

In our study, *S. aureus* was found to be the most frequently isolated pathogen with a rate of 19.6% among all isolates, followed by *Streptococcus* spp. (13.7%), *Pseudomonas* spp. (12.4%), and *E. coli* (11.8%). *S. aureus* has been reported to be the most common pathogen in several studies as in our study (14). In addition, some other studies have reported *Pseudomonas* spp. (28), *E. coli* (14), enterococci (29), and coagulase-negative staphylococci (30) as the most common pathogens in diabetic foot wounds.

The prevalence of MRSA in all types of infections has increased during the past few decades in Western countries and has become an emerging problem. The prevalence of MRSA in DFIs varies between 12% and 30% in several studies from Europe and the United States (9,21,31). In our country, MRSA prevalence is not as high as in Western countries. A large systematic review showed that MRSA prevalence in DFIs was not in high levels and had actually decreased in 2007–2011 compared to 1989–2011 period (respectively 5.7% and 7.8%) (26). In this study, the prevalence of MRSA in DFIs was 4.5%, which is compatible with the data in Turkey.

The resistance rates of bacteria isolated from DFI cultures are at substantial levels. In our study, MDR bacteria were detected in nearly one-fourth of all patients. Infection with MDR bacteria may cause a longer hospital stay, higher treatment costs, and a worse outcome. Patients with DFIs have several risk factors that lead to MDR bacteria. Frequent hospitalization, previous broad-spectrum antibiotic use, osteomyelitis, ulcer duration, size and type (ischemic ulcer), and diabetes duration have

been found to be associated with MDR bacteria in DFIs (9,32,33). In this study, PAD was found to be associated with MDR bacteria ($P < 0.05$). PAD may potentially lead to the selection of resistant microorganisms by reducing the transition rates of antibiotics to the tissue (34). However, a considerable proportion of these patients with MDR bacteria had frequent antibiotic use, previous DFI, and osteomyelitis. Therefore, we believe that these underlying conditions are the main contributing factors to bacterial resistance.

We also compared the PAD and the non-PAD groups in terms of the four most isolated pathogens, and *Pseudomonas* spp. were found to be more frequent in the PAD group ($P = 0.02$). There were no significant differences in terms of other bacteria (*S. aureus*, *Streptococcus* spp., *E. coli*). We came across only one study in the literature investigating the relationship between PAD and causative microorganisms. In that study, the causative pathogens of DFIs were assessed for the presence of ischemia, and no difference was detected in terms of microorganisms (14). Further investigation using larger patient groups is needed on this subject. However, we believe that our finding may be useful for clinicians dealing with the treatment of DFIs, especially in the planning of empirical treatment.

In our study, we could not evaluate the prevalence rate of anaerobic cultures and their effect on the patients' clinical course because anaerobic tissue cultures are not routinely applied in our hospital. This is an important limitation as anaerobic bacteria are believed to have a significant role in DFIs, especially in patients with PAD.

In conclusion, we found that PAD is very common in patients with PEDIS grade 3–4 DFIs and its frequency increases with age. The prevalence of MDR organisms and *Pseudomonas* spp. is also elevated in this group. Medical therapies and wound debridement procedures are not always sufficient, and the need for amputation, which is known to reduce the quality of life and shorten life expectancy, is high. Therefore, PAD should be investigated especially in elderly adults with DFIs, and medical and surgical-vascular interventions should be performed immediately to prevent lower extremity limb loss. Clinicians should consider *Pseudomonas* spp. and MDR bacteria in this group of patients and start therapy with broad spectrum antipseudomonal antibiotics.

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