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Pattern and microbiological features of diabetic foot ulcers

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Abstract
Aim: Diabetic Foot Ulcers (DFUs) are a common complication of type-1 and type-2 diabetes and a significant cause of morbidity and mortality. About 10-15% of patients with diabetes develop foot ulcers. Due to insufficient diagnosis and treatment, amputations in patients occurs and treatment costs increase, which brings a serious financial burden to the patient and the country. This study aimed to evaluate the clinical and microbiological data on diabetic foot infections in our clinic.
Material and Methods: In this study, 62 diabetic foot patients who were followed up with the diagnosis of diabetic foot in our internal medicine clinic between 2016 and 2022 were retrospectively analyzed. Bacterial identification, antimicrobial therapy, and ulcer classification data, such as cultures and biopsies, were obtained from patient files and electronic records. Patients were divided into two groups: medical treatment and surgical treatment. Surgical interventions and amputations were made in line with the decisions of the diabetic foot council.
Results: A total of 62 patients, 40 (64,5%) male, and 22 (35,5%) female, were included in the study. Diabetic chronic microvascular complications were found in 49 patients and osteomyelitis in 43 patients. All of these 43 cases were amputated. The distribution of DFUs according to pathophysiological etiology was most frequently neurogenic (46.7%). Bacteria were isolated from 24 patients, and the most frequently isolated bacteria was Staph. Aureus. Other patients who did not detect growth in the culture received empirical antibiotic therapy. When patients in medical and surgical treatment groups were compared, hypertension, hyperlipidemia, bacterial growth in culture, gender, and oral antidiabetic drug or insulin use properties were statistically similar. The presence of peripheral vascular disease, poor glycemic control, osteomyelitis, sedimentation >70 mm/hr, and diabetic complications were statistically significantly higher in the surgical group.
Discussion: Poor glycemic control and diabetic chronic complications increase the risk of diabetic foot infections. It may be possible to protect patients from DFUs through glycemic control, prevention and early treatment of chronic complications.

Keywords
Diabetic Infections, Microbiological Data, Amputation

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Introduction

Diabetic foot infection usually starts as a superficial infection. If it is not treated, it can quickly progress to the subcutaneous tissues and affect muscles, tendons, bones, and joints. It can progress to septic gangrene and lower extremity amputation. Approximately 60% of non-traumatic lower extremity amputations occur in diabetic patients [1].

Major risk factors for diabetic foot and lower extremity amputations are neuropathy, nephropathy, ischemia (peripheral vascular disease), hypertriglyceridemia, smoking, and poor glycemic control [2]. Especially, diabetic neuropathy and peripheral artery disease are the two most important risk factors playing a role in developing diabetic foot ulcers. About 25% of diabetic patients have lower extremity infections. Furthermore, about 50% of them result in hospitalization for severe infection, and 25% result in lower extremity amputation [3]. It is reported that the probability of having a second amputation within 1-3 years in a diabetic patient who has had one amputation is 22-42%. The cost of treatment, which is already high, increases 5-8 times with amputation [4]. In diabetic foot ulcer cultures, gram-positive aerobic bacteria; Staph. Aureus, Coagulase-negative Staphylococcus, Enterococcus spp., Group D streptococcus are isolated at a rate of 52%, and gram-negative bacilli, Pseudomonas aeruginosa, Serratia marcescens, Acinetobacter spp. in a rate of 42 % [5].

Diabetic foot is a severe cause of morbidity and mortality. Due to the delay and inadequacies in treatment, the hospitalization period of patients is prolonged. This results in loss of labor, disability, and psychosocial trauma, and treatment costs bring a severe financial burden to the patient and the country. This study aims to evaluate the clinical and microbiological data and amputation rates of complex cases discussed in the diabetic foot council and compare them with other literature studies.

Material and Methods

Our study is a cross-sectional descriptive study in which the data of 62 patients, followed in our internal medicine clinic between 2016-2022, were obtained retrospectively from patient files and electronic records. Those who underwent surgery and/or amputation in external center clinics with the diagnosis of DFUs, patients with superficial infection (stage 1) on an outpatient basis, those who received empirical treatment without culture and further examination in the follow-ups, patients under the age of 18, pregnant women, cancer patients, kidney and liver patients were excluded. Patients with insufficiency were not included in the study. Patients with stages 2-5, whose DFUs classification was made in our clinic, those who had deep tissue culture, and complex cases who underwent further examination were included in the study.

DFUs were established in patients with clinical, laboratory, and radiological findings, and it was staged according to the Wagner-Meggitt classification. The approval of the ethics committee was received from the ethics board of the university with the number 2018/167 on 12/07/2018. Routine biochemical parameters, C-Reactive protein (CRP), Erythrocyte Sedimentation rate (ESR), and complete blood count were evaluated. Culture samples were taken only as deep tissue culture and abscess culture. Swab cultures were not evaluated within the scope of the study. At the time of culture taking, 39 patients received empirically prescribed oral antibiotic therapy. Deep tissue cultures and other chronic complications related to diabetes were screened. Radiological imaging (direct X-rays, MRI, and bone and leukocyte-marked scintigraphy) findings were obtained retrospectively from patient files.

Patients' age, gender, smoking, diabetic comorbidities, growth in wound culture, hypertension, hyperlipidemia, and treatment methods were recorded. Patients were divided into a medical treatment group and surgical treatment (debridement, amputation, and medical treatment) group. It was seen that the decision of amputation was taken together with orthopedics and traumatology, cardiovascular surgery specialists, dermatology, endocrinology, plastic and reconstructive surgery specialist, and infectious diseases specialists.

Statistical Analyses

Analysis of data was performed using SPSS 22.0 statistical package program. The Kolmogorov-Smirnov test was used to determine whether the continuous variables showed normal distribution or not. The t-test was used in the comparison of two independent groups. Pearson's chi-square test and Fisher's exact chi-square test analyzed categorical data. Statistical significance level was determined as $p<0.05$.

Results

Sixty-two patients were included in the study. Patients were 52.4 ± 6 years old, and 40 (%64.5) were male, 22 (%35.5) were female. In the treatment of diabetes 67.7% (n=42) used insulin and %32.2 (n=20) oral antidiabetic drugs. The mean duration of diabetes was 14.2 ±5 years. Diabetic chronic microvascular complications were found in 79% (n=49), peripheric vascular disease in 40% (n=25), hypertension in 56.4% (n=35), and hyperlipidemia in 38.7% (n=24) of subjects when diabetic

Table 1. Evaluation of clinical data of medical treatment and surgical treatment groups

Parameters	Surgical treatment group (n=43)	Medical treatment group (n=19)	P value
Gender, male, % (n)	%45,1 (28)	%19,3 (12)	
Age, year ± SD	52.1±4	52.3±5	
Peripheric arterial disease	%32,2 (n=20)	%8,0 (n=5)	0,001
Diabetic chronic complications	%61,2 (n=38)	%17,7 (n=11)	0
Hypertension	%20,9 (n=18)	%27,4 (n=17)	0,568
Hyperlipidemia	%22,5 (n=14)	%16,1 (n=10)	0,582
Sedimentation >70 mm	%49,3 (n=43)	%12,9 (n=8)	0,004
Bacterial growth in tissue culture (+)	%25,8 (n=16)	%12,9 (n=8)	0,039
A1c, %	12.3	9.8	0

Table 2. Wagner-Meggitt classification in diabetic foot infection cases

Stage 0: Bone spur and/or callus formation with intact skin, the risk for ulcer	0
Stage 1: Superficial ulcer without extension to deep tissues	0
Stage 2: Deep ulcer involving the tendon, bone, ligament, or joint	6
Stage 3: Deep ulcer including abscess and/or osteomyelitis	49
Stage 4: Gangrene involving the fingers and/or metatarsal	7
Stage 5: Gangrene of the heel and/or entire foot that is unrecoverable and requires amputation	-

complications were examined. Osteomyelitis was detected in 69.3% (n=43) of patients radiologically and clinically. All patients with osteomyelitis had a high erythrocyte sedimentation rate (ESR>70 mm/hr).

When the patients in the medical treatment and surgical treatment groups were compared, no statistically significant difference was found between surgical treatment and parameters such as hypertension, hyperlipidemia, bacterial growth in the culture, gender, use of oral antidiabetic drugs or insulin. A statistically significant difference was found between surgical treatment and the presence of peripheral vascular disease, osteomyelitis, sedimentation >70 mm/hr, diabetic microvascular complications, and poor glycemic control (Table 1).

All patients were classified according to the Wagner-Meggitt classification at the time of admission to the hospital (Table 2). Thirty-six of the 43 patients who underwent amputation were stage 3, and 7 were stage 4. The distribution of diabetic wounds according to the pathophysiological etiology was as follows: %46.7 (n=29) neurogenic, %25.8 (n=16) neuroischemic and %27.4 (n=17) ischemic. Deep tissue culture with a cotton swab was taken during debridement from all patients. Bacteria were isolated from 24 patients (%38.7). The distribution of these patients was as follows: Staph. aureus 12, P. aeruginosa 5, E. coli 5, Citrobacter 1, Strep. agalactiae 1. It was seen that patients with no growth in the culture received antibiotic treatment. No bacterial growth was detected in the tissue cultures of these patients.

Staphylococcus aureus (50%) was the most common bacteria, while Pseudomonas aeruginosa (20.8%) and Escherichia coli (20.8%) bacilli were found to be the most common gram-negative bacteria. Of the 12 Staph aureus isolates, 4 (16.6%) were Methicillin-resistant Staph aureus (MRSA). Extended Spectrum Beta-Lactamase (ESBL) positivity was 32.5%. Staph. aureus (vancomycin, linezolid, Teicoplanin, trimethoprim-sulfamethoxazole, levofloxacin, Fusidic acid) were sensitive, and oxacillin, cefoxitin, cefazolin were resistant. Pseudomonas aeruginosa (imipenem, meropenem, piperacillin-tazobactam, cefoperazone -sulbactam, ceftazidime) was detected as sensitive. Escherichia coli (amikacin, trimethoprim-sulfamethoxazole, imipenem, meropenem, Ertapenem) was sensitive, and ciprofloxacin, piperacillin-tazobactam were resistant. Strep. agalactiae (vancomycin, Teicoplanin) and Citrobacter (amikacin, imipenem) were sensitive.

Discussion

Diabetic foot lesions may present as lesions ranging from simple superficial hyperemia to ulceration, osteomyelitis, and gangrene. Diabetic lower extremity ulcers can be classified as non-leg-threatening, leg-threatening, and life-threatening ulcers [6]. In our study, we found foot and/or finger threatening ulcers in 43 patients in the surgical treatment group and life-threatening ulcers in 4 patients in the medical treatment group. A retrospective evaluation of 24 isolates detected in 62 diabetic foot ulcer patients was performed in our study. Most of the isolates were gram-positive bacteria, and most were staphylococcus strains (50%). Among Gram-negative bacteria, Pseudomonas aeruginosa (20.8%) and Escherichia coli (20.8%) bacilli predominated.

Isolation of causative bacteria is very important for determining the suitable treatment protocol in diabetic foot ulcers. Multiple bacteria may be responsible for the presence of deep tissue infection and osteomyelitis. Therefore, in addition to swab culture, the microbiological examination should be performed by taking samples from purulent drainage in deep tissues, necrotic tissues, and, if necessary, bone tissue during debridement [7]. Gram-positive bacteria gram-negative aerobic and anaerobic bacteria are isolated in diabetic foot ulcers. Generally, the causative agents in severe ulcers are polymicrobial bacteria such as P. aeruginosa, Staph. aureus and Enterococcus spp. It has been reported that polymicrobial infections develop more frequently in patients receiving empirical antibiotic therapy. Because of this, it is imperative to use appropriate antibiotics in the treatment of diabetic foot infections.

Drug selection should be made by evaluating the severity of the infection, duration of the diabetic wound, and previous antibiotic exposure [8]. In our study, bacterial isolation was achieved in deep tissue culture in %38.7 (n=24) of the cases. The isolated bacteria were Staph. aureus (%50), E. coli (%20 .8), Pseudomonas (%20.8), Citrobacter (%4.1), Strep. Agalactiae (%4.1), which is consistent with literature. It is shown in the studies that Staph. aureus (%47.5), Pseudomonas spp. (%16.9), E. coli (%10.2), Streptococcus spp. (%8.5), Enterobacter spp. (%7.0), Proteus spp. (%6.7) and Acinetobacter spp. (%3.2) are isolated in the cultures [9]. Our study detected bacterial isolation in tissue cultures at a much lower rate than in the literature. The data of culture positivity lower than expected can be explained by the fact that most of our patients received antibiotic treatment before coming to our clinic. The patients were difficult cases referred to our clinic from primary and secondary medical centers. There was no statistically significant difference between bacterial isolation in culture, medical treatment, and surgical treatment. There was no statistically significant difference between medical and surgical treatment in terms of bacterial growth in tissue culture.

Although DFUs are usually polymicrobial infections, they were monomicrobial infections in our study [10,11]. DFUs have been reported as polymicrobial infections in studies [12,13]. In our study, most patients with DFUs had a history of antibiotic use, and sensitive bacteria could not be detected due to antibiotic use. However, only resistant organisms could be detected, which may explain monomicrobial dominance. S.aureus strains that we detected in the etiology in our study showed 100% sensitivity to Teicoplanin, Linezolid, and Vancomycin. S.aureus Cefoxitin, Oxacillin, Cefazolin were resistant to a certain extent (27.3-89%). All strains of MRSA were susceptible to Teicoplanin, Vancomycin, and Levofloxacin. E. coli strains Amikacin, Imipenem, Meropenem showed 90-95.4% sensitivity. Pseudomonas aeruginosa was 80.4-100% sensitive to Imipenem, Cefoperazone-Sulbactan, Piperacillin-Tazobactam, Ceftazidime.

Diabetic neuropathy, peripheral vascular disease, foot deformity, previous diabetic foot ulcer, and amputation are the most critical risk factors for diabetic foot [14]. In our study, at least one diabetic neuropathy, retinopathy, and nephropathy were present at a rate of %79 (n=49). Various studies have shown

that 40-60% of patients undergoing amputation because of the diabetic foot have neuropathy. In our study, diabetic chronic complications were 61.2% (n=38) in the surgical treatment group and 17.7% (n=11) in the medical treatment group. We found more chronic complications in the surgical treatment group than in the medical treatment group. Studies have shown that poor glycemic control is a significant risk factor for diabetic foot and amputation [15]. In our study, the relationship between the presence of poor glycemic control and amputation was statistically significant. Atherosclerosis begins at an earlier age in diabetics and tends to progress more rapidly. Foot ulcer and amputation are the primary macrovascular complications associated with diabetes. Studies have shown that peripheral vascular disease is a predictive factor for diabetic foot ulcers [16]. In our study, we found peripheral vascular disease at a very high rate of 40.3% (n=25), hypertension at a rate of 56.4 % (n=35), and hyperlipidemia at a rate of 38.7% (n=24). The relationship between the presence of peripheral vascular disease and surgical treatment and undergoing amputation was statistically significant. There was no statistically significant difference between hypertension and hyperlipidemia and surgical treatment. The International Diabetes Federation recommends reducing such diabetes-related complications as the primary goal since diabetic patients have a high lifetime risk of developing foot ulcers of 25% [17].

In our study, osteomyelitis was found at a rate of 69.3% (n=43). At least one finger amputation was performed in cases with osteomyelitis. The amputation rates reported in Turkey were found to be 36.7-37 % [18]. In developed countries, the prevalence of diabetic foot has been reported as 10-15%, and amputation prevalence is 15% [19]. In our research, amputation rates are higher than in developed countries, which is compatible with the data of our country. The reason for the high rate of amputation is the insufficient number of centers where diabetic foot patients can be treated and the delay in the admission of patients to these centers. Studies have shown that the best inflammatory marker for osteomyelitis is sedimentation rate [20]. In our study, a significant correlation was found between sedimentation >70 mm/hr, the presence of osteomyelitis, and undergoing amputation. Studies have found that 5% of diabetic patients with DFUs required major amputation during the 12-month follow-up period. It shows that the most important cause of non-traumatic lower extremity amputation is diabetic foot ulcers [21,22].

Studies indicate that most diabetic foot ulcers can be prevented with a multidisciplinary approach. Follow-up and treatment of diabetic foot ulcers by a team lead to successful results [23]. All patients in our study were diagnosed and treated in our clinic with a multidisciplinary approach. Together with appropriate antibiotic therapy, surgical drainage, debridement, and resection of dead tissue should be performed, appropriate wound care should be applied, and metabolic disorders should be corrected. The treatment of diabetic foot infections is usually started empirically, and there is no standard approach to antibiotic use and duration of treatment. The antibiotic regimens used were not superior to each other. However, there is a common view that antibiotic treatment is unnecessary in uninfected ulcers [24].

Our study showed that empiric antibiotic treatment was started before culture at a rate of %51.6 (n=32). Ampicillin-sulbactam, piperacillin-tazobactam, ertapenem, and moxifloxacin were given to 12 patients with a moderate infection piperacillin-tazobactam, imipenem, and meropenem treatment were given to 26 patients having a more severe infection, even if there was no growth in the culture. The aim of surgery in the treatment of diabetic foot ulcers is to clean the infected and necrotic tissue to a level that will allow the granulation tissue to provide secondary healing to come out [18]. In our study, early local surgical procedure (incision, debridement) was performed in 19 patients, and at least one finger amputation was performed in 43 patients.

This study has some limitations. The study was designed retrospectively and should be supported by prospective studies. The number of patients in the study is low. In addition, because the study was performed retrospectively, patients' backgrounds and other comorbid diseases could not be evaluated in detail.

Conclusion

In our study, Staph was most commonly found in DFUs infections. E.coli and Pseudomonas aeruginosa are the most common gram-negative bacillus when detecting aureus. In line with these findings, it can be said that antibiotics (Teicoplanin, Vancomycin, and Linezolid) with 100% sensitivity in gram-positive infections will be an appropriate choice in the empirical treatment of DFUs. High sensitivity to Vancomycin and Teicoplanin should be considered in MRSA infections. In the empirical treatment of gram-negative infections, it may be more appropriate to use Amikacin and Imipenem, Meropenem. Dominant isolated organisms and local antimicrobial susceptibility patterns should be considered when in selecting antibiotic therapy.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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