CLINICAL AND MICROBIOLOGICAL EVALUATION IN PATIENTS WITH DIABETIC FOOT INFECTION

Petek Konya*, **Neşe Demirtürk****, **Mehmet Nuri Konya**** **and Recep Keşli*****

*Afyon Saglık Bilimleri University Department Of Infection Disease And Clinical Microbiology, Afyon, Turkey., **Afyon Saglık Bilimleri University Department of Orthopaedics and Traumatology, Afyon, Turkey., ***Selcuk University, School of Medicine, Department of Medical Microbiology, Turkey.

ABSTRACT AIM: The present study aimed to evaluate clinical and microbiological findings of the patients with a diabetic foot infection. **METHODS:** The present study was carried out retrospectively between March 2015 and September 2016. Eighty diabetic foot infection attacks of 68 patients with foot lesions at the grade of $\geq$ PEDIS 3 and $\geq$ WAGNER 2 were included in the present study. Demographic data of the patients, type of diabetes, staging of a diabetic foot according to PEDIS and Wagner classification, the treatments they have received, laboratory parameters, culture results, and treatment outcome have all been recorded to prepared forms. Deep tissue culture was obtained from all patients and sampling was carried out. The treatment they received and prognoses were evaluated. **RESULTS:** In samples obtained from 80 attacks included in the study, 30 (% 53,6) GRAM (-) bacteria 24 (%42,9) GRAM(+) bacteria, 2 (%3.5) Candida spp, overall 56 (%70) bacteria were isolated. The most commonly growing bacteria were; S.aureus (n=16,%28.6),Proteus spp (n=9,%16) and E.coli (n=6, %10). In 7 (%91.25) attacks, surgical treatment was administered apart from antibiotic treatment, and the rate of amputation was found to be %33.75. The rate of recovery was found to be higher in patients whose antibiotic treatment remained unchanged from the onset than that found in patients whose treatment was changed. In 70% of diabetic foot infection attacks evaluated in the present study, growth was detected, and most of the agents were established to be GRAM negative bacteria. **CONCLUSION:** Antimicrobial treatment providing cover against probable microbial agent increases the probability of recovery. Therefore, determination of active spectrums according to regions and the choice of suitable antibiothreapy will render prognosis better.

KEYWORDS Diabetic foot, Infection, culture

Introduction

One of the most important complications of diabetes is diabetic foot infection (DFI). In the aetiology of DFIs, infections also play a part in addition to peripheric angiopathy and neuropathy.

Moreover, the development of infection is closely associated with the frequency of extremity amputations in patients with diabetes. Over half of foot amputations, which are not associated with trauma, occur in diabetic patients. DFIs are an essential economic and social public health issue not only for being a significant health problem threatening lower extremities and lives of patients but also for leading to hospitalisations of patients for an extended period. [1].

DFI requires a multidisciplinary approach. Hence, coordinated action of plastic surgery, orthopaedics, endocrinology, radiology, microbiology and infectious diseases departments is necessary for treatment [2]. One of the most critical parameters of infection is the elimination of infections. Microbiological diagnosis is critical for proper infection treatment and the prevention of unnecessary antibiotic use [3].

The present study aimed to determine the demographic char-

Table 1 Demographic data of patients.

	Number of patients (n)	Percentage (%)
Sex		
Female	23	33.8
Male	45	66.2
Age		
65 age >	30	44.1
65 age ≤	38	55.9
Type of Diabetes		
Type 1	2	2.9
Type 2	66	97.1
Antidiabetic treatment		
İnsulin	28	41.2
Oral Antidiabetic Drug (OAD)	11	16.2
İnsulin + OAD	29	42.6

Figure 1: Distribution of DAI attacks according to Wagner and PEDIS classification.

acteristics of patients followed with the diagnosis of DFI and the distribution of microbial agents using an accurate sampling of cultures and to evaluate the suitability of empiric antibiotics administered.

Material and Method

The present study was carried out retrospectively between the dates of March 2015- September 2016 after local ethics committee approval. All patients, included in the study, were informed about the aim and method of the study. The signed consent form was obtained from all individuals participating in the study. Patients with DFI, who were followed in Infectious Diseases and Clinical Microbiology clinic were included in the present study. Patients whose foot lesion was ≥ class 3 according to PEDIS classification and ≥ class to 2 according to Wagner classification were included in the study. In patients who referred with more than one DFI attack at different times, each DFI attack was evaluated separately. A thorough history and family history was obtained from all patients. All underwent systemic examination and findings were recorded. Demographic data of the patients, clinical examination findings of foot lesions(purulent drainage, erythema, fluctuation, heat increase, pain or tenderness, induration, spread to deep tissues and bone), laboratory findings (hematological and biochemical blood examinations, CRP and sedimentation), results of imaging studies and findings of foot lesions according to classification techniques employed in the present study were recorded .

In the patients included in the study, to determine DFI agents, the sample for tissue culture was obtained by punch biopsy needle from wound bed of infected lesions after debridement, and in closed lesions that did not require debridement, the culture was sampled by needle aspiration. Clinical samples from patients were inoculated into media with suitable transportation for anaerobe and aerobe cultures and sent to the microbiology laboratory.
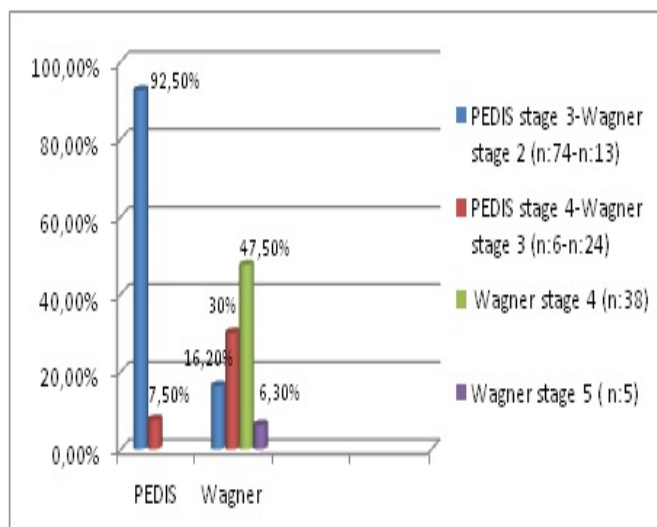
Previous antibiotic treatment received by the patients were recorded at first evaluation. Ampiric antibiotic treatment initiated after sampling was recorded and the patients were followed up for response to treatment via clinical and laboratory parameters . When there was no response to treatment, and empiric treatment did not cover the agents isolated in the culture, antibiotic treatment regimen was replaced with another one. Surgical interventions carried out in patients who did not respond to treatment only with antibiotics and wound care were evaluated as well. All medical and surgical interventions were evaluated, and prognoses were determined.

Statistical Analysis

For the analysis of findings, IBM SPSS Statistics 20.0 program was used. Whether the data were distributed normally was evaluated with Shapiro-Wilk test. In the comparison of normally distributed data between groups, Student-t-test and the comparison of data not normally distributed, the Mann-Whitney U test was utilized. In the comparison of categorical data, Chi-square analysis was used. In descriptive statistics, percentage, mean, standard deviation, median, minimum and maximum values were used. P-value of <0.05 was considered statistically significant.

Results

Eighty DFI attacks identified in 68 patients with DFI were included in the present study. Forty-five patients were male (%66.2) while 23 were (%33.8) female. Mean age was 61.59±11.93. The number of patients with Type 1 DM was 2 (%2.9), while that of patients with Type 2 DM was 66 (%97.1) Mean duration of diabetes was found to be 16.7±8 years. Demographic data of the patients are demonstrated in Table 1.

The most common localization of 80 DAI attacks was 35 fingers (%43.75)with ulcers localized in this region. Other localisations are respectively 14 metatarsal(17.5%), 11 heel(13.75%), 11 foot ridge(13.75%), 9 foot base(11.25%). The distribution of DFI attacks included in the study according to Wagner and PEDİS classification is demonstrated in Figure I.

Of 80 cultures obtained from 80 DFI attacks of 65 patients, 73 (%91.2) was tissue culture and 7 (%8.8) abscess aspiration
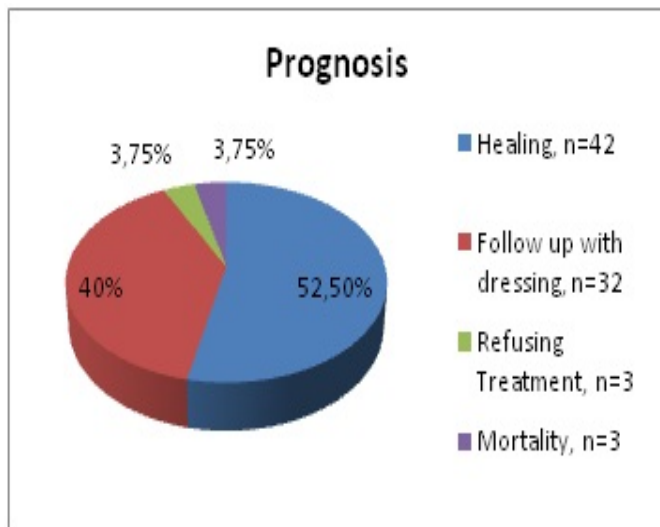
Figure 2: Prognosis in DFI attacks.

Table 2 Distribution of isolated agents.

	n	%
GRAM positive agents		
S.aureus (MSSA)	13	23.2
Enterococcus spp.	5	8.9
Streptococcus spp.	3	5.4
MRSA	3	5.4
Overall	24	42.9
GRAM negative agents		
Proteus spp.	9	16
E.coli	6	10.7
Klebsiella spp.	5	8.9
Morganella morganii	4	7.1
Acinetobacter spp.	3	5.4
GSBL(+) E.coli	2	3.6
P.aeruginosa	1	1.8
Overall	30	53.6
Candida spp.	2	3.5
Overall	56	100

culture. No microorganism was isolated in 24 out of 80(%30) cultures. Fifty-six microbial agents were isolated from remaining 56 (%70) cultures. There was no polymicrobial growth. Anaerobic microorganism growth occurred in none of the samples. The distribution of isolated microorganisms was shown in Table II. Most commonly isolated microorganisms were S.aureus, Proteus spp, and E.coli. In 47 of 80 evaluated attacks (%58.7), patients were on antibiotics before the presentation, while in 33 (%41.3), there was no previous history of antibiotic use. Of antibiotic treatment regimes, 44 was monotherapy and three combined antibiotic treatment. Antibiotics used were determined to be in decreasing order of frequency amoxicillin-clavulanic acid, cephalosporin, moxifloxacin, ciprofloxacin, fusidic acid and rifampicin. While there was the previous history of antibiotic use in 33.3% of attacks in which there was GRAM positive agent growth, such history was present in 66.6% of attacks with GRAM negative agent growth, with a statistically significant difference (p=0.009).

In all DFI attacks evaluated in the present study, after microbiological sampling was carried out, empiric antibiotic treatment was commenced. Options preferred in ampiric treatment were in decreasing order of frequency ampicillin-sulbactam, moxifloxacin, ertapenem, daptomycin, tigecycline, cefazolin, ciprofloxacin, piperacillin-tazobactam, meropenem, cefepime and trimethoprim-sulfamethoxazole. In 70 attacks monotherapy and in 10 attacks combined treatment was commenced. It was established in the follow up treatment that in 48 attacks (%60), treatment continued with empiric treatment used at onset, in 17 (%21.25), treatment was altered according to the growth results in the sample and 14 (%17.5), treatment was changed in spite of the absence of growth in the culture, as there was no clinical response to treatment. In one case (%1.25) Candida spp. Isolated in the culture was not considered as a causative agent clinically and treatment was changed, taking clinical response into consideration.

In the evaluation of empiric antibiotic regimes administered in DAI attacks with growth in their cultures, it was established that in 22 of 56 (39.2%) cases with growth in their cultures, empiric treatment was continued with the present antibiotic, while in 34(%39.2), treatment was changed according to culture results.

When all Wagner stages were evaluated, of 32 DFI attacks in which treatment was changed, complete improvement or complete infection control was achieved in 36 (75%). Of 48 attacks whose treatment was continued with original empiric treatment, complete improvement or infection control was achieved in 36 (%75) (p=0.02).

It was established that in 3 of 80 attacks (%3.75) patients refused treatment. In 42 out of remaining 77 DFI attacks, (%52,5), the improvement was observed with medical or surgical treatments. In 32 (%40) attacks, present infection picture partly resolved, but the diabetic wound did not close, and the patient was followed with foot care. Mortality occurred in 3 of 80 attacks (% 3,75). Mortality occurred due to pulmonary embolism during admission to hospital in one patient and complications associated with DFI in two patients. The prognoses of 80 patients followed in the present study are displayed in Figure II.

Discussion

Diabetic foot infection is a significant health problem requiring a multidisciplinary approach. In patients with whom DFI developed, rapid control of infection and prevention of amputations

secondary to DFI and of deaths associated with sepsis and septic shock are most important targets. To reach these targets, rapid intervention to DFIs, early and accurate debridement and proper antibiotic treatment are of great significance [3,14].

It is known that DFI's occur more commonly in male patients and those over the age of 40 and that the prevalence increases with advancing age[4]. In a multicenter study carried out in Turkey with 455 patients, by Saltoğlu et al., it was established that the male/female ratio was 2.1 and the mean age of patients was 61 [5]. Consistent with their results, the male/female ratio was found to be 1.95 and mean age 61.5 in the present study.

In the present study, it was established that DFIs are localized most commonly in toes. In the literature, similarly, it has been reported that diabetic foot ulcers occur most commonly in the front part of the foot (70-90%) to be followed by the heel and mid part of the foot. The reason for this localization is the foot region, which is first influenced by peripheral neuropathy and exposed to trauma most commonly [6].

The distribution of DFI attacks evaluated in the present study according to Wagner classification was as follows: Wagner 2 %16.2; Wagner 3 %30, Wagner 4 %47.5 and Wagner 5 %6.3, indicating that the rate of attacks classified as Wagner 3 or over was 83.7%. As to PEDIS classification, the distribution of our study group was PEDIS 3 %92.5 and PEDIS 5 %7.5. When these distributions are evaluated, it can be seen that the majority of our cases consists of those with severe infection. In a study, the rate of patients at Wagner stage 3 or over was found to be 70%, which is quite lower than our results [7]. The high rate of patients at Wagner stage $\geq$ 3 in the present study may be attributed to the low sociocultural level of patient population, and hence to the delay in presentation to hospital for diabetic foot injuries.

Microorganisms which are agents of DFI are quite variable. In the literature, DFI's are evaluated as having polymicrobial aetiology [8]. The distribution of microbial agents varies according to the severity of underlying disease, characteristics of the hospital and regional variations between countries. The most commonly detected agents are GRAM-positive aerobes such as S. aureus, and streptococcus and Gram-negative microorganisms belonging to Enterobacteriaceae family. In a study conducted by Ertuğrul et al. in 2017, the results of the studies performed between 2000-2014 in Turkey has been investigated, and the change in the distribution of microorganisms leading to DFI was evaluated with five year periods, and %45.8 of the agents were found to be GRAM-positive, %53.7 GRAM-negative and %0.05 Candida spp. The most commonly isolated organisms were Staphylococcus aureus (%22.8), Pseudomonas aeruginosa (%16.7) and Escherichia coli (%12.9). Overall 28 studies were evaluated and the increase in GRAM negative agents and a decrease in GRAM negative agents in five year periods was found to be significant. Also, decrease in MRSA rate within the last five years was also found to be substantial. Another observation was that the prevalence of isolation of P. aeruginosa agents decreased over the years [9]. Unlike the data in the present study, GRAM negative organisms account for 53.6% of isolated agents. Also, P. Aeruginosa was found to be isolated only the rate of %1.8, and our findings are discordant with those of Ertugrul et al. Given our results, care should be taken to cover GRAM negative bacteria when administering empiric antibiotic treatment, and as suggested in the literature, anti-pseudomonal spectrum should be chosen only in risky conditions.

In the present study, microbiological sampling was carried out in all 80 DFI attacks we evaluated. Seventy-three of the samples (%91.2) was tissue culture and 7(%8.8) abscess aspiration culture. In 24 out of 80 (30%)cultures obtained, no agent could be isolated. In a study by Mutluoğlu et al, no agent was isolated in 20 of 89 patients (22.4%) with deep tissue culture, which is compatible with our results [10].

The microbial agent was isolated in 70% of the samples obtained in the present study. The distribution of overall 56 agents isolated was similar to that in the study of Saltoğlu et al., and 24 (%42.9) were found to be GRAM-positive bacteria, 30 (%53.6) GRAM-negative bacteria and 2 (%3,5) fungi. [5]. The most commonly growing three microorganisms among all isolated agents were; S.aureus (n=16,%28.6),Proteus spp (n=9,%16) and E.coli (n=6, %10.7). In the literature, among GRAM positive bacteria playing a part in the aetiology of DFI, Proteus species are among the least common ones [11,12]. However, in a study performed in Brazil, similar to our results, the most commonly isolated GRAM negative agent in DFI has been reported to Proteus[13], which is among the enteric bacteria, is commonly found in water, soil and stool and can be an agent in wound infections by colonizing open wounds. These bacteria, which can grow easily in moist environments, possess the characteristics of producing inducible beta-lactamase and GSBL: Recently, it has been reported that the prevalence of GSBL positive Proteus has increased[14]. Therefore, when initiating antibiotic treatment in DFIs, it should be kept in mind that resistant Proteus species can be common agents. S. aureus isolates methicillin-resistant rates were evaluated in various studies and the study of Ertuğrul et al., the rate of MRSA among GRAM positive bacteria was found to be %10.3, and in the study of Saltoğlu et al, this rate was %11.9 and in the present study %12.5 [5,9]. In view of our results, it was concluded that empiric antibiotic treatment does not have to cover MRSA strains as the rate of isolation of MRSA was only 12.5% and that the addition of drugs protecting cover against these microorganisms would be indicated only if high-risk factors present for MRSA as defined in the literature, if the lesion is > stage 4 according to Wagner classification and if MRSA is isolated in the culture.

In the studies especially from our country investigating active agents in DFI's, Pseudomonas has been reported to be the leading agent among GRAM negative bacteria. [5,12]. However, in the present study, Pseudomonas accounted for only one of the isolated GRAM negative agents. When culture results obtained with swab method are compared with those obtained with deep tissue culture, it can be seen that the former method remains inadequate in establishing the true rate of GRAM-negative microorganisms [15]. In the present study, all cultures were obtained from deep tissue, which may be associated with low rates of Pseudomonas. Nevertheless, the likelihood of the changes in the spectrum of agents in patients with DFI should be taken into account, and exciting results we obtained should serve as a guide for future studies.

In DFI, when necrotic wounds coursing with gangrene, anaerobes may turn out to be agents[4]. In spite of many studies carried out on DFI within the last two decades, the actual prevalence of anaerobic pathogens in DFI remains uncertain. In DFI, the growth of microbial agent in the culture obtained depends upon many parameters such as the method of obtaining a culture, type of sample, transfer method of sample to microbiology laboratory and method and methods used in the laboratory. In the present study, no anaerobic agent was isolated, and it was thought that this might be associated with the difficulties mentioned above in isolation.

The probability of the isolation of GRAM-negative agents is higher in patients who have used extended spectrum antibiotics previously [16]. Similarly, in the present study, in 33.3% of DFI attacks with GRAM positive agent growth, there was a history of previous antibiotic use, while antibiotic use history was present in 66.6% of attacks with GRAM negative agent growth The difference between them was found to be statistically significant (p=0.009).The results obtained in the present study support the idea that previous history of antibiotic use increases the probability of GRAM-negative agents in DFI's.

In antibiotic selection at the onset of DFIs, care should be taken to cover commonly encountered microorganisms and severity of infection, and in patients hospitalized previously, local sensitivity results of the hospital (if present) should be taken into consideration [17]. In the present study, in DFI attacks, ampiric antibiotic treatment was instituted immediately after microbiological sampling. In the evaluation of empirical treatments, it was seen that improvement was observed in 34 of 48 patients (70.8%) who continued ampiric treatment unchanged, while improvement occurred in 19 of 32(59%) patients whose treatment regime was replaced with another(p=0.02). This result indicates the importance of suitable empiric antibiotic treatment in the prognosis of the patient. The suitability of the antibiotic agent chosen at the onset of treatment to the probable agent significantly increases the likelihood of recovery.

In the approach to DFI's, where antibiotic treatment and wound care are present, early surgical intervention in infected ulcer is significant. Surgical treatments for these patients include a wide range of simple debridement of soft tissues, ranging from significant amputation. [17] In our study, the rate of recovery was significantly higher in patients who underwent surgical treatment with concomitant antibiotic therapy. (Tablo EKLENEBİLİR). When we look at the studies conducted in our country, it was reported that the rate of amputation was 37% in the follow-up of 574 patients who applied between 1998 and 2008 in the study of Yeşil et al. [18]. Again in our country, Durgun and friends in their study of patients with DAI amputation rate was 33.3% [19]. In our study, the rate of amputation was 33.75% by the literature.

In conclusion; in the follow-up patients at an advanced age and of the male sex, it is essential to inform patients about DFI that may involve toes and to warn patients before lesion development so that the development of DFI can be prevented. In patients who refer after the development of DFI, especially if the stage of the wound is advanced, GRAM negative agents should be taken into account in empiric treatment, and due to the varying spectrum of agents, the repetition of similar studies periodically is of great significance.

Competing Interests

The authors declare that they have no competing interests.

Funding

References

1. Aydoğan Ü, Akbulut H DY. Diyabetik Ayak. TAF Prev Med Bull. 2010;9:375–82.

2. Konya MN. Diyabetik ayak yaralarında negatif basınçlı yara kapama sistemi etkili midir ? Retrospektif karşılaştırmalı çalışma, Kocatepe Tıp Dergisi 2018;19:48-53.

3. Saltoglu N, Kılıçoglu Onder, Baktıroglu S, Osar-Siva Z, Aktas S, Altindas, et al. Diyabetik ayak yarasi ve Infeksiyonunun tanisi, tedavisi ve Onlenmesi: Ulusal uzlasi raporu. Klimik Derg. 2015;28:2–34.

4. Zukic E, Gojak R, Novakovic A, Gazibera B. Predictive role of preventive measures in preventing the progression of diabetic foot. Mater Sociomed. 2015;27:234–6.

5. Saltoglu N, Yemisen M, Ergonul O, Kadanali A, Karagoz G, Batirel A, et al. Predictors for limb loss among patient with diabetic foot infections: An observational retrospective multicentric study in Turkey. Clin Microbiol Infect. 2015;21:659–64.

6. Riaz M, Miyan Z, Zaidi SI, Alvi SFD, Fawwad A, Ahmadani MY, et al. Characteristics and Outcomes of Subjects With Diabetic Foot Ulceration. Diabetes Care. 2012;35:63–63.

7. Pitter D, Wyssa B, Herter-Clavel C, Kursteiner K, Vaucher J, Lew D. Outcome of diabetic foot infections treated conservatively. Arch Intern Med 1999; 159(26): 851-6.

8. Schaper NC. Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in research studies. Diabetes Metab Res Rev. 2004;20:90–5.

9. Ertugrul BM, Ozturk B, Oncul O, Tulek N, Willke A, Sacar S, et al. A prospective, multi-center study: Factors related to the management of diabetic foot infections. Eur J Clin Microbiol Infect Dis. 2012;31:2345–52.

10. Mutluoglu M, Sivrioglu AK, Eroglu M, Uzun G, Turhan V, Ay H, et al. The implications of the presence of osteomyelitis on outcomes of infected diabetic foot wounds. Scand J Infect Dis. 2013;45:497–503.

11. Lipsky BA, Sheehan P, Armstrong DG, Tice AD, Polis AB, Abramson MA. Clinical predictors of treatment failure for diabetic foot infections: data from a prospective trial. Int Wound J. 2007;4:30–8.

12. Frykberg RG, Armstrong DG, Giurini J, Edwards A, Kravette M, Kravitz S, et al. Diabetic foot disorders: a clinical practice guideline. American College of Foot and Ankle Surgeons. J Foot Ankle Surg. 2000;39:1–60.

13. Perim MC, Borges J da C, Celeste SRC, Orsolin E de F, Mendes RR, Mendes GO, et al. Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections. Rev Soc Bras Med Trop. 2015;48:546–54.

14. Chaubey M, Shenoy S. Occurrence of TEM, SHV and CTX-M β lactamases in clinical isolates of Proteus species in a tertiary care center. Infect Disord Drug Targets. 2018;18(1):68-71.

15. Huang Y, Cao Y, Zou M, Luo X, Jiang Y, Xue Y, et al. A Comparison of Tissue versus Swab Culturing of Infected Diabetic Foot Wounds. Int J Endocrinol. 2016;8198714.

16. Motta RN, Oliveira MM, Dias AM, Forti AC, Carvalho CBM. Plasmid-Mediated Extended-Spectrum β-lactamase-Producing Strains of Enterobacteriaceae Isolated from Diabetes Foot Infections in a Brazilian Diabetic Center. Braz J Infect Dis. 2003;7:129–34.

17. Akcay S, Satoglu I, Harman E, Kurtulmus A, Kazimoglu C. A Retrospective analysis of amputation rates and co-morbidity in patients with Diabetic Foot Ulcer. Med Sci. 2012;1:331–40.

18. Yesil S, Akinci B, Yener S, Bayraktar F, Karabay O, Havit-cioglu H, et al. Predictors of amputation in diabetics with foot ulcer: single center experience in a large Turkish cohort. Hormones. 2009;8:286–95.

19. Durgun O, Durgun AG, Ersoy CÖ, Almacioğlu S, Karadayi D, Özkaya G, et al. Retrospective Analysis of the Factors Determining Amputation Necessity in Patients with Diabetic Foot Advanced. Uludağ Üniversitesi Tıp Fakültesi Derg. 2012;38:59–62.