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Original Article

Microbial Infections in Diabetic Foot Ulcers at Al-Azhar University Hospital, New Damietta, Egypt

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ABSTRACT

Background: Worldwide, diabetes mellitus [DM] continues to be a major source of morbidity and mortality and is a critical public health issue. Diabetic foot ulcers are significant sources of distress and discomfort for many diabetic patients, with frequent resistance to treatment**.**

Aim of the work: This study's objective was to assess the frequency of microbes that cause diabetic foot ulcers, in order to reduce morbidities associated with this condition.

Patients and methods: One hundred diabetic patients with infected foot ulcers who were undergoing surgery in the outpatient clinic at Al-Azhar University Hospital in New Damietta were included in this study. Samples were obtained from each patient for microbiological testing and antibiotic sensitivity.

Results: The most found organism was staphylococcus aureus [19%] among gram-positive and E. coli [13%] among gram-negative, and longer diabetes duration and T1DM were significant risk factors for microbial growth.

Conclusion: From the results of the study we can conclude that the most found organism was staphylococcus aureus among gram-positive and E. coli among gram-negative. Longer diabetes duration and T1DM were significant risk factors for microbial growth.

Keywords: Microbial; Infections; Diabetic Foot; Ulcers; Organisms.

INTRODUCTION

Diabetes mellitus [DM], a major global source of morbidity and mortality, is a severe public health concern. Foot infection is the most typical diabetes-related hospitalisation cause, which is a serious consequence of diabetes that can eventually induce gangrene and lower extremity amputation [1, 2]. In 2015, the number of persons worldwide who had diabetes mellitus increased to 8.8%, or 415 million people [3]. As a result, there are now more patients suffering from foot illness and other diabetic effects. According to epidemiological research, persons with diabetes account for up to 75% of lower-extremity amputations [LEAs] [4].

The presence of ulcers [superficial or deep] on inspection, indications of inflammation, such as cellulitis or purulent discharge, or evidence of necrosis, with or without osteomyelitis or systemic toxicity, were all considered indicators of diabetic foot infection [5].

As the frequency of diabetes mellitus increases, the issue of diabetic foot infection is getting worse. Lower limb amputation is 15 to 46 times more likely to occur in diabetes than in non-diabetics [6].

In the course of their lives, about one-fourth of diabetics may experience an ulcer, and up to half of these ulcers will become infected [7]. Each year, more than a million diabetic individuals require limb amputations [8].

The presence of multidrug-resistant microorganisms in diabetic foot ulcers can be influenced by a number of variables, such as inappropriate antibiotic administration, the chronic nature of the lesion, and frequent hospitalizations [9].

Peripheral neuropathy, macro and micro-angiography, which are common and cause ischemia of the foot tissues and wounds to become infected 5 times more frequently in diabetic patients than in non-diabetic ones, are significant contributing factors to the emergence of "Diabetic foot," which results in sensory impairment, deterioration of the intrinsic muscles of the foot and joint, and formation of foot deformities [7].

The most common complication of diabetic foot ulcers is infection, which significantly raises the risk of amputation. While mild infections are commonly monomicrobial, severe diabetic foot infections typically produce polymicrobial isolates. Three to five organisms may be cultured when a diabetic foot infection is really serious [7].

The Wagner classification divides tissue injury severity and depth into five categories. Wagner grades I and II have a predominance of aerobic bacteria [staphylococcus spp., streptococcus spp., and enterobacteriaceae] while grades III to V have a predominance of anaerobic bacteria [10]. Effective antibiotic treatment for these illnesses should contribute to a decrease in morbidity [11].

Due to the frequency of diabetes mellitus and the morbidity associated with diabetic foot infections, this study aims to evaluate the prevalence of microbial pathogens in diabetic foot ulcers.

PATIENTS AND METHODS

In this study, which was conducted in the outpatient clinic at the Al-Azhar University Hospital in New Damietta, 100 diabetic patients with infected foot ulcers who were receiving surgery were included.

Each patient had a thorough personal and clinical history taken, which included details on the type and duration of their diabetes, the length of each ulcer, any prior amputations or ulcers, as well as information on the location and size, depth, margin, colour, and grade of the ulcer, glycemic control status, use of oral hypoglycemic/insulin, and the type and duration of antibiotics they had been taking, which had to be stopped 48 hours before sample collection.

Microscopic examination: Direct film from samples was done and the organism was identified by a direct Gram stain.

Culture of Aerobic Bacteria: An incubator was used to keep the inoculated Brain heart infusion broth at 37 °C overnight. The following day, broth was used to make a smear for a Gram stain, and subculture was carried out on the appropriate culture media. For 24 hours, at 37 °C, the media plates and broth were incubated.

Culture of Anaerobic Bacteria: It took at least 48 hours for the injected Robertson boiled meat broth to get turbid. Smear was created using gram-stained broth. Subculture was carried out on a Blood Agar plate, and an indicator of anaerobic bacteria will be a metronidazole disc. In an anaerobic jar at 37 °C, for 48 hours, the blood agar plate was incubated anaerobically [12].

Blood culture: was done for every patient and positive isolate was identified.

Fungal culture: By inoculating the fungal isolates into Sabouraud's dextrose agar and incubating them for 3–4 weeks at 25 °C and 37 °C, the fungi were identified.

Antibiotic sensitivity: Antibiotic sensitivity was assessed using the Kirby Bauer disc diffusion method and the lowest inhibitory concentration strategy [MIC]. Standard microbiological techniques were used for isolation, identification, and susceptibility testing [13].

Statistical analysis: Data were checked, entered and analysed using SPSS version 23 for data processing. The following statistical methods were used for analysis of results of the present study. Data were expressed as number and percentage for qualitative variables and mean + standard deviation [SD] for quantitative one. Student "t" test for comparison of means of two independent groups. Mann Whitney test was used to calculate difference between quantitative variables in not normally distributed data in two groups. Chi- square test was used to find the association between row and column variables. Z-test for percentage: to compare percentage of outcome between the two groups. Odds ratio [OR] compares the odds or the risk that a disease will occur among individuals who have a particular characteristic or who have been expressed to a risk factor to the Odds that the disease will occur in individuals who lack the characteristic or have not been exposed. For all above-mentioned statistical tests done, the threshold of significance was fixed at 5% level [P-value].

RESULTS

Table [1] shows that patients' mean age was 48.56 ± 10.74 years with mean BMI of 27.35 ± 2.61 kg/m^2. 69% of the patients were males. 36% of the patients were smokers.

Table [2] shows that mean duration of DM 10.26 ± 5.14 years. Majority of patients were type 2 DM [64%] and 61% of the patients took oral antidiabetic drugs and 39% of the patients uses insulin.

Table [3] shows that the most prevalent grade was grade III [35%] followed by grade II [24%].

Table [4] shows that the most found organism was staphylococcus aureus [19%] among gram-positive and E. coli [13%] among gram-negative.

The majority of cases had positive results on culture results; comparison between patients on antibiotics and those with no history of antibiotic use revealed that positive culture was more frequent in case of no history of antibiotics [91.5% vs. 72.4%; P=0.012]. Also, there was a significant difference regarding Wagner classification in relation to antibiotic use.

Table [6] shows that longer diabetes duration and T1DM were significant risk factors for microbial growth.

Table [1]: Demographic data of the studied patients

Variable		Studied patients [n=100]
Age [years] Mean ±SD		56.48 ± 8.72
Sex	Male	69 [69%]
	Female	31 [31%]
Body mass index [kg/m^2] Mean ±SD		27.35 ± 2.61
Smoking		36 [36%]

Table [2]: Clinical characteristics among the studied patients

Variable		Studied patients [n=100]
Duration of Diabetes mellitus [years] Mean ±SD		10.26 ± 5.14
Diabetes mellitus type	Type I	36 [36%]
	Type II	64 [64%]
Medications	Oral hypo-glycaemic drugs	61 [61%]
	Insulin	39 [39%]

Table [3]: Wagner classification distribution among the studied patients

	Studied patients [n=100]	
	No.	**%**
Grade I	15	15%
Grade II	24	24%
Grade III	35	35%
Grade IV	17	17%
Grade V	9	9%

Table [4]: Organisms frequency and distribution according to culture among the studied patients

	Studied patients [n=100]	
	No.	**%**
Negative growth	14	14%
Staphylococcus aureus	19	19%
Staphylococcus epidermidis	6	6%
Staphylococcus hemolyticus	2	2%
Enterococcus faecalis	5	5%
Streptococcus	7	7%
E. coli	13	13%
Klebsiella pneumonia	9	9%
Pseudomonas	11	11%
Proteus	4	4%
Acinetobacter	2	2%
Candida albicans	3	3%
Others	5	5%

Table [5]: Clinical data according to history of antibiotic use among the studied patients

	History of AB use [n=29]		No history of AB use [n=71]		
	No.	**%**	**No.**	**%**	
Culture					
No growth	8	27.6	6	8.5	**.012**
Growth	21	72.4	65	91.5	
Wagner classification					
Grade I	7	24.1%	8	11.3%	**.015**
Grade II	12	41.4%	12	16.9%	
Grade III	5	17.2%	30	42.3%	
Grade IV	3	10.3%	14	19.7%	
Grade V	2	6.7%	7	9.9%	

Table [6]: Multivariate regression analysis of factors influencing microbial growth

	Odds Ratio	Wald	Sig.	95% CI
Longer diabetes duration	1.215	1.199	**0.023**	.139 - 2.679
Type I Diabetes mellitus	0.465	0.363	**0.047**	.039 - 5.592
Insulin	0.573	0.187	0.666	.046 - 7.167
≥ 1 month	3.725	0.555	0.456	.117 - 8.321

DISCUSSION

Some investigations have revealed that the presence of anaerobic microbes is related to deeper diabetic foot infections [DFIs]. Antimicrobial-resistant organisms are frequently observed in DFIs, which may be an indication of how frequently patients interact with hospital settings or as a result of repeated exposure to antibiotic treatments. Additionally, bacteria typically create biofilms that hinder immune system clearing and increase antimicrobial resistance; in one study, 78.2% of chronic wounds displayed biofilm development [14].

Regarding demographic data; the mean age was 48.56 ± 10.74 years with mean BMI of 27.35 ± 2.61 kg/m^2. 69% of the patients were males. 36% of the patients were smokers. Our results were supported by study of **Mashaly *et al.*** [15] as they reported that the present study included 104 type 2 diabetic patients who presented with foot ulcers. There were 41 women and 63 men. They were between the ages of 42 and 66. While in the **Anafo *et al.*** [16] trial, sixteen participants included one hundred [100] people with active diabetic foot ulcer [DFU]. The majority of participants [57.0%] were female [54.0%] and above the age of sixty. In contrast, **Abd Al Hamead *et al.*** [17]'s study had 75 patients, with a male to female ratio being nearly equal at 37 to 38. The median age was 48 years, with ages ranging from 27 to 72. The age group of 41–60 years and 51–60 years had the highest rates of diabetic foot infections, respectively.

The present study showed that mean duration of DM 10.26 ± 5.14 years. Majority of patients were type 2 DM [64%] and 61% of the patients took oral antidiabetic drugs and 39% of the patients uses insulin. Our results were supported by study of **Ismail *et al.*** [18] According to their research, a total of 120 DFI patients were examined; the average age was 56.1 9.9 years, with a male to female ratio of 1.1:1. Of all the patients , 108 [90%] had type 2 DM, whereas only 12 [10%] patients had type 1 DM, and more than half of them [64; 53.3%] were smokers. The duration of diabetes ranged from 0.4-32 years with a mean of 14.6±6.4 years.

The present study showed that as regard Wagner classification distribution among the studied patients; the most prevalent grade was grade III [35%] followed by grade II [24%]. In accordance with our results study of **Ismail *et al.*** [18] as they reported that DFUs were graded according to Meggitt Wagner's grade into grade 2 in 30 patients [25%], grade 3 in 60 patients [50%], and grade 4 in 30 patients [25%]. Whereas in the study of **Abd Al-Hamead *et al.*** [17] regarding Wagner classification distribution among the studied patients; Second grade was the most common [26.7%], followed by third grade [24%]. Aerobic gram-positive cocci, particularly Staphylococcus aureus, are the main microbiological causes of diabetic foot infections [DFIs] in developed [primarily North American and European] countries, and over the past 15 years, the prevalence of methicillin-resistant S. aureus [MRSA] has increased significantly. Additionally, recent research has shown that the aetiology of DFIs varies significantly among global locations. Studies from those locations show that aerobic gram-negative bacteria, particularly Pseudomonas aeruginosa, are far more prevalent in warm temperatures in Asia and Africa than in Western nations. Recently, it has been discovered that gram-negative microorganisms possess enzymes like extended-spectrum beta-lactamases or carbapenemases that render them incredibly resistant to antibiotics, despite the fact that they are often sensitive to them. Polymicrobial illnesses are also rather typical everywhere [19].

The current study showed that as regard Organisms frequency and distribution according to culture among the studied patients; the most found organism was staphylococcus aureus [19%] among gram-positive and E. coli [13%] among gram-negative. Our results were supported by study of **Smith *et al.*** [20] They noticed that while only 55% of the patients had good growth on conventional laboratory culture, 75% of the patients had positive growth on 16S AS [16S amplicon sequencing] [41 unique taxa, representing 82 different operational taxonomic units [OTUs]]. Corynebacterium spp., Anaerococcus spp., and Peptoniphilus spp. were the most common bacteria discovered in all ulcers, with Staphylococcus spp. being introduced in new ulcers. In 72% of samples with positive cultures, S. aureus was identified. Additionally, **Anafo *et al.*** [16] found that coagulase-negative Staphylococci [CoNS] [54.0%] were the most common bacteria found in the participant's foot ulcers. Following S. aureus and Pseudomonas spp., which each recorded a prevalence of 19% [n = 19], Escherichia coli came in second with a prevalence of 24.0%. MRSA infection rates in the ulcers ranged from 6% to 6%, with 31.6% of the individuals having S. aureus foot ulcer infections. Furthermore, **Banu *et al.*** [21] showed that there were no polymicrobial illnesses seen. In all, 20 organisms [24.4%] and 62 organisms [75.6%] were, respectively, gram-positive and gram-negative. Staphylococcus aureus and Escherichia coli were the most frequently isolated species [24.4% each], followed by Citrobacter sp. [12.1%], Klebsiella oxytoca [12.1%], Pseudomonas aeruginosa [17.1%], and Proteus sp [9.8 per cent]. However, in the study of **Mashaly *et al.*** [15] the most frequently isolated species were K. pneumoniae [22, 26.8%], S. aureus and CoNS [18, 22% for each], and P. mirabilis [14, 17.1%], followed by P. aeruginosa [6, 7.3%]. On the other hand, each of the E. coli and Raoultella ornithinolytica was isolated from only 2.4% of the DFU cultures. S. epidermidis, S. haemolyticus, S. hominis, and S. simulans accounted for 38.9%, 27.8%, 22.2%, and 11.1% of the isolated CoNS, respectively. In addition, **Al-Joufi *et al.*** [22] 48.5% of the patients had polymicrobial diseases, according to the data.

Anaerobes [7.9%] and Gram-negative pathogens [38.6%] were outnumbered by the discovered Gram-positive pathogens [46.7%]. The most common infections were caused by S. aureus [22.2%], methicillin-resistant S. aureus [7.7%], Enterococcus spp. [12.8%], Pseudomonas aeruginosa [9.4%], E. coli [7.9%], and Klebsiella spp. Vancomycin and clindamycin had no effect on Gram-positive bacteria in the study by **Al-Joufi *et al*.** [22]. Ipenem and meropenem, however, showed strong efficacy against the isolates that were Gram-negative. In the study of **Palomo *et al*.** [23] detected oxacillin-sensitive S. aureus in 47% of patients, ampicillin-sensitive enterococci in 89% of cases, and coagulase-negative staphylococci in 20% of cases. With P. aeruginosa being 76% susceptible to ceftazidime and meropenem, the susceptibility profile of Gram-negatives was excellent. Except for K. pneumoniae, which exhibited 75% meropenem susceptibility, the other predominant Enterobacterales were highly susceptible to ceftazidime, piperacillin-tazobactam, and meropenem. Additionally, **Ismail *et al*.** [18] reported that 86 patients [87.7%] had isolates that were multidrug and extensively drug resistant. The most effective antimicrobial drugs against Gram-positive bacteria were vancomycin, teicoplanin, and linezolid, whereas colistin, the antibiotics piperacillin-tazobactam, imipenem, and meropenem were efficient against Gram-negative bacteria. Amikacin, tigecycline, and meropenem for gram-negative bacteria [GNB], and linezolid and vancomycin for staphylococci, were the most effective antibiotics, according to **Mashaly *et al*.** [15] Additionally, the most effective antibiotics against Gram-negative bacteria were found to be imipenem and amikacin, while vancomycin was most effective against Gram-positive bacteria, according to **Abd Al-Hamead *et al*.** [17].

Our results showed that as regard Multivariate regression analysis of factors influencing microbial growth; longer diabetes duration and T1DM were significant risk factors for microbial growth. In the study of **Mashaly *et al*.** [15] peripheral neuropathy, hospital admissions within the previous month, Extended-spectrum beta-lactamase [ESBL]-producing organism infections were found to be substantially correlated with prior antibiotic use, DM duration of 10 years, and past antibiotic intake. On the other hand, peripheral neuropathy was the only significant independent risk factor for DFUs to be infected with ESBL producers that was discovered [adjusted OR = 15.5, 95% CI = 2.104-114.28]. **Khalifa** [24] The presence of smoking [P = 0.040], poor glycemic control [HbA1c cut-off of 10%] [P = 0.010], peripheral neuropathy with lost ankle reflex [P = 0.0001], peripheral arterial disease [P = 0.0001], and previous ulcer location [P = 0.050] were also found to be significant independent potential risk factors for the recurrence of foot ulcers.

Conclusion: From the results of the study we can conclude that the most found organism was staphylococcus aureus among gram-positive and E. coli among gram-negative. Longer diabetes duration and T1DM were significant risk factors for microbial growth. The abundance of anaerobic bacteria has important implications for treatment as it suggests that the microbiome of each ulcer and that, although diverse, are not distinctly different from one another with respect to new or recurrent ulcers. Therefore, when considering antibiotic therapy, the duration of current ulceration may be a more important consideration than a history of healed ulcer. We recommend the combination between microbial infections in diabetic foot ulcers and other diagnostic methods for better accuracy.

Conflict of interest and Financial Disclosure: None

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