

STUDY TO DETERMINE THE BACTERIOLOGICAL ANALYSIS OF DIABETIC FOOT ULCERS

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ABSTRACT

Aim: This study was conducted to evaluate the bacterial flora according to the Wagner classification of the diabetic foot disease. Determine aerobic bacterial pathogens and their drug sensitivity model from their ESBL (Extended Spectrum Beta-Lactamase) production for isolation. **Methods:** A prospective study was conducted among diabetic patients with diabetic foot infection in a tertiary care hospital in the orthopedic department. All recorded cases were classified according to the Wagner classification and socio-demographic data were recorded. Ulcer samples were collected and processed for pathogen isolation and identification according to standard CLSI (Clinical and Laboratory Standards Institute) guidelines. The isolates were sensitized to antibiotics and ESBL production was demonstrated according to standard guidelines. **Place and Duration:** In the Orthopedic Department, Al Qassimi Hospital Sharjah, United Arab Emirates (UAE). for one-year duration from March 2019 to March 2020. **Results:** The study included 370 cases in which Wagner's grade 3 ulcers predominated. The majority was male, the common age group was 46-55 years, and the mean age was 55.3 ± 1.4 years. The most common disease was peripheral arterial disease and the second common problem was peripheral neuropathy, both were significantly associated with the development of diabetic foot ulcers. Methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa were the most common isolates. Vancomycin, clindamycin and linezolid shows maximum susceptibility against MRSA. Meropenems and piperacillin-tazobactam showed maximum susceptibility against gram-negative strains. The frequency of ESBL producers in the study was 16.24%. **Conclusions:** Diabetic foot infections are very serious problem in diabetic patients and require appropriate treatment. The outcome of an infection depends on the classification of the ulcer according to the Wagner classification as well as the nature of the infection, whether it is due to single organism or multi-organism. Our study highlights and recommends that proper multicenter studies are required to evaluate the proper antibiotic therapy for diabetic foot ulcers and to apply appropriate antibiotic treatment to reduce the incidence and growth of multi-drug resistant microbes.

Key words: Diabetic Foot Infections, Diabetic Foot Ulcer, Staphylococcus Aureus, ESBL, Pseudomonas Aeruginosa, Wagner's Classification.

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INTRODUCTION

Diabetes is recognized worldwide one of the rapidly growing non-communicable diseases requiring the highest priority¹⁻². The incidence of diabetes has increased tenfold worldwide, from 1.2% to 12.1% between 1971–2000, and continues to rise. The annual cost of diabetes in UAE was approximately \$ 38 billion. 10-25% of diabetics in America in 2011, develop foot infections [DFI] during periods of illness, ranging from simple calluses to severe osteomyelitis. Diabetic foot injuries are an important medical, social and economic problem and the main cause of hospitalization of diabetic patients. The main cause of DFI is peripheral neuropathy, affecting 30% of the diabetic population, leading to loss of protective pressure and pain sensation with limited joint mobility⁴. The presence of macrovascular disease and accompanying peptic ulcer infection increases the likelihood of lower limb amputation. Treatment of microbial infections caused by diabetic foot ulcers is difficult due to a number of associated factors in the response, including general glycemic control, related complications

such as vascular disease and neuropathy⁵⁻⁶. Ulcer depth is an important determinant of the outcome of a diabetic foot ulcer. Various wound classification systems are used to assess the severity of diabetic foot ulcers which include area, depth, presence of neuropathy, ischemia, etc. However, an easy-to-use classification system that provides a uniform definition of ulceration and infection will guide the appropriate treatment plan strategy and predict outcome and amputation⁷. The Wagner classification system is the most widely used classification system for diabetic foot ulcers. Most authors assume that the treatment of infections requires the identification of the pathogenic microbial flora and the initiation of appropriate antibiotic therapy in accordance with the susceptibility report⁸. Most of these infections are multi-microbial and the pathogens depend on metabolic factors, foot hygiene and the body's immune pattern. The development of multi-drug resistance is an important factor in preventing recovery from DFI⁹.

This study was conducted to evaluate the bacterial flora according to the Wagner

classification of the diabetic foot. Determine the aerobic bacterial pathogens and their drug sensitivity model in relation to their ESBL production for isolation.

METHODS

The study was held for one-year duration from March 2019 to March 2020. The study included all patients who came to the orthopedic department for diabetic foot. The cases included in the study were confirmed by history and laboratory tests as diabetes. All cases were admitted to the ward and followed until discharge. Associated complications (intermittent claudication with or without peripheral pulse) duration of diabetes, duration of ulceration, type of ulcer, glycemic control, signs of peripheral ischemia were recorded. Ulcer size was measured by multiplying the longest and widest diameters and expressed in square centimeters. Ulcer assessment and Wagner classification were performed (Table 1). Ulcers were classified as infected if there was inflammation or purulent discharge with symptoms of lymphangitis / lymphadenopathy or edema. Based on suggestive results from radiographs and blood investigations, all cases were followed up.

Microbiological examination

Samples were taken from the ulcers after the site was thoroughly rinsed with sterile normal saline and cleaned. A sterile swab was used to collect the sample with a sterile spoon at the base of the ulcer or deep inside the wound. The obtained soft tissue samples were directly processed to detect aerobic bacteria. All samples were processed according to standard guidelines and isolates were isolated and identified according to Clinical and laboratory standards institute (CLSI) guidelines. Antibiotic susceptibility testing of aerobic bacterial isolates was performed using the standard Kirby-Bauer disc diffusion method in accordance with CLSI guidelines. Gram-negative bacterial isolates were tested for ESBL production by double disc diffusion, methicillin resistant oxacillin disc (1 Table II shows the demographic features of the patients.

µg) and oxacillin screen agar (6 µg / ml). Vancomycin resistant isolates were also detected using Vancomycin Screen Agar (6 µg) / ml according to the standard guidelines recommended by CLSI.

Statistical analysis

All data was analyzed on SPSS version 20. Quantitative variables are expressed as mean ± SD and quantitative variables as percentages.

RESULTS

In our study, a total of 386 patients attended the diabetic foot clinic. Those patients who met inclusion criteria were admitted for this study. During hospitalization, basic socio-demographic features, characteristics of foot ulcers and ulcer classification were made. The socio-demographic features included gender, age, duration of diabetes, type of diabetes, related risk factors such as HbA1c, smoking, alcoholism, hypertension, and a long history of DFI (Table:2). Risk factors for the development of foot ulceration, such as peripheral neuropathy, peripheral arterial disease, ulcer size and ulcer site, have been evaluated in a clinical trial and reported.

Table 3 summarizes the risk factors in the cases studied. Overall glycemic control was poor in 60.3% of the cases in the study, indicating HbA1c levels > 6.5%.

Table 4: Profile of bacteria isolated from diabetic foot infections.

Pseudomonas aeruginosa was the major isolate among Gram-negative with a frequency of 20.3% and *Klebsiella pneumoniae* 9.8%, *Escherichia coli*, *Acinetobacter baumannii* (6.6 and 8.9%). Antibiotic susceptibility results for Gram-positive strains are summarized in Table 5.

Table 5 summarizes the antibiotic susceptibility pattern of Gram-negative strains in the study.

Table 1: Wagner’s classification of diabetic foot disease.

Grades	Symptoms
Grade-0	High risk foot and No ulceration
Grade-1	Superficial ulceration
Grade-2	Deep ulcer (Cellulitis)
Grade-3	Osteomyelitis with ulceration or abscess
Grade-4	Gangrenous patches
Grade-5	Gangrene of entire foot

Table 2: Socio-demographic data of cases enrolled in study.

Data Character	Number	Percentage
Sex		

Male	258	69.7%
Female	112	30.3%
Age group (years)		
35-45	82	22.2%
46-55	156	42.2%
>56 years	132	35.7%
Type of diabetes		
Type-I	28	7.6%
Type-II	342	92.4%
Duration of diabetes		
≤ 5 years	67	18.1%
6-10 years	161	43.5%
>10 years	142	38.4%
Smoking		
Yes	224	60.5%
No	146	39.5%
Alcoholism		
Yes	214	57.8%
No	156	42.2%

Table 3: Risk factors of cases in the study.

Risk factor	Number	Percentage
HbA1c		
<6.5%	147	39.7%
>6.5%	223	60.3%
	370	
Hypertension		
Yes	310	83.8%
No	60	16.2%
	370	
Site of ulcer		
Fore foot	132	35.7%
Mid foot	62	16.8%
Hind foot	176	47.6%
	370	
Complications		
PN (peripheral neuropathy)	108	29.2%
PAD (peripheral arterial disease)	148	40.0%
Both	114	30.8%
Size of ulcer		
≤2.5cm	138	37.3%
≥2.5cm	232	62.7%
Duration of ulcer		
≤3 months	238	64.3%
>3 months	132	35.7%

Table 4: Profile of bacteria isolated from diabetic foot infections.

Name of the isolate	Number	Percentage
Gram positive organisms		
Methicillin sensitive-Staphylococci aureus	37	8.4%
Methicillin resistant Staphylococcus aureus	89	20.3%
Enterococcus faecalis	29	6.6%
Group B Streptococci	27	6.2%
Coagulase Negative Staphylococci	67	15.3%
Gram negative organisms		
<i>Escherichia coli</i>	29	6.6%
<i>Klebsiella pneumoniae</i>	43	9.8%
<i>Pseudomonas aeruginosa</i>	89	20.3%
<i>Acinetobacter baumannii</i>	39	8.9%
<i>Proteus sp</i>	17	3.9%
<i>Citrobacter sp</i>	27	6.2%

Table 5: Antibiotic susceptibility pattern of isolates in the study.

Antibiotic	Proportion susceptible (No) (%)					
	MSSA	%	MRSA	%	CONS	%
Penicillin	33	89.2%	49	55.1%	47	70.1%

Amikacin	33	89.2%	73	82.0%	63	94.0%
Ciprofloxacin	31	83.8%	64	71.9%	53	79.1%
Erythromycin	29	78.4%	65	73.0%	61	91.0%
Tetracycline	25	67.6%	63	70.8%	55	82.1%
Clindamycin	37	100.0%	85	95.5%	67	100.0%
Cotrimoxazole	25	67.6%	49	55.1%	51	76.1%
Netilmicin	35	94.6%	75	84.3%	63	94.0%
Linezolid	37	100.0%	89	100.0%	67	100.0%
Ticarillin	37	100.0%	89	100.0%	67	100.0%
Mupirocin	35	94.6%	85	95.5%	65	97.0%
Amoxyclovanic acid	33	89.2%	61	68.5%	59	88.1%
Vancomycin	37	100.0%	89	100.0%	67	100.0%

DISCUSSION

Diabetic foot infections are one of the most serious and difficult complications faced by a person with diabetes, along with other complications associated with uncontrolled glycemic control. Various classification systems are available for DFI classification, such as Wagner and the University of Texas¹⁰⁻¹¹. In this study, the Wagner classification was used to classify ulcers with DFI and assess the ulcer bacteriological profile by grade.

In this study, males are more dominant than females, as has been observed in many studies around the world¹². The male population is subject to more difficult work in the workplace than women, who are at greater risk of injury¹³. Men in our study accounted for 69.7%, which, like the Jeffcoate EJ results, reported a 67% higher incidence of DFI in men in the study report¹⁴. Most of the cases in our study were placed in grade 3 in our study, which is in line with the findings of Armstrong DJ and Shea JD that 48% of the ulcers were Grade 3 in their study¹⁵. However, the results of our study contradict the Mayfeild JA reports, which found 52% of ulcers to be second degree ulcers. As observed in many studies, patients with type II diabetes were common in our study and the incidence was around 95.4%, which is almost similar to many studies in India. In our study, DFU was more common in patients with diabetes > 10 years of age, and in Yonem A, the longer the duration, the greater the development of DFI. In our study, poor glycemic control with high HbA1c levels, long-term hypertension were important risk factors for the development of DFI and a statistically significant association with these risk factors was found¹⁶. Lavery LA and Oyibo SO reported similar results in their studies. In our study, the incidence of peripheral neuropathy was 25.4%, similar to Gershater MA, who reported 27% and 40% in their own study with peripheral arterial disease, which is close to 37% in our study. Our study found a statistically significant association with peripheral arterial disease, as in others. Other factors such as smoking, alcoholism, ulceration, and a history of DFI treatment did not have a significant association with the development of DFI in our study. Few studies have shown that cigarettes and ulcers in pressure zones such as the front and rear legs are the more common sites of development and are statistically significant. Most studies indicate that the size

and depth of the ulcer are important factors in determining the DFI score. Osteomyelitis is considered to be an important factor associated with high amputation risk and poor DFI outcomes¹⁷.

Multi-drug resistance is a serious problem among gram-negative isolates due to the production of extended-spectrum B-lactamases. In our study, the main producer of ESBLs was *Pseudomonas aeruginosa* (15%), followed by *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Most of the research conducted commonly deals with the development of drug resistance depending on the ESBL production observed in our study.

CONCLUSION

As a result, DFUs are a serious problem in uncontrolled diabetics and require appropriate treatment. The outcome of an infection depends on the classification of the ulcer according to the Wagner classification as well as the nature of the infection, whether it is a multi-organism or a single organism infection. This study looked at the bacteriological profile of diabetic foot infections according to the Wagner ulcer classification, and related risk factors for ulcer treatment and treatment outcomes. This study highlights and recommends that prospective multicentre studies are required to evaluate the appropriate antibiotic regimen for diabetic foot ulcers and to implement appropriate antibiotic treatment to reduce the incidence and development of multi-drug resistant microorganisms.

ETHICS APPROVAL: The ERC gave ethical review approval

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin

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