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PATTERN OF MICROORGANISM PRESENTATION AND THEIR INFLUENCE ON SURGICAL OUTCOMES FOLLOWING MANAGEMENT IN DIABETIC FOOT ULCERS

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ABSTRACT

Objective: To determine pattern of anaerobic microbial infection in patients with diabetic foot ulcers and the effect of those infections on surgical outcomes following management.

Study Design: Analytical (Descriptive) Study.

Setting: Department of Medicine, Hayatabad Medical Complex, Peshawar, Khyber Pakhtunkhwa.

Subjects and Methods: This study was conducted from 1st July 2023 to 31st December 2024 at the Department of Medicine, Hayatabad Medical Complex, Peshawar, Khyber Pakhtunkhwa. A total of 125 patients of both genders with DM Type II diabetic foot ulcers were included in the study. The following parameters were considered: age, gender, education, MR number, contact number, locality, duration of T2DM, outcomes, complications and culture reports from swabs. The data was added to Microsoft Excell and transferred to SPSS version 23.0 for data analysis. The Pearson chi-square test was utilized to measure the correlation. Results are shown in the form of description, tables and charts.

Results: The study recorded 100% infection rate among the diabetic foot ulcer subjects Age range in this study was from 20 to 80 years with mean age of 57.752±6.89 years. Pepto streptococcus was found in 22.4% patients, Bacteroides fragilis in 12%, Clostridium perfringens in 15.2% and E. coli was seen in 16%. In terms of outcomes following management, healing was seen in 80.8% patients, toe Amputation in 13.6% and limb amputation in 5.6%.

Conclusion: Pepto streptococcus and Bacteroides were the most common microorganisms found in culture swabs from diabetic foot ulcers and most patients from these microbes ultimately had to undergo amputation.

INTRODUCTION

Diabetes mellitus is among the most common non-communicable diseases. Type 2 diabetes mellitus is one of the most common public health issues worldwide and its incidence is on the rise, particularly in middle-income and low-income countries. (1) When associated with complications, type 2 diabetes can have a profound impact on the person with consequences also for society. Diabetes was previously thought to be a disease of the affluent and mostly prevalent in urban areas but due to urbanization change in nutrition and a more sedentary lifestyle for many people, it has affected middle-income and low-income nations, including Pakistan. (2) Among the various complications of diabetes, one of the most dreadful and difficult complications to treat is diabetic foot ulcers. The predisposing factors for diabetic foot ulcers are poorly controlled diabetes and peripheral neuropathy. (3) In such conditions these foot ulcers are prone to colonize with various organisms with a tendency to invade deeper tissues leading to gangrene. (4)

These infections usually polymicrobial involving multiple aerobic and anaerobic infections usually considered starting the empirical treatment. (5) Among anaerobic infections, Bacteroides, Pepto streptococcus, Pepto coccus are common organisms. (6) Many researchers have presented a picture of mixed infections with both aerobic and anaerobic bacteria. The antibiotic susceptibility pattern also shows a lot of variation among different geographical places and also with various periods of time. The multidrug resistant bacteria have been reported in many diabetic foot infections. (7,8). Since the presence of anaerobic organisms has been shown to be a risk factor for severe infections of diabetic foot ulcers as already described in literature, the emergence of resistance among anaerobic pathogens poses a problem in the choice of empirical antibiotic regimens. (9,10)

The prevalence of foot ulcers is reported to range from 4.0% to 10.0% in patients with diabetes, which suggests that lifetime risk of developing foot ulcers in these patients may be as high as 25.0%.(11) Of all the non-traumatic lower limb amputations, 8 out of 10 amputations are done in patients with diabetes, out of which 85.0% follow a foot ulcer.(5,9) Studies from Pakistan indicate that the prevalence of diabetes foot ulcers ranged from 4.0%(12) to 10.0%; the amputation rate in Pakistan is reported to be high ranging from 21.0%9 to 48.0%. (11)

In a study conducted on 160 patients about 73 of the patients had positive anaerobic microorganisms, in which Bacteroides species 21(28.8%), followed by Pepto streptococcus species 15(20.5%). In the same study the frequency of the Clostridium species was 4(5.5%) (12). The annual

incidence of foot ulcers in diabetes is approximately 2% in most Western countries, although higher rates have been reported in certain populations with diabetes, including Medicare beneficiaries (6%) and U.S. veterans (5%) (13). Although the lifetime risk of foot ulcers until recently was generally believed to be 15–25%, recent data suggest that the figure may be as high as 34% (14).

Many studies have reported on the microbiology of diabetic foot infections' over the past 25 years, but the role of anaerobes in the prognosis of these infections is particularly unclear.(14) The study would enlighten the patterns of anaerobic infection in Diabetic foot ulcer which would farther help the expert to know the prevalence of such infections and would be helpful in treatment.

MATERIALS AND METHODS

After attaining approval from the hospital ethical and review committee, the following study was conducted from 1ˢᵗ July 2023 to 31ˢᵗ December 2024 at the Department of Medicine, Hayatabad Medical Complex, Peshawar, Khyber Pakhtunkhwa. A total of 125 patients of both genders with DM Type II diabetic foot ulcers were selected through non-probability consecutive sampling techniques. The sample size of 125 was calculated with 95% confidence level, 4% margin of error and taking prevalence of Clostridium species as 5.5% (15) according to a latest study. All DM Type II Patients with diabetic foot ulcers, age 20-80 years of either gender with at least one year history of diagnosis of diabetes were included while those with prolonged steroids use and patients with chronic diseases were excluded. Peshawar. Patients were explained about the purpose and the benefits of the study, and a written informed consent was taken. The following parameters were considered: age, gender, education, MR number, contact number, locality, duration of T2DM, outcomes, complications and culture reports from swabs.

Initially 3cc venous blood sample was obtained by using disposable syringe. All samples were stored in sterile vials and were sent to the laboratory of the hospital for assessment of HbA1c. Reports were obtained and HbA1c level was noted, if HbA1c >7%, then poor glycemic control was labeled (as per operational definition).

After local debridement of devitalized tissue, the ulcer wound was scrubbed thoroughly with normal saline to remove surface colonizers. Sample collection was then done using sterile cotton swabs for all cases. Scrapings of the ulcer base were collected in a sterile manner where necrotic tissue is present. Pus was aspirated where appropriate (presence of any deep abscess). Anaerobic isolation was done when clinically suspected and for these the overlying and adjacent areas was carefully disinfected with 70% ethanol to eliminate contamination with indigenous flora. Then swabs and tissue scrapings were collected and immediately inoculated into the transport media. Specimens were transported in a sterile bottle for anaerobic studies in Robertson cooked meat (RCM) media.

The tubes were immediately overlaid with sterile liquid paraffin and transported to the lab without delay. The sample was inoculated onto 5% sheep blood agar plates and Bacteroides Bile Esculin Agar as the selective medium for identification of B. fragilis. Reduced methylene blue was used as the indicator. After incubation, the primary plates were examined for colony morphology, hemolysis, and pigmentation. The individual colonies were identified by Gram-stain and subculture to the purity blood agar plate. The following antibiotic discs were placed on the first quadrant of the purity plate. Vancomycin 5 µg, kanamycin 1000 µg and colistin sulfate

10 µg. Metronidazole 5 µg discs and nitrate discs were placed in the second quadrant. The plates were then incubated anaerobically as mentioned above for 48 h at 35°C. Required results were then obtained from labs and a pre-designed Performa was used to extract the data at time of discharge.

Data was entered and analyzed using SPSS version 23. Mean and SD were calculated for numerical variables like age, BMI, duration of diabetes, HbA1c levels and RBS level, Frequency and percentage were calculated for categorical variables like gender, glycemic status (good/poor), classes of the BMI index, Wegner grading, anti-diabetic used, antibiotic used, history of previous amputation and outcome. Data was stratified for dependent/outcome (isolated anaerobes) and independent (age, gender, BMI, glycemic control and other) variables. Post-stratification, chi-square test was applied to compare the results between the converted categorical variables. P-value ≤0.05 was taken as significant.

RESULTS

Age range in this study was from 20 to 80 years with a mean age of 57.752±6.89 years, BMI 29.368±3.38 Kg/m^2, duration of diabetes 4.808±1.47 years, HbA1c level 7.012±0.48%, RBS level 227.784±59.08 mg/dl, size of ulcer 2.648±2.648 cm^2, depth of ulcer 4.200±1.65 mm and duration of ulcer was 4.712±2.27 months. Male patients were 75.2% and females were 24.8%.

The Pepto streptococcus species was found in 22.4% patients, Bacteroides fragilis 12%, Clostridium perfringens 15.2% and E. coli was seen in 16% patients.

Healing was seen in 80.8% patients, Toe Amputation 13.6% and Limb Amputation was 5.6% as. Stratification of outcomes with respect to age, glycemic control and other variables are shown in Tables-4.

Table- 1: Mean ± SD of patients according to age, BMI, Duration of diabetes, HbA1c level, RBS level.

Demographics		Mean ± SD
1	Age (years)	57.752±6.89
2	BMI (Kg/m^2)	29.368±3.38
3	Duration of diabetes (years)	4.808±1.47
4	HbA1c level (%)	7.012±0.48
5	RBS level mg/dl	227.784±59.08

Table 2- : Frequency and %age of patients according to microorganism spp.

Microorganism species		Frequency	%age
1	Pepto streptococcus	28	22.4%
2	Bacteroides Fragilis	15	12%
3	Clostridium perfringes	19	15.2%

4	E. coli	20	16%
	Total	125	100%

Table-3: Frequency and %age of patients according to outcome.

Outcome		Frequency	%age
1	Healing	101	80.8%
2	Toe Amputation	17	13.6%
3	Limb Amputation	7	5.6%
	Total	125	100%

Table 4: Stratification of outcomes with respect to various variables.

Age	Healing	Toe amputation	Limb amputation	P-value
20-50	18(94.7%)	1(5.3%)	0(0%)	0.230
51-80	83(78.3%)	16(15.1%)	7(6.6%)	
Glycemic status				0.474
Poor	65(78.3%)	12(14.5%)	6(7.2%)	
Good	36(85.7%)	5(11.9%)	1(2.4%)	
Antibiotic use				0.353
Oral	74(84.1%)	10(11.4%)	4(4.5%)	
Injectable	27(73%)	7(18.9%)	3(8.1%)	
Wagner grade				0.653
1	5(100%)	0(0%)	0(0%)	
2	43(79.6%)	9(16.7%)	2(3.7%)	
3	43(81.1%)	5(9.4%)	5(9.4%)	
4	8(80%)	2(20%)	0(0%)	
5	2(66.7%)	1(33.3%)	0(0%)	

DISCUSSION
According to a study, the rise of the prevalence of diabetic mellitus is associated with the increasing problem of infections among diabetic patients. Diabetic foot infection accounts for 20% of hospital admission (16). Diabetic foot infection is generally polymicrobial and both aerobic anaerobic bacteria were isolated from these infections (17). In our study, Pepto streptococcus species was found in 22.4% patients, Bacteroides fragilis 12%, Bacteroides species 23.2%, Porphyromonas 11.2%, Clostridium perfringens 15.2% and E. coli was 16% in diabetic patients suffering from diabetic foot infection, which is like another study (18). Results of our study are also comparable with a study conducted on 160 patients, about 73 of the patients had positive anaerobic microorganisms, in which Bacteroides species 21(28.8%), followed by Pepto streptococcus species 15(20.5%). In the

same study the frequency of the Clostridium species was 4(5.5%). (16)

Staph aureus was regularly cultured from 26 samples obtained from patients in multiple analysis conducted by El-Tahawy[19], Abdulrazak et al[17]. Contrary to this E. coli has been observed in diabetic foot ulcers in research conducted by Ako-Nai et al[20] while Pseudomonas aeruginosa was seen by Shankar et al[24] and his colleagues in culture isolates. Some variables that can affect the source of infection in diabetic foot ulcers(DFIs) include the immune status of the patient, choice of empirical antibiotic given and sample collection methods[17,19,20,22]. The following study also isolated few samples of gram-positive cocci such as coagulase negative staphylococci and enterococcus from certain patients mimicking the findings of some researchers[19,23]. Patients infected with gram positive bugs had milder clinical presentations in terms of pain and fever while gran negative microbes were relatively more aggressive[17].

Circumstances became alarming when wounds became infected with an extended spectrum beta lactamase (ESBL) producing bacteria, which was reported to be 31.3% in the following study. Shoba et al reported the highest prevalence of ESBL in *Acinetobacter* spp. (50%) followed by *E. coli* (36%), *P. aeruginosa* (33%) and *Enterobacter* spp. (25%)[24]. Similar outcomes were seen by Zubair et al[25] in their research. One theory behind this might be the extended use of broad-spectrum antibiotics that may influence antibiotic resistance among organisms such as MRSA or vancomycin resistant enterococcus species (VRE) in diabetic foot infections.

Among gram negative organisms E. coli was also one of the regular pathogens isolated which is in accordance with the work of Hadadi Azar et al[26]. The pedigree of E. coli was sensitive to fluoroquinolones with some level of resistance observed to certain antibiotics as reported in international reviews[18,26]. Antibiotic resistant organisms have the potential to be transmitted from infected individuals with diabetic foot ulcers through health care staff[27]. Surprisingly Bacteroides fragilis has also been disclosed as a familiar anaerobe in the work of Shankar et al in diabetic foot ulcers[21]. In the following study the concentration of anaerobic bacteria was relatively less compared to other studies, the reason being most patients did not have any chronic infection as illustrated by Zubair et al[13] in his paper.

It was observed that linezolid and vancomycin were good choices to provide broad spectrum coverage against methicillin resistant staph aureus and enterococcus species. These drugs proved to be highly efficacious specifically for resistant bugs and as empirical treatment for diabetic foot ulcers. Development of resistant groups of bacteria made clindamycin and ciprofloxacin less responsive as empirical treatment for DFIs. The statistics showed that imipenem was also potent enough to be considered as alternative for empirical treatment. The study's diverse microbial profile and antibiotic resistance highlights the importance of obtaining culture materials from infected ulcers for microbial characterization and antibiotic susceptibility testing.

CONCLUSION

The study found that all the participants with diabetic foot ulcers were infected. Most anaerobic organisms were Pepto streptococcus species and Bacteroides species. Gram-negative bacteria predominated among isolates, according to this investigation. The necessity to monitor resistance is necessitated by the various antibiotic resistance of the bacterial isolates. Antibiotic susceptibility testing is recommended before treatment.

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