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Pattern of Anaerobic Microbial Infection in Diabetic Foot Ulcers and Its Outcome

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Abstract

Objective:
To determine pattern of anaerobic microbial infection in patients with diabetic foot ulcers and surgical implication and outcome.

Methodology:
This Descriptive Study was conducted in surgical D unit department of Surgery, Khyber Teaching Hospital, Peshawar from 5th October 2020 to 5th April 2021. A total of 125 patients, selected through non-probability consecutive sampling, for both genders with Diabetes mellites Type II having diabetic foot ulcers were included in the study. Required results were then obtained from labs and a pre-designed Performa was used to extract the data at time of discharge.

Results:
Age range in this study was from 20 to 80 years with mean age of 57.752±6.89 years. Pepto streptococcus species was found in 22.4% patients, Bacteroides fragilis 12%, Bacteroides spp 23.2%, Porphyromonas 11.2%, Clostridium perfringens 15.2% and E.coli was 16%. Healing was seen in 80.8% patients, Toe Amputation 13.6% and Limb Amputation was 5.6%.

Conclusion:
The study recorded 100% infection rate among the diabetic foot ulcer subjects. Peptostreptococcus spp and Bacteroides spp were mostly found anaerobic organisms.

Keywords: Diabetes Mellitus, Diabetic Foot Ulcers, Anaerobic Microbial Infection, Outcomes

Introduction

Diabetes mellites is a global public health problem and its incidence is on the rise especially as a non-communicable illness. Initially its was thought to be a disease of people belonging to the urban population but now due to a decrease in the regional and demographic gap between societies specifically related to changes in diet and a sedentary lifestyle, it's now prevalent in rural middle income communities as well [1]. If not treated on time, diabetes can have a significant negative influence on the individual and the society as a whole [2]. The American Diabetes association (ADA) formulated a criterion to diagnose diabetes mellites, which required a fasting plasma glucose hence it made It a bit bothersome [3, 4]. The breakthrough came when, in 2009, the international expert committee on diabetes suggested that glycated hemoglobin was a better indicator of chronic glucose exposure [5]. An amendment to this rule by ADA stated that an HbA1c level of 6.5% (48 mmol/mol) be used as the borderline value for final diagnosis of diabetes mellitus [6].

One alarming sequalae of uncontrolled diabetes mellites is diabetic foot which is often very difficult to treat. The pathological phenomenon behind this complication is microangiopathy, arthropathy and peripheral neuropathy [7]. If there is superimposed microbial infection of deeper tissues in the ulcer, that can lead to gangrene formation as well [8]. Multiple microorganisms both aerobic and anaerobic are responsible to cause such infections [9], the most prominent ones include Bacteroides, Pepto streptococcus, Pepto coccus [10] etc. Before culture and sensitivity confirms the true bug, patients are initially put on empirical antibiotic treatment. One indicator of such variation among the microorganisms is the antibiotic susceptibility pattern observed in various geographical locations. The multidrug resistant bacteria have been reported in many diabetic foot infections [11-12].

These factors have made it very difficult to decide upon the choice of empirical antibiotic regimens [13, 14].

The prevalence of foot ulcers among diabetics is estimated to range from 4% to 10% and some studies suggest the overall lifetime risk of developing this complication to be as high as 25% [15]. It has been observed that 80% of lower limb amputations are done in diabetics secondary to foot ulcers being neglected, not following trauma [5, 9]. The amputation rate due to diabetic foot ulcers in Pakistan is reported to range from 21% [9] to 48% [15].

In a study conducted on 160 patients about 73 of the patients had positive anaerobic microorganisms, in which Bacteroides spp 21(28.8%), followed by Peptostreptococcus spp 15(20.5%). In the same study the frequency of the Clostridium spp was 4(5.5%) [16]. In most Western nations, the annual incidence of foot ulcers among people with diabetes is around 2%, while greater rates have been noted in other populations with diabetes, including Medicare recipients. (6%) and U.S. veterans (5%) [17]. 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% [18].

Research has already been done microbiology of diabetic foot infections for over 30 years however the role of anaerobes in the overall prognosis of such patients is still not understood [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.

Objective

To determine pattern of anaerobic microbial infection in patients with diabetic foot ulcers and surgical implication and outcome.

Operational Definitions

Type 2 Diabetic: Fasting blood glucose >126mg/dl or random blood glucose >200mg/dl and history of taking anti-diabetic drugs for >1year

Poor glycemic control: It was labeled as poor if three-month HbA1c >7% at time of presentation.

Pattern of Anaerobes Isolated from Diabetic Foot Ulcer:

The following anaerobes were isolated from the diabetic foot ulcer which were used in study:

- Peptostreptococcus spp.
- Bacteroides fragilis
- Bacteroides spp.
- Veillonella spp.
- Porphyromonas spp.
- Clostridium perfringens
- E. Coli (Facultative Anaerobe)

Outcome:

- **Healing:** Clean and clear margins, no slough, no discharge, no necrotic tissue, and formation of granulation tissue
- **Toe amputation**: A person who had gone through toe amputation with head of meta tarsal at time of discharge.
- **Limb amputation:** A person who had gone through limb amputation either above knee or below knee at time of discharge.

Materials and Methods

Study Design:

Descriptive Study.

Setting:

Unit (D), Department of Surgery, Khyber Teaching Hospital, Peshawar.

Duration:

This study was conducted from 5th October 2020 to 5th April 2021.

Sample Size:

Sample size of 125 cases were calculated with 95% confidence level, 4% margin of error and taking prevalence of Clostridium spp as 5.5% [16] according to a latest study.

The formula used is $^{16}n = \frac{z^2 \times p(1-p)}{e^2}$ (CI=95%, p=0.139%, e=4%, z=1.96)

Sampling Technique:

Non-probability consecutive sampling

Sample Selection

Inclusion Criteria:

1. DM Type II Patients with diabetic foot ulcers, age 20-80 years of either gender.
2. At least one year history of diagnosis of diabetes.

Exclusion Criteria:

1. Patients with prolonged steroids use.
2. Patients with chronic diseases.

Data Collection Procedure

125 patients fulfilling selection criteria were enrolled in the study admitted to Department of Surgery, Unit (D), Khyber Teaching Hospital, Peshawar. Patients were explained about the purpose and the benefits of the study and a written informed consent was taken. Baseline parameters like age, gender, duration of diabetes, Anti-diabetic medications, and history of medical illness was noted.

Initially 3cc venous blood sample was obtained by using disposable syringe. All samples were stored in sterile vials and was 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). The body mass index (BMI) is the metric currently in use for defining anthropometric height/weight characteristics in adults and for classifying (categorizing) them into groups which is being categorized 4 different categories.

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 was 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 over laid 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. Incubation was done in anaerobic Gaspak jar at 35-37°C for 48 h. Reduced methylene blue was used as the indicator. After incubation, the primary plates was 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 for48 h at 35°C. Required results were then be obtained from labs and a pre-designed performa was used to extract the data at time of discharge.

Data Analysis

Data was entered and analyzed using SPSS version 23. Mean and SD was calculated for numerical variables like age, BMI, duration of diabetes, HbA1c levels and RBS level, Frequency and percentage was 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 as shown in Table-1.

Male patients were 75.2% and females were 24.8% as shown in Table 2.

Peptostreptococcus spp was found in 22.4% patients, Bacteroides fragilis 12%, Bacteroides spp 23.2%, Porphyromonas 11.2%, Clostridium perfringens 15.2% and E. coli was 16% as shown in Table 3.

Frequency and %age of patients according to glycemic status, Wegner grading, anti-diabetic used, and antibiotic used are shown in Table 4.

Healing was seen in 80.8% patients, Toe Amputation 13.6% and Limb Amputation was 5.6% as shown in Table-5. Stratification of outcomes with respect to age, glycemic control and other variables are shown in Tables 6-10 respectively.

Tables

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 gender

	Gender	Frequency	%age
	Male	94	75.2%
	Female	31	24.8%
	Total	125	100%

Table 3: 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	Bacteroides species	29	23.2%
4	Porphyromonas	14	11.2%
5	Chlostridium perfringes	19	15.2%
6	E. coli	20	16%
	Total	125	100%

Table 4: Frequency and %age of patients according to glycemic status, Wagner classification, antidiabetic used, antibiotic used

1	Glycemic Status	Frequency	%age
	Good	83	66.4%
	Poor	42	33.6%
	Total	125	100%
2	**Wagner Grade**	**Frequency**	**%age**
	1	5	4%
	2	54	43.2%
	3	53	42.4%
	4	10	8%
	5	3	2.4%
	Total	125	100%
3	**Antidiabetic Used**	**Frequency**	**%age**
	Oral	88	70.4%
	Injectable	37	29.6%
	Total	125	100%
4	**Antibiotic Used**	**Frequency**	**%age**
	Yes	22	17.6%
	No	103	82.4%
	Total	125	100%

Table 5: Frequency and %age of patients according to outcome

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

Table 6: Stratification of outcomes with respect to age

Age (years)	Outcomes			p-value
	Healing	Toe Amputation	Limb Amputation	
20-50	18(94.7%)	1(5.3%)	0(0%)	0.230
51-80	83(78.3%)	16(15.1%)	7(6.6%)	
Total	101(80.8%)	17(13.6%)	7(5.6%)	

Table 7: Stratification of outcomes with respect to Glycemic Status

Glycemic Status	Outcomes			p-value
	Healing	Toe Amputation	Limb Amputation	
Good	65(78.3%)	12(14.5%)	6(7.2%)	0.474
Poor	36(85.7%)	5(11.9%)	1(2.4%)	
Total	101(80.8%)	17(13.6%)	7(5.6%)	

Table 8: Stratification of outcomes with respect to Wagner Grade

Wagner Grade	Outcomes			p-value
	Healing	Toe Amputation	Limb Amputation	
1	5(100%)	0(0%)	0(0%)	0.653
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%)	
Total	101(80.8%)	17(13.6%)	7(5.6%)	

Table 9: Stratification of outcomes with respect to Antidiabetic Used

Antidiabeic Used	Outcomes			p-value
	Healing	Toe Amputation	Limb Amputation	
Oral	74(84.1%)	10(11.4%)	4(4.5%)	0.353
Injectable	27(73%)	7(18.9%)	3(8.1%)	
Total	101(80.8%)	17(13.6%)	7(5.6%)	

Table 10: Stratification of outcomes with respect to Antibiotic Used

Antibiotic Used	Outcomes			p-value
	Healing	Toe Amputation	Limb Amputation	
Yes	19(86.4%)	1(4.5%)	2(9.1%)	0.318
No	82(79.6%)	16(15.5%)	5(4.9%)	
Total	101(80.8%)	17(13.6%)	7(5.6%)	

Discussion

According to a study, the rise of the prevalence of diabetic mellitus is associated with the increasing problem of infections among diabetic patients. Especially diabetic foot infection accounts for 20% of hospital admission [19]. Diabetic foot infection is generally polymicrobial and both aerobic anaerobic bacteria were isolated from these infections [20]. In our study, Peptostreptococcus spp was found in 22.4% patients, Bacteroides fragilis 12%, Bacteroides spp 23.2%, Porphyromonas 11.2%, Clostridium perfringens 15.2% and E. coli was 16% in diabetic patients suffering from diabetic foot infection, which is similar to another study [21]. 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 spp 21(28.8%), followed by Peptostreptococcus spp 15(20.5%). In the same study the frequency of the Clostridium spp was 4(5.5%) [16].

Staph aureus was regularly cultured from 26 samples obtained from patients in multiple analysis conduced by El-Tahawy [22], Abdulrazad *et al* [20]. Contrary to this E. coli has been observed in diabetic foot ulcers in research conducted by Ako-Nai *et al* [23] 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 [20, 22, 23, 25]. 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 [22, 26]. Patients infected with gram positive bugs had milder clinical presentations in terms of pain and fever while gran negative microbes were relatively more aggressive [20].

Circumstances became alarming when wounds became infected with extended spectrum beta lactamase (ESBL) producing bacteria, which was reported to be 31.3% in the following study. Shoba *et al* reported highest prevalence of ESBL in *Acinetobacter* spp. (50%) followed by *E. coli* (36%), *P. aeruginosa* (33%) and *Enterobacter* spp. (25%) [27]. Similar outcomes were seen by Zubair *et al* [28] 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 spp (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* [29]. The pedigree of E. coli was sensitive to fluoroquinolones with some level of resistance observed to certain antibiotics as reported in international reviews [21, 29]. Antibiotic resistant organisms have the potential to be transmitted from infected individuals with diabetic foot ulcers through health care staff [30]. Surprisingly Bacteroides fragilis has also been disclosed as a familiar anaerobe in the work of Shankar *et al* in diabetic foot ulcers [24]. In the following study the concentration of anaerobic bacteria was relatively less compared to other studies, 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 highlight the importance of obtaining culture materials from infected ulcers for microbial characterization and antibiotic susceptibility testing.

Conclusion

The study found that all of the participants with diabetic foot ulcers were infected. Most anaerobic organisms were Pepto streptococcus species and Bacteroides species. Gram-negative bacteria predominated among the 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.

Declaration and Conflict of Interest

I declare that this article has been composed solely by the authors of this study and that it has not been submitted, in whole or in part, in any other journal for publication. There was no conflict of interest among the authors.

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