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Study of clinico-microbial profile of diabetic foot ulcers

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Abstract: Background: Diabetic foot ulcer (DFU) is a major complication of Diabetes Mellitus. The International Working Group on Diabetic Foot defines DFU as a "full-thickness lesion of the skin distal to the malleoli in a person with Diabetes Mellitus".
Objective: The objective of this study was to evaluate the clinico-microbial profile of patients with diabetic foot ulcers and the pattern of antimicrobial susceptibility.
Methods: This hospital-based, prospective observational study was conducted among patients with diabetic foot ulcers in the Department of General Surgery at Era's Lucknow Medical College & Hospital, Lucknow, after taking written informed consent.
Results: Pseudomonas was found in 39 (18.2%) cases, coagulase-negative Staph aureus was found in 23 (10.7%) cases, Acinetobacter was found in 80 (37.3%) cases, E. coli was found in 56 (26.1%) cases, Proteus spp was found in 13 (6%) cases, Staph aureus was found in 23 (10.7%) cases, Acetobacter was found in 1 (0.46%) case, and Enterococcus was found in 2 (0.93%) cases. A single organism was involved in 48 (47.1%) cases, while multiple organisms were involved in 54 (52.9%) cases in our study. In our study, 83 (81.3%) cases were gram-negative and 11 (10.7%) cases were gram-positive.
Conclusions: According to microbial culture, Pseudomonas, Klebsiella, E. coli, and coagulase-negative Staph aureus were the most commonly found organisms in diabetic foot ulcers. These findings suggest that appropriate management of diabetic foot ulcers should involve selecting an appropriate antimicrobial agent based on microbial culture and susceptibility testing.

Keywords: Clinico-microbial profile; DFU; HBA1C levels; Wagner's scale grade.

1. Introduction

Diabetes mellitus (DM) is a severe health issue and the leading cause of morbidity and mortality. According to estimates, someone dies every six seconds due to this terrible condition [1]. In low- and middle-income countries, there were reportedly five million diabetes-related fatalities in 2015 [2]. The diabetes epidemic is said to have its epicentre in Asia. The number of diabetics in South Asian countries was 80 million in 2017; by 2045, that figure is expected to rise to 151 million [3]. In India, the prevalence of diabetes mellitus is 8.8% among those aged 20 to 79 [4]. One of the most prevalent and dangerous consequences of diabetes mellitus is diabetic foot, which frequently results in hospitalization and disability [5]. The International Working Group on Diabetic Foot defines DFU as a "full-thickness lesion of the skin distal to the malleoli in a person with Diabetes Mellitus." In India, 4.54% of individuals with type 2 diabetes mellitus were discovered to have diabetic foot ulcers; of them, 46.1% had neuropathic, 19.7% had ischemic, and 34.2% had neuro-ischemic foot ulcers [6].

Patients who do not manage their diabetes properly are more likely to develop complications such as peripheral arterial disease (PAD), coronary artery disease, and gangrenous and non-gangrenous ulcers in their foot, where there is sensory loss due to neuropathy. The three factors that predispose to ulcers are neuropathy, PAD, and pressure overload [7]. About 15-25% of diabetics get diabetic foot ulcers as the most common infection, which primarily affects elderly individuals with poor diabetic control [8]. For diabetic foot ulcer patients, who are at a considerably higher risk, the worst-case scenario is when the consequences necessitate limb amputation. Each year, hundreds to thousands of DM patients experience this [9].

Studies on the types of foot infections and the responsible organisms in DM patients suggest that there is typically only one bacterium responsible for the illness, with Staphylococcus aureus and Streptococcus species being the most prevalent pathogens [10–12]. The infections with the worst prognosis, on the other hand, are those that have more than one causal pathogen. Escherichia coli, Klebsiella spp., and Pseudomonas spp. are a few of the species that are commonly reported [12].

The right care, the application of policies and procedures, patient counselling, and patient awareness of the potential complications of DM can considerably reduce the need for amputations, the length of hospital stays, and admissions. Prompt and timely interventions by healthcare professionals in DFU will result in a significant improvement in clinical outcomes. However, in our area, there is a lack of information available on this topic. Studies are necessary to lower the prevalence of diabetic foot ulcers, their costs, and to enhance patient quality of life.

Therefore, the goal of this hospital-based prospective observational study is to evaluate the bacterial profile of diabetic foot patients and the pattern of antimicrobial susceptibility in a tertiary care hospital setting.

2. Materials and Methods

This hospital-based prospective observational study was conducted among patients with diabetic foot ulcers in the Department of General Surgery at Era's Lucknow Medical College and Hospital, Lucknow after taking written informed consent. The study was approved by the institutional ethics committee and was conducted from February 2021 until the desired sample size of 102 cases was reached using consecutive sampling.

2.1. Inclusion criteria

Patients of either sex above 18 years of age diagnosed with diabetes mellitus and foot ulcers who provided consent were included.

2.2. Exclusion criteria

Malignant ulcers were excluded from this study.

2.3. Sample size

The sample size was calculated based on the prevalence of microorganisms among cases of diabetic foot ulcers using the formula:

$$n = \frac{z_{\alpha}^{pq}}{L^2},$$

where p=58.3% the prevalence of microorganisms among cases of diabetic foot ulcer as per a study [13] and q=100-p. The Type I error α was set at 5% for the significance level of 95%, with an allowable error L of 10% absolute for detecting the results with 80% power of the study, and data loss of 10%. The minimum sample size required was calculated to be 102.

2.4. Sampling technique

Consecutive sampling was used to recruit all available subjects sequentially until the desired sample size was reached, keeping in mind the given duration of the study and the patient flow in the setup.

2.5. Data collection

A pre-designed proforma was used to collect data from the study population. The study included patients with Diabetic foot ulcers admitted to the Department of General Surgery at Era's Lucknow Medical College & Hospital in Lucknow who met the selection criteria. Diabetic foot ulcers were classified according to Wagner's Grading/Classification of Ulcer during the physical examination.

2.6. Wagner's Grading/Classification of Ulcer

Diabetic foot ulcers were classified according to Wagner's grading/classification system, which includes the following grades:

- Grade 0 – pre-ulcerative lesion/healed ulcer
- Grade 1 – superficial ulcer
- Grade 2 – ulcer deeper to subcutaneous tissue exposing soft tissues or bone
- Grade 3 – abscess formation underneath/osteomyelitis
- Grade 4 – gangrene of part of the tissues/limb/foot
- Grade 5 – gangrene of entire one area/foot

2.7. Plan of Study

This was a prospective cross-sectional study that evaluated diabetic foot ulcer patients who were admitted to the Department of General Surgery.

After obtaining approval from the ethics committee of the hospital, 102 consecutive cases were included in the study. Patients or their parents provided written informed consent.

A detailed medical history was taken from each patient using a pre-designed proforma. A thorough physical examination and laboratory tests were conducted to record the following data for all subjects:

1. Complaints at the time of admission
2. Examination and laboratory findings
3. Culture and sensitivity findings

2.8. Statistical analysis:

Methods for analysis involved descriptive statistics such as mean, standard deviation and frequencies, and inferential statistics including correlation coefficient, independent sample t-test and Chi square test were used. The data was compiled in MS Excel, and other relevant softwares. The data was presented in table & graphs where ever applicable. Data was analyzed as per objectives. P value <0.05 was considered as significant. Inferences were drawn with the help of appropriate of significance.

3. Results

In this study, the age distribution of patients showed that the highest number of patients belonged to the age group of 50-59 years, comprising 34 (33.3%) of the total participants. Diabetic foot ulcers were found to be more common in men, with 89 (87.2%) male patients in the study. Most of the patients, i.e., 91 (89.3%), had Type-2 Diabetes Mellitus, and 67 (65.6%) of them used oral medications for diabetes management. The majority of patients with Diabetes Mellitus in the study, i.e., 81 (79.5%), had HbA1c levels between 6.4-9.

Among the microbial cultures obtained from the foot ulcers, Klebsiella was the most commonly isolated organism, found in 80 (37.3%) cases, followed by E. coli in 56 (26.1%) cases. The other microorganisms isolated were Pseudomonas in 39 (18.2%) cases, Staphylococcus aureus in 23 (10.7%) cases, coagulase-negative Staphylococcus aureus in 23 (10.7%) cases, Proteus species in 13 (6%) cases, Enterococcus in 2 (0.93%) cases, and Acetobacter in 1 (0.46%) case. In 48 (47.1%) cases, a single organism was involved, whereas in 54 (52.9%) cases, multiple organisms were involved. Among the isolates, 83 (81.3%) were gram-negative, and 11 (10.7%) were gram-positive.

In terms of the duration of the ulcer, 83 (81.4%) patients had ulcers lasting for more than two weeks. According to Wagner's scale, grade 3 ulcers were the most common, found in 38 (37.2%) patients. The most common type of infection causing foot ulcers was polymicrobial, i.e., caused by more than two organisms in two or more pus cultures of the same patient, which was found in 54 (52.9%) cases

Table 1. Distribution according to HBA1C Levels

Hba1c levels	No of patients(n=102)	Percentage
<5.7	0	0%
5.7-6.4	0	0%
6.4-9	81	79.5%
>9	21	20.5%
Total	102	100%

Table 2. Distribution according to duration of ulcer

Duration of ulcer	No of patients(n=102)	Percentage
≤2 weeks	19	18.6%
> 2 weeks	83	81.4%
Total	**102**	**100%**

Table 3. Distribution according to Wagner's scale grade

Grade	No of patients(n=102)	Percentage
Grade 1	6	5.8%
Grade 2	25	24.5%
Grade 3	38	37.2%
Grade 4	19	18.6%
Grade 5	14	13.7%
Total	**102**	**100%**

Table 4. Distribution according to type of organism involved

Organism involved	Number(n=102)	Percentage
Klebsiella	80	37.3%
Pseudomonas	39	18.2%
Coagulase negative staph.aureus	23	10.7%
E coli	56	26.1%
Proteus spps	13	6%
Enterococcus	2	0.93%
Acinetobacter	1	0.46%

Table 5. Distribution of cases according to nature of organisms involved

Nature of organism	Number(n=102)	Percentage
Gram -ve	83	81.3%
Gram +ve	11	10.7%
Mixed	8	7.9%
Total	**102**	**100%**

Table 6. Distribution of cases according to Infection type

Infection type	Number(n=102)	Percentage
Mono-microbial	48	47.1%
Poly-microbial	54	52.9%
Total	102	100%

4. Discussion

In the study, the age group with the most number of patients was 50-59 years old, accounting for 34 (33.3%) cases. Previous studies by Shankar Rao AG et al. [14], Ahmad S et al. [15], and Anand A et al. [16] reported mean ages of 59 years, 58.12 years, and 52.42 years, respectively, with age ranges varying from 24 to 92 years.

Of the study participants, 89 (87.2%) were males and 13 (12.8%) were females. Other studies by Shankar Rao AG et al. [14] and Anand A et al. [16] reported male-to-female ratios of 83.07% to 16.92% and 3.5:1, respectively.

Among the patients, 91 (89.3%) had type 2 diabetes while 11 (10.7%) had type 1 diabetes. Mohanty DP et al. [17] noted that foot ulcers were commonly observed in elderly men with type 2 diabetes (NIDDM).

In our study, 81 (79.5%) patients with diabetes mellitus had HbA1c levels between 6.4-9. Previous studies by Ahmad S et al. [15] and Khan DM et al. [18] reported mean HbA1c levels of 9.33% and >7.0, respectively.

Regarding the duration of diabetes mellitus, most patients (46) had a duration of less than 5 years, comprising 45% of the total. Shankar Rao AG et al. [14] reported an average duration of diabetes of 9.4 years, with 52.4% of patients having it for more than 10 years. Ahmad S et al. [15] reported an average age of onset of 12.3 years.

Regarding the duration of the ulcer, 83 (81.4%) patients had ulcers lasting more than 2 weeks. Shankar Rao AG et al. [14] reported that 62.5% of patients had ulcers for 3 months prior to visiting the hospital. Atmakuri D et al. [19] reported that diabetes mellitus was the most common comorbidity (n = 50) and that 30 (29.7%) of the total instances of secondary pyodermas were diabetic foot ulcers, with the majority of lesions being between 2 and 5 cm in size (n = 47) and lasting less than a week (n = 39) or between 1 and 6 weeks (n = 35).

As per Wagner's scale, 6 patients (5.8%) had grade 1, 25 (24.5%) had grade 2, 38 (37.2%) had grade 3, 19 (18.6%) had grade 4, and 14 (13.7%) had grade 5. Grade III was seen in 48% of patients, grade I in 16%, grade II in 14%, grade IV in 14%, and grade V in 8%.

According to Anand A et al. [16], amputation was required for 100% of patients with grades IV and V, 8.3% of patients with grade III, 4.16% of patients with grade II, and none of the patients with grade I. Patients with septic diabetic foot who were admitted with fever and a high Wagner's grade were more likely to harbor anaerobic infections. For wounds worse than Wagner's grade III, anaerobic coverage medications should be taken into consideration [20]. Wagner grades of the lesions ranged from Grades II to V, with Grade IV predominating, according to Amaefule KE et al. [21].

According to microbial culture, Pseudomonas was seen in 39 cases (18.2%), coagulase-negative Staphylococcus aureus was found in 23 cases (10.7%), Klebsiella was found in 80 cases (37.3%), E. coli was seen in 56 cases (26.1%), Proteus spp. was seen in 13 cases (6%), Staphylococcus aureus was seen in 23 cases (10.7%), Acinetobacter was seen in 1 case (0.46%), and Enterococcus was seen in 2 cases (0.93%).

Pseudomonas aeruginosa was the most common pathogen in 23.3% of cases (n=15), followed by Staphylococcus aureus, E. coli, Acinetobacter baumannii, and Klebsiella pneumoniae in 15.38% of cases each, and Burkholderia cepacia, which accounted for 10% of cases. 11 instances (16.92%) involved the isolation of several organisms, according to Ali SQ et al. [22].

Pseudomonas spp. were the most frequently reported bacteria (15.9%), while methicillin-resistant Staphylococcus aureus was the most frequently reported gram-positive bacteria (20.7%), according to Ahmad S et al. [15].

According to Anand A et al. [16], Proteus mirabilis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus were all recovered from the patients who had amputation.

According to Chaudhry WN et al., [23], Staphylococcus aureus was the most prevalent isolate (25%). Pseudomonas aeruginosa (18.18%), Escherichia coli (16.16%), Streptococcus species (spp.) (15.15%), Proteus spp. (15.15%), Enterococcus spp. (9%) and Klebsiella pneumonia were also identified as prevalent isolates.

Staphylococcus aureus and Pseudomonas aeruginosa were the two most frequently isolated bacterial pathogens, accounting for 25% and 18.18% of isolates, respectively, according to Gopi A et al. [24]. Of the samples tested, 21.3% (32/150) were positive for fungi, with Candida albicans accounting for 25% (08/32) of all isolated fungi.

According to Shanmugam P et al., [25], the three most prevalent isolates were Pseudomonas spp. (16%), Escherichia coli (14.6%), and Staphylococcus aureus (13.3%).

Pseudomonas aeruginosa (39.6%), Escherichia coli (17.46%), Acinetobacter species (15.41%), Proteus species (12.47%), and Klebsiella species (9.75%) were the most frequently detected isolates, according to Khan DM et al., [18].

According to Mukkunnath SN et al., [26], Pseudomonas was the most prevalent isolate, followed by Klebsiella (21.7%). The most common gram-positive organisms were Enterococcus (17.5%) and Staphylococcus aureus (16.6%).

In our study, 48 (47.1%) cases involved a single organism, while 54 (52.9%) involved multiple organisms. Shankar Rao AG et al., [14], observed that multiple organisms were isolated in 11 cases (16.92%). Mohanty DP et al., [17], observed that diabetic foot infections are polymicrobial in nature. Mukkunnath SN et al., [26], observed that mono-microbial infection was more common than poly-microbial infection

Out of 102 isolates, 83 (81.3%) were gram-negative and 11 (10.7%) were gram-positive, while mixed organisms were seen in 8 (7.9%) cases. According to Shanmugam P et al., [25], gram-negative bacilli were more prevalent (65.1%) than gram-positive cocci (34.9%). Similarly, Mukkunnath SN et al., [26], observed that gram-negative bacilli were more prevalent than gram-positive cocci.

Gram-positive organisms contributed to 11% and Candida spp. to 1% of the swab cultures. Ali SQ et al., [22], observed that isolated gram-positive bacteria were more common than isolated gram-negative bacteria. Ahmad S et al., [15], reported that 96 (66.2%) isolates were gram-negative bacteria, while 49 (33.8%) were gram-positive bacteria.

Atmakuri D et al., [19], observed that Methicillin Susceptible Staphylococcus aureus (n=38) among gram-positive bacteria and Pseudomonas aeruginosa (n=17) among gram-negative bacteria were the most common organisms isolated.

Prasant HA et al., [27], observed that Staphylococcus aureus (26%), E. coli (19.2%), and Pseudomonas aeruginosa (19.2%) were the most frequently isolated organisms. Mohanty DP et al., [17], reported that gram-negative bacteria comprised the major group of 73 (54.1%), followed by gram-positive bacteria 54 (40%), and fungus 08 (5.9%), which were observed on culture.

5. Conclusion

Based on the results of microbial culture, Pseudomonas, coagulase-negative Staphylococcus aureus, Klebsiella, Escherichia coli, Proteus, and Staphylococcus aureus were identified as the most commonly found organisms. Acinetobacter and Enterococcus were also observed. In our study, single organisms were involved in 48 (47.1%) cases while multiple organisms were involved in 54 (52.9%) cases. Gram-negative organisms accounted for 83 (81.3%) of the isolates, while gram-positive organisms accounted for 11 (10.7%). It is necessary to conduct further large-scale studies on microbiological studies in DFU to identify treatment response and recurrence rates. This will help to develop consensus guidelines for the effective management of diabetic foot.

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