

RESEARCH ARTICLE

CLINICO-BACTERIOLOGICAL PROFILE IN DIABETICS ADMITTED FOR FOOT ULCERS: RETROSPECTIVE OBSERVATIONS FROM A TERTIARY CARE HOSPITAL

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ABSTRACT: **Aim:** To study the clinical profile with co-morbidities of patients admitted with Diabetic foot ulcer (DFU) infection. Also, to study the microbiological flora causing the infection, along with antibiotic susceptibility pattern of the pathogens isolated from these ulcers. **Material and method:** A prospective study was carried out on 95 diabetic patients with foot ulcers for a period of 1½ years. The demographic details of the patients with Diabetic foot ulcer (DFU) along with Wagner's grade and treatment profile were also collected. The tissue sample were collected and processed as per standard microbiological procedure and antibiotic susceptibility testing was done as per Clinical & Laboratory Standards Institute (CLSI). The data was analyzed by percentage and correlated. **Results:** A total of 95 patients were included in the study with 83 males. The majority of patients with DFU were below the age of 40 years. Around 36.48% patients developed DFU following 6-10 years of diagnosed as Diabetes mellitus. Hypertension (26.88%) and neuropathy (20%) were the most common co-morbidity and complications among these patients. A total of 92 pathogens isolated from these patients, with Gram negative pathogens being predominant. The most common pathogens isolated were *Pseudomonas aeruginosa* (30%), followed by *Escherichia coli* (28.8%) and *Staphylococcus aureus* (72.5%) among Gram negative and Gram positive pathogens, respectively. Minimal resistance was noted for high end antimicrobials like Beta lactam- Beta lactamase inhibitors & carbapenems. But, for empirical therapy, fluoroquinolones and aminoglycosides may be considered as alternative for cephalosporins. **Conclusion:** Diabetic foot ulcer infection is mainly seen in individuals with > 5 years of diabetes and complications especially peripheral neuropathy and nephropathy. Gram negative bacteria, coliforms are predominant pathogens isolated. Fluoroquinolones and aminoglycoside group of antimicrobials can be considered for empirical therapy provided antibiogram is monitored on regular basis.

KEYWORDS: Diabetic foot infection, Diabetes mellitus, Wagner's grade, antibiogram

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INTRODUCTION:

India is considered as the Diabetic Capital of the world. It is estimated that in India, the people with Diabetes mellitus will be close to 69.9 million and 80 million by 2025 and 2030, respectively. Diabetic foot, is the most common and feared complication among the patients with Diabetes mellitus, greater than retinopathy, nephropathy, heart attack and stroke combined^[1,2]. In diabetic patients, when adequate care is not taken, the foot ulcer may progress to severe infection / necrosis, finally leading to amputation of the limb. The Diabetic foot is associated with high morbidity and mortality^[2], and also immense consequences which affects the psycho-social and economic status of the patient and their family. In India, the Diabetic foot ulcer (DFU) and associated condition is the second most common cause of lower limb amputations. In diabetic patients, foot disorders such as ulcerations, infection and gangrene are the leading causes of hospitalization^[3]. Billions of dollars is involved in direct medical expenditure as well as lengthy hospital stay and period of disability in diabetic foot treatment^[4].

The ulceration in Diabetic foot ulcer is multifactorial, even a trivial breach in the skin lining can lead to infection and gangrene. The major complications of diabetes mellitus which leads to DFU are mainly, peripheral neuropathy and vascular disease, which causes reduced sensation and vascular supply, especially to lower limb^[5]. Various studies, in animals and in-vitro, have demonstrated that the hyperglycemia-induced metabolic abnormalities and atherosclerosis are the major cause for peripheral neuropathy and vascular disease in Diabetes mellitus^[6]. These predisposing factors lead a trivial break in the skin to infection and gangrene, leading to amputation. It is very essential to categorize the patients who are at high risk to develop complications of DFU. Therefore, a detailed history and examination of the diabetic patient with regard to foot injury, clinical features and microbial flora contributing to ulcerations, needs to be studied

extensively, for optimal therapy and prevention of complications^[2]. Hence, this study was conducted to investigate the impact of various risk factors like neuropathy, nephropathy, bacterial infections, in the prognosis of patients with Diabetic foot.

MATERIALS AND METHODS:

A prospective study was carried out on 95 diabetic patients with foot ulcers for a period of one and half years at a tertiary care teaching hospital. Principles of convenience sampling were applied for collecting data in the study. Diabetic foot infection is defined as the presence of ulcers (superficial to deep) on examination or evidence of inflammation, i. e. cellulitis or purulent discharge or evidence of necrosis, with or without osteomyelitis or systemic toxicity. Diabetics were diagnosed on the basis of fasting plasma glucose of 126 mg/dl and above or if symptoms were present (i. e polyuria, polydipsia, polyphagia, weight loss, and blurred vision) and a random plasma concentration of 200 mg/dl or more on 2 different occasions.

A semistructured questionnaire was developed to record the medical history, examination details and investigation reports. Detailed medical history and physical examination included demographic data, duration of diabetes, treatment compliance, method of glycemic control, history of previous amputation, awareness about complications, personal habits like smoking and alcohol consumption, history of ischemic heart disease, hypertension or cerebrovascular disease, nephropathy, neuropathy, duration of wound, history of antibiotic intake prior to admission. Neuropathy was assessed with 128 Hz tuning fork, ischemia by pulsations of dorsalis pedis and posterior tibialis, osteomyelitis (to assess bone involvement) was diagnosed on X- rays, categorization of foot ulcers into five types based on Wagner's classification. The extent of foot infection

was assessed based on Wagner's classification as follows: Grade 1 when ulceration involving only the dermis; Grade 2 when ulceration involving tendons and/or joint capsules; Grade 3 when ulceration is extending to bone, usually causing osteomyelitis; Grade 4 when localized gangrene is present and Grade 5 when gangrene is extensive and involving a major part of the foot.

Microbiological investigations:

Wound was thoroughly washed with saline to remove the slough and the local antiseptic application applied during previous dressing. Specimen was collected from the edge of the wound and sent to the lab immediately. Gram's staining was done followed by culture on 5% Sheep blood agar, Mac Conkey agar, incubated at 37°C. After 24-48 hours of incubation, bacterial growth was identified by standard microbiological methods and antimicrobial susceptibility tests were performed using Kirby-Bauer disc diffusion test according to Clinical & Laboratory Standards Institute (CLSI) guidelines. The data was subjected to frequency and percentage.

Statistical analysis:

The data were entered in to Microsoft Excel 2013 and then imported into SPSS version 20 (SPSS INC, Chicago, IL, USA). The data were analyzed for frequency and percentage and represented in Tables or in graphs accordingly.

RESULTS:

Of the 95 patients involved for the study, 83 were males and 12 females. Among them, 83 were of less than 40 years age group and the rest of the population is above 40 years of age. Out of these 95 patients, 48 of them were from town, 37 of them were from the city and the rest of them belonged to a village (Table 1).

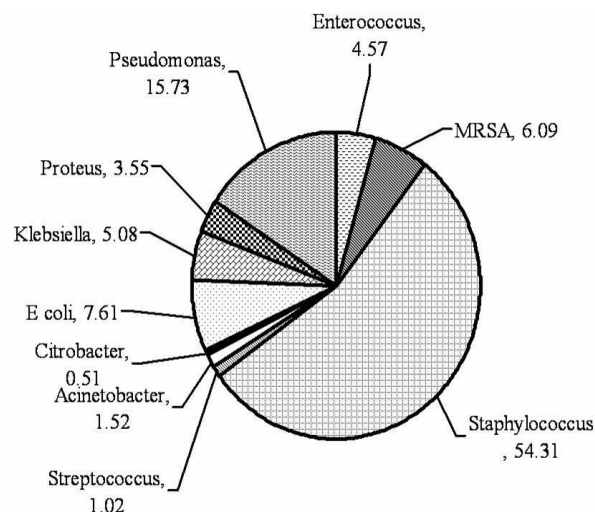


Figure 1: The percentage of microorganisms isolated from the diabetic foot samples

Table 1: Demographic details of the diabetic foot patients in our study

	Choice	Frequency	%
Place	Village	10	9.6
	Town	48	46.08
	City	37	35.52
Age	Less than 40	83	79.68
	41 to 50	22	21.12
	51 to 60	33	31.68
	61 to 70	29	27.84
	Greater than 7	08	7.68
Gender	Female	12	11.52
	Male	83	79.68

As enlisted in Table 2, patients who were diabetic for more than 10 years were 20.16%, patients suffering

from diabetes since 6-10 years were 36.48% and 34.56% were diabetic for less than 5 years. Among the patients studied, 52 patients(49.92%) were with no co-morbidities and the rest of the people suffered from various co morbidities (CKD - 4.8%, HTN-26.88%, COPD -0.96%, PAOD -7.68%, IHD – 13%, varicose veins – 0.96%).The patients were also classified based on Wagner's grade into five groups: 27.55% of patients were grade 1, 15.2% grade 2, 25.65% grade 3; 21.85% grade 4 and none were grade 5 (Table 3). Among the 95 patients, 11 people had 2 co morbidities, one patient had 3 co morbidities and rest of the patients had either one (31) or no comorbidities at all. Patients that were on oral hypoglycemic agents were 61 and on insulin were 20 and that were on both were 14. Patients with associated diabetic complications like retinopathy (16.32%), nephropathy (12.48%) and neuropathy (20%).

Table 2: Diabetic status of patients in our study

	Choice	Frequency	%
Diabetes duration (in years)	Less than 5	36	34.56
	6 to 10	38	36.48
	More than 10	21	20.16
Comorbidities	No co morbidities	52	49.92
	Chronic Kidney Disease	5	4.8
	Hypertension	28	26.88
	Chronic Obstructive Pulmonary Disease	1	0.96
	Peripheral arterial Obstructive disease	8	7.68
	Ischemic heart Disease	13	12.48
	Varicose veins	1	0.96

Number of co morbidities (along with DF)	0	52	49.92
	1	31	29.76
	2	11	10.56
	3	1	0.96
Drug	Oral Hypoglycemic	61	58.56
	Insulin	20	19.2
	Both	14	13.44
Other associated diabetic Complications	Retinopathy	17	16.32
	Nephropathy	13	12.48
	Neuropathy	20	19.2

Table 3: Condition and treatment provided for diabetic foot

	Choice	Frequency	%
Wagner	Grade 1	29	27.84
	Grade 2	16	15.36
	Grade 3	27	25.92
	Grade 4	23	22.08
Presentation	Gangrene	23	22.08
	Ulcer	43	41.28
	Cellulitis	20	19.2
	Abscess	29	27.84
	Osteomyelitis	3	2.88

	Maggots	4	3.84
	Incised wound	1	0.96
	Necrotizing fasciitis	8	7.68
Number of diabetic Complications	Only diabetic foot	66	63.36
	diabetic foot + 1 more complication	13	12.48
	diabetic foot + 2 more complications	9	8.64
	diabetic foot + 3 more complications	6	5.76

	Spilt thickness skin grafting	12	11.52
	Disarticulation	25	24
	Amputation below knee	4	3.84
	Amputation Above knee	2	1.92
Number of treatment/ procedures done	1	61	58.56
	2	30	28.8
	3	4	3.84

Table 4: Treatment given to the diabetic patients

	Choice	Frequency	%
Pre-treatment duration of stay	Same day	49	47.04
	1 to 3 days	14	13.44
	4 to 7 days	10	9.6
	More than 7 days	5	4.8
Post-treatment duration of stay	Less than 7 days	8	7.68
	8 to 14 days	25	24
	15 to 21	18	17.28
	22 to 30	14	13.44
	31 to 45	18	17.28
	More than 46	9	8.64
Treatment	Dressing	16	15.36
	Incision and drainage	25	24
	Fasciotomy	6	5.76
	Debridement	43	41.28

Among the 95 patients the diabetic foot cases 23 cases presented as gangrene of the foot, 43 of those cases presented as ulcers, 20 patients presented with cellulitis and 29 people presented with abscess. Few patients presented with necrotizing fasciitis (8), Maggots (4), Osteomyelitis (3), and incised wound (1).

With regard to the treatment given to the diabetic patients, dressing was done for 16 people out of the 95 patients, incision and drainage was done for 25 people, fasciotomy was done for 6 people, 43 people underwent debridement, skin grafting was done for 12 people, disarticulation was done for 25 people, below knee amputation was done for 4 people, above knee amputation was done for 2 people. Duration of stay pre-treatment as enlisted in table 4 is as follows on the same day (49), for 3 days (14), 4 to 7 days (10), more than 7 days (5). Post treatment stay for less than 7 days (8), 8 to 14 days (25), 15 to 21 days (18), 22 to 30 (14), 31-45 (18), more than 46 days (9). The total number of treatment and procedures on patients done are as follows, one procedure was done for maximum number of patients 61(58.56%), 2 procedures in 30(28.8%) and 3 procedures in 4(3.84%)(Table 4).

Among the sample received from these diabetic patients, a total of 92 pathogens were isolated. The Gram negative pathogens were predominant 52 (56.6%), among which *Pseudomonas* sp 16(30%) was the most common pathogen, followed by

Escherichia coli 15(28.8%) and *Klebsiella* sp 10(19.2%) Table5. Among Gram positive pathogens, *Staphylococcus aureus* was the commonest pathogen, accounting for 29(72.5%), followed by *Enterococcus* sp 9 (22.5%). Among the total *Staphylococcus aureus* (29) isolated, 12 were Methicillin resistance and MRSA isolation rate being 41.3%.

The drug resistance pattern was studied in detail. Among the *Pseudomonas aeruginosa* isolated, more than 50% were resistant to 3rd generation cephalosporin, gentamicin, ciprofloxacin; none of the strains were resistant to carbapenems. Secondly, among *Escherichia coli* and *Klebsiella* isolates amikacin, piperacillin/tazobactam and carbapenem were sensitive in more than 90% of the isolates. Among MRSA, there was no resistance observed with linezolid which is the drug of choice in Skin and soft tissue infection (Table 5).

Table 5: Antibiogram of the various organisms isolated from the diabetic foot in the study

Name of organism (N)	A	AMC	CP	CU	CT/CZA	COT	G	AK	CF	LF	A	CD	VA	TP	IZ	FT	CBS	MR	IM	AT
Gram negative																				
<i>Pseudomonas</i> (16)	-	-	-	-	10	14	8	2	12	3	-	-	-	-	-	7	7	0	0	5
Percent resistant	-	-	-	-	62.5	87.5	50	12.5	75	18.75	-	-	-	-	-	43.75	43.75	0	0	31.25
<i>E. coli</i> (15)	14	13	13	13	12	10	7	1	7	4	-	-	-	-	-	2	2	1	1	4
Percent resistant	93.33	86.67	86.67	86.67	80	66.67	46.67	6.67	46.67	26.67	-	-	-	-	-	13.33	13.33	6.67	6.67	26.67
<i>Klebsiella</i> (10)	10	7	9	8	5	3	2	0	5	1	0	0	-	-	-	1	1	0	0	0
Percent resistant	100	70	90	80	50	30	20	0	50	10	0	0	-	-	-	10	10	0	0	0
<i>Proteus</i> (7)	6	6	5	5	1	4	4	0	3	3	-	-	-	-	-	0	0	0	0	0
Percent resistant	85.71	85.71	71.43	71.43	14.29	57.14	57.14	0	42.86	42.86	-	-	-	-	-	0	0	0	0	0
<i>Acinetobacter</i> (3)	3	-	2	3	3	3	3	2	3	1	-	-	-	-	-	3	3	1	1	0
Percent resistant	100	-	66.67	100	100	100	100	66.67	100	33.33	-	-	-	-	-	100	100	33.33	33.33	0
<i>Citrobacter</i> (1)	1	1	1	1	1	0	0	0	1	0	-	-	-	-	-	1	1	0	0	0
Percent resistant	100	100	100	100	100	0	0	0	100	0	-	-	-	-	-	100	100	0	0	0
Gram Positive																				
<i>Staphylococcus</i> (17)	14	9	3	1	0	9	3	0	7	1	2	0	0	0	0	-	-	-	-	-
Percent resistant	82.35	52.94	17.65	5.88	0	52.94	17.65	0	41.18	5.88	11.76	0	0	0	0	-	-	-	-	-
<i>MRSA</i> (12)	12	12	12	12	12	8	11	3	10	5	7	2	0	0	0	-	-	-	-	-
Percent resistant	100	100	100	100	100	66.67	91.67	25	83.33	41.67	58.33	16.67	0	0	0	-	-	-	-	-
<i>Enterococcus</i> (9)	7	3	0	0	0	4	2	1	4	2	2	2	0	0	0	-	-	-	-	-
Percent resistant	77.78	33.33	0	0	0	44.44	22.22	11.11	44.44	22.22	22.22	22.22	0	0	0	-	-	-	-	-
<i>Streptococcus</i> (2)	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	-	-	-	-	-
Percent resistant	0	0	0	0	0	50	50	50	0	0	0	0	0	0	0	-	-	-	-	-

DISCUSSION:

This study evaluated the risk factors / co-morbid conditions in patient admitted with Diabetic foot ulcer. In our study, the patients with number of diabetic complications like nephropathy, neuropathy correlated well with the Wagner's grade of ulcers, i.e., more complications are seen with higher Wagner's grade. Few parameters like patient's age, gender, BMI, duration of diabetes, HbA1c and smoking did not have significant correlation with risk of diabetic foot ulcer. As observed by various studies worldwide, the incidence of distal sensory neuropathy increased with higher grade of diabetic foot, i.e., 21.5% in grade 2; 26.3% in grade 3; while 52.6% in grade 4 [7-9].

Few of the factors like patient's age, male gender, duration of diabetes, HbA1c had significant association with high risk of diabetic foot [10], which was not observed in our patients. In United States patients, male gender, duration of diabetes mellitus, previous history of foot injury, high plantar pressure, was associated with increased risk of foot ulcer [11]. Similarly, in Iranian patients, smoking was a risk factor among the diabetics to cause complications in foot ulcer [12], which was not observed among our 95 patients with DFU. Amputation rate was higher with advanced age in Swedish patients DFU [13], which was contrary to few other studies [14,15], but we have not found any association of age with amputation in our study. In North Indian patients, risk factor for amputation were leukocytosis, neuropathy and higher HbA1c levels [16], such correlation with either leukocytosis or neutrophil lymphocyte ratio on Wagner's grade is not evident in our study population. However, neuropathy was significant, as the patient presented with worse Wagner's grade had decreased sensory input which contributed to foot ulcer following trivial trauma and bad foot care which again worsened the ulcer. It should also be noted that together with neuropathy, visual impairment is one of the risk factors for the development of diabetic foot ulcers as they hamper the foot care and noticing trivial ulcer in

view reduced sensations.

When the infection among the DFU was analyzed by a United Kingdom based study under the consortium of Concordance in Diabetic Foot Ulcer Infection revealed gram positive cocci (70.6%) was isolated in majority of the patients, followed by gram negative bacilli (36.7%). The most common pathogen among Gram positive cocci was *Staphylococcus aureus* (43.8%) and Enterobacteriaceae i.e. Coliforms (26.6%) and *Pseudomonas aeruginosa* (8.6%) among Gram negative bacteria^[17]. A study from a tertiary care hospital from North India, in which 80 patients with infected DFU were studied, 183 pathogens were isolated. The gram negative to gram positive ratio was 3: 2, with Gram negative aerobic bacilli accounted for 94 total isolates with *Proteus* sp 23 (12.6%) was predominant, and among gram positive cocci, *Staphylococcus aureus* accounted for 25 (13.7%). MRSA isolation rate was as high as 56% i. e. 14 MRSA of total 25 *Staphylococcus aureus*^[18]. A study in South Indian population^[19], which involved, 386 patients, 438 pathogens were isolated and both Gram positive and negative were almost equally isolated. The isolation of MRSA was around as high as 72%. Among the gram-negative bacilli, *Pseudomonas aeruginosa* was the predominant isolate among the Gram negative similar to our study. In another Indian study^[20], with 170 isolates from 104 patients with Diabetic foot infections, Gram negative bacilli *Escherichia coli* (37, 21.7%) was commonest followed by *Pseudomonas aeruginosa*. Only one patient was isolated with MRSA among 104 patients with a very low incidence of around 10% (1 in 11 *Staphylococcus aureus* isolates). These study emphasis that the microbial flora causing infection in Diabetic foot can vary in different population under study, depending on the various factors like age, gender, co-morbidity and previous antibacterial exposure.

Various studies have showed vary percentage of Gram-positive pathogens isolation^[18, 20]. The MRSA isolation among the *Staphylococcus aureus* is higher compared to patients from other centers^[18, 20]. Treating

these patients with MRSA is a challenge, as vancomycin, which is nephrotoxic, cannot be administered in most of the patients with Diabetic foot they have already compromised renal functions. Only linezolid can be prescribed in these individuals. The presence of infection can hamper the quick healing of the Diabetic foot and infection with MDR pathogen can increase the duration of morbidity.

The pathogenesis of neuropathy in diabetic patients is explained by polyol pathway, where the enzyme aldose reductase and sorbitol dehydrogenase activity is increased due to hyperglycemic state, leading to conversion of intracellular glucose to sorbitol and fructose, this causes reduced myoinositol production in nerve cell. The reduced myoinositol, which is required for normal nerve conduction, causes neuropathy. The neuron injury or death occurs due to increased oxidative stress and vasoconstriction, due to deficiency of nicotinamide adenine dinucleotide phosphate, which is required for detoxification of reactive oxygen species and production of vasodilator, nitric oxide. All the components of nervous system, i.e., sensory, motor and autonomic, are affected in diabetic patients. The sensory loss, especially in lower extremities, due to peripheral neuropathy patient are unable to detect even the trivial trauma. With the presence of retinopathy, many wounds go unnoticed and progressively worsen as the affected area is continuously subjected to repetitive pressure and shear forces from ambulation and weight bearing. When the various therapeutic procedures were compared with Wagner's scale of Diabetic ulcer, no significant difference in our study population.

Proteinuria, an indicator of nephropathy, was observed in majority of our patients. A strong positive correlation between proteinuria and Wagner's scale in neuropathic ulcers was observed, similar to other study^[21]. Nephropathy reflects wide vascular damage at the glomeruli and also reflects damage at the retina and intima of the arteries. In addition, nephropathy is associated with the development of hypertension and dyslipidemia, both

well-known risk factors for CAD. Moreover, patients at all stages of diabetic nephropathy are at a particularly high risk of foot ulceration as neuropathy is particularly common in the early stages of nephropathy [22]. Recent data have shown higher mortality rates in patients with foot ulcers²³. The key players in mortality of diabetic foot ulcers include neuropathy, ischemia, and infection. The roles played

by the participating factors can vary^[24]. The limitations of this study were lesser number of patients and this study didn't have any patients under grade 5 which probably would have made us understand the pathophysiology and diabetic complications better.

CONCLUSION:

Diabetic foot ulcer infection is mainly seen in individuals with > 5 years of diabetes and with complications mainly peripheral neuropathy and nephropathy. Patient's age, male gender, smoking duration of diabetes, HbA1c had no significant association with high risk of diabetic foot ulcer. Gram negative bacteria, coliforms are predominant pathogens isolated. Flouroquinolones and aminoglycoside group of antimicrobials can be considered for empirical therapy provided antibiogram is monitored on regular basis.

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