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A Clinico-Bacteriological Study of Diabetic Foot Ulcers in a Tertiary Care Hospital

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A B S T R A C T

Diabetic foot is one of the most feared complications of diabetes and is the leading cause of hospitalization in diabetic patients. Diabetic patients have a lifetime risk as high as 25% for developing into foot ulceration. The reasons for the increased incidence of diabetic ulcer involve the interaction of several pathogenic factors like neuropathy, abnormal foot biomechanics and peripheral arterial disease. The study was performed to determine the microbial etiology (aerobic and anaerobic) of diabetic foot ulcer and their susceptibility pattern in a tertiary care hospital. A total number of 120 diabetic patients were studied over a period of two year. Pus and tissue samples were collected for bacteriological study. The specimens were processed in the Microbiology laboratory for Gram stain, aerobic culture, and anaerobic culture. The organisms isolated were identified by standard procedures and antimicrobial susceptibility was done by Kirby-Bauer disc diffusion method. Out of 120 samples, a total of 227 organisms were isolated in the present study, with an average of 1.87 organisms per specimen. Among them, aerobic organisms were 166 (73.1%) and anaerobic organisms were 61 (26.9%). Out of 166 aerobic organisms isolated, the most common isolates were *Pseudomonas aeruginosa* 41(24.70%) followed by *Staphylococcus aureus* 33(19.88%). Amongst 61(26.9%) anaerobes, *Peptostreptococcus magnus* (27.8%) was the commonest isolated organism. Aerobes were found to be susceptible mainly to antibiotics like Vancomycin, Imipenem, Piperacillin - Tazobactum while anaerobes were susceptible to Metronidazole. *Pseudomonas aeruginosa* was predominant isolate in aerobes while *Peptostreptococcus magnus* was commonest among anaerobe. This study will help the clinician for proper management of ulcer and thus reduces dreaded complication of it.

Introduction

Diabetes mellitus is a chronic disorder and affects large segments of population and also a major public health problem[1]. The worldwide prevalence of diabetes now exceeds 200 million and is predicted to rise to more than 300 million in the next 20 years.[2]The incidence of diabetes in India was estimated to be 31.7 million in the year 2000

and projected to reach 79.4 million in 2030, according to the World Health Organisation.[21]

Diabetic foot is one of the most feared complications of diabetes and is the leading cause of hospitalization in diabetic patients. Diabetic foot is characterised by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, leading to development of gangrene and even necessitating limb amputation.[18]

Diabetic patients have a lifetime risk as high as 25% for developing foot ulceration.[19] The reason for the increased incidence of diabetic ulcer involve the interaction of several pathogenic factors like neuropathy, abnormal foot biomechanics and peripheral arterial disease[3]. Neuropathy plays the central role with disturbances of sensory, motor and autonomic functions leading to ulceration due to trauma or excessive pressure on a deformed foot that lacks protective sensations[12]. Susceptibility to infections increases due to hyperglycemia and poor vascular supply to the foot. Hence diabetic foot infections are the most common reason for hospital admissions in diabetic patients[20].

The most important characteristic of the diabetic ulcer is that, it is polymicrobial in nature, comprising of both aerobic and anaerobic microorganisms[4]. In superficial wounds, aerobic bacteria are predominant pathogens. Anaerobic organisms are found more frequently in deeper wounds[17].

The present study was undertaken to assess the role of aerobic and anaerobic bacteria in the causation of diabetic foot ulcer and their antibiotic susceptibility pattern which would assist clinicians in therapy of this dreaded complication of diabetes.

Materials and Methods

A prospective study was carried out over a period of two year in Department of Microbiology, at a tertiary care hospital. A total of 120 patients with diabetic ulcer, attending the surgical OPD or admitted in surgical wards, were included in the study. These patients were known diabetic or detected to be diabetic by blood glucose estimation at the time of their visit to the hospital.

A detailed history of the patient regarding age and sex, type of diabetes, duration of diabetes, smoking, hypertension, duration of foot ulcer was taken and recorded. The adequacy of peripheral circulation was done by palpating the posterior tibial artery and the dorsalis pedis artery. Foul smell, local rise of temperature, discharge and discolouration of the surrounding area was noted. Foot ulcers in diabetic patients were categorized into six grades (grade 0 - grade 5) based on Meggit Wagner Classification System[10]. The criterion for inclusion was a diabetic patient with foot ulcer of grade 2 or more.

Samples like pus or necrotic tissues (in case of suspected anaerobic wound) were collected. Before that the surface of the ulcer was rinsed with sterile normal saline, superficial exudates was debrided using a sterile instrument[3].

Pus was collected from the oozing wound or collected from the infective centre of the wound with the help of sterile cotton tipped swab which was moistened with normal saline or aspirated with the help of sterile needle and syringe (In case of abscess)[15,3]. In case of suspected anaerobic condition, necrotic tissue sample was taken with a sterile blade/knife in wedge shape, including base and margin of ulcer and transported in thyoglycollate broth[4].

For aerobic culture, specimen was inoculated on MacConkey's agar and blood agar plates and incubated at 37 °C. For anaerobic culture, Specimen was inoculated on Neomycin blood agar, Kanamycin-vancomycin blood agar and Robertson's cooked meat medium. The inoculated plates were incubated immediately in anaerobic gas pack jar at 37°C for 48 hours. The organisms isolated were identified by standard procedures[6].

Antimicrobial susceptibility testing of aerobic and anaerobic isolates was performed by the Kirby Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI)[11,5].

Results and Discussion

Out of 120 patients, 71(59.1%) were males and 49(40.8%) were females with male to female ratio was 1.4:1.Most of the patients with diabetic foot were in the age group 51 to 60 (34.1%) years. About 37 (30.8%) patients had diabetes mellitus for 6-10 years. Only one (0.84%) case was insulin-dependent diabetes mellitus and the others were non-insulin-dependent diabetes mellitus (NIDDM) cases, 119 (99.16%). Forty one (34.16%) patients presented with ulcer of 6-8 month duration, followed by 37 (30.83%) patients had ulcer of 3-5 months duration. The commonest clinical findings seen were fever (56.6%), followed by neuropathy (50.8%), vasculopathy (48.3%), crepitation (37.5%) and foul smell (34.1%).Sixty nine (57.5%) patients were smokers and fifty eight (48.4%) patients were associated with hypertension. Majority of the patients presented with the ulcer of Wagner grade II, 71(59.2%), followed by grade III, 27 (22.5%).Eighty four (70%) patients had poor glycolic control (BSL> 200 mg/dl), while 36(30%) patients had blood sugar level less than or equal to 200 mg/dl (Table 1).

A total of 227 organisms were isolated in the present study, with an average of 1.87 organisms per specimen. Among them, aerobic organisms isolated were 166 (73.1%) and anaerobic organisms were 61 (26.9%).Out of 166 aerobic organisms isolated, the most common isolates were *Pseudomonas aeruginosa* 41(24.70%) followed by *Staphylococcus aureus* 33(19.88%), *Escherichia coli* 30(18.08%), *Klebsiella pneumoniae* 21(12.65%), and *Proteus spp.* 17(10.2%) (Table 2). And out of 61 anaerobic organisms, the most common isolates were *Peptostreptococcus magnus* 17(27.8%), followed by *Bacteroides fragilis* 15(24.5%), *Prevotella melaninogenica* 12(19.6%) (Table 3).

Monomicrobial flora was present in 42 (35%) cases, of which aerobes were in 39 (32.5%) cases and anaerobes were in 3 (2.5%) cases. Polymicrobial flora were present in 78 (65%) cases, of which 48 (40%) cases were aerobic organisms and aerobic along with anaerobic organisms were isolated in 30 (25%) cases.

The results of the test for susceptibility to the commonly used antibiotics are shown in Table 4. Almost all the strains of *Pseudomonas aeruginosa* was sensitive *to* imepenem (95%), piparacillin/tazobactum (87%) and least sensitive to ciprofloxacin (31%), co-trimoxazole (26%). *Staphylococcus aureus* were most often susceptible to vancomycin (100%), clindamycin (84%), Cefoxitin (66%), but were relatively less susceptible to co-trimaxazole (36%) and penicillin (33%). *Escherichia coli* were 93% sensitivity to imipenem, 90% to piparacillin-tazobactum while 36% and 30% resistance to cotrimoxozole and penicillin respectively. *Klebsiella pneumoniae* was found to be sensitive to Imipenem(100%), piparacillin/ Tazobactum (85%) and less sensitive to cotrimoxozole(33%) and penicillin (28%). Almost all the anaerobes were 100% sensitive to metronidazole. The antibiotic sensitivity pattern of anaerobic organism shown in Table 5.

Table.1 Showing patients' demographic data and clinical characteristics

Sr.No.	Patient characteristics	Number
a.	Male	71 (59.16%)
b.	Female	49 (40.84%)
	Age wise distribution of patients	
a.	21-30 yr	3 (2.5%)
b.	31-40 yr	7 (5.83%)
c.	41-50 yr	24 (20.0%)
d.	51-60 yr	41 (34.17%)
e.	61-70 yr	27 (22.5%)
f.	71-80 yr	18 (15.0%)
	Duration of diabetes mellitus	
a.	Detected on Admission	9 (7.5%)
b.	1-5 years	33(27.5%)
c.	6-10 years	37 (30.8%)
d.	11-15 years	26(21.7%)
e.	16-20 years	15 (12.5%)
	Type of diabetes mellitus	
a.	IDDM	1(0.84%)
b.	NIDDM	119(99.16%)
	Poor glycemic control (BSL>200 mg/dl)	84 (70%)
	Smoking	69 (57.5%)
	Hypertension	58(48.4%)
	Clinical findings associated with ulcer	
a.	Fever	68 (56.6%)
b.	Neuropathy	61 (50.8%)
c.	Vasculopathy	58 (48.3%)
d.	Crepitation	45 (37.5%)
e.	Foul smell	41 (34.1%)
f.	Cellulitis	25 (20.8%)
g.	Osteomyelitis	17 (14.1%)
h.	Gangrene	8 (6.6%)
	Duration of diabetic foot ulcer in months	
a.	0-2	19 (15.84%)
b.	3-5	37 (30.83%)
c.	6-8	41 (34.16%)
d.	9-11	18 (15.0%)
e.	12-14	5(4.17%)
	Grading of lesions in the diabetic patients	
a.	Grade -2	71 (59.2%)
b.	Grade-3	27 (22.5%)
c.	Grade-4	15 (12.5%)
d.	Grade-5	7 (5.8%)

Table.2 Number and percentage of aerobic organisms isolated in the study

Name of organism	Number of organisms	Percentage (%)
Pseudomonas aeruginosa	41	24.70
Staphylococcus aureus	33	19.88
Escherichia coli	30	18.08
Klebsiella pneumonia	21	12.65
Proteus mirabilis	9	5.42
Proteus vulgaris	8	4.82
Coagulase-negative Staphylococcus	7	4.22
Streptococcus pyogenes	5	3.01
Citrobacter freundii	5	3.01
Enterococcus	3	1.81
Enterobacter	2	1.20
Acinetobacter	2	1.20
Total	**166**	**100 %**

Table.3 Number and percentage of anaerobic organisms isolated in the study

Name of organisms	Number of organisms	Percentage (%)
Peptostreptococcus magnus	17	27.88
Bacteroides fragilis	15	24.59
Prevotella melaninogenica	12	19.67
Peptostreptococcus asaccharolyticus	7	11.48
Fusobacterium	5	8.19
Clostridium tetani	3	4.92
Clostridium perfringens	2	3.27
Total	**61**	**100 %**

Table.4 Showing antibiotic sensitivity pattern of aerobic organisms

Name of organism	P		AMC		CFX		IMP		PIT		Va		E		G		Cf		CAZ		CO		CD	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Pseudomonas spp.(n=41)	-	-	26	63	-	-	39	95	36	87	-	-	-	-	19	46	13	31	23	56	11	26	-	-
S. aureus (n=33)	11	33	-	-	22	66	-	-	-	-	33	100	16	48	-	-	14	42	-	-	12	36	28	84
E.coli (n=30)	9	30	12	40	-	-	28	93	27	90	-	-	-	-	14	46	-	-	15	50	11	36	-	-
K.pnaemonae (n=21)	6	28	10	47	-	-	21	100	18	85	-	-	-	-	11	52	-	-	15	71	7	33	-	-
P.mirabilis (n=9)	4	44	6	66	-	-	9	100	7	77	-	-	-	-	3	33	-	-	5	55	4	44	-	-
P.vulgaris (n=8)	3	37	6	75	-	-	8	100	7	87	-	-	-	-	5	62	-	-	4	50	3	37	-	-
CONS (n=7)	3	42	-	-	5	71	-	-	-	-	7	100	5	71	-	-	4	57	-	-	3	42	6	85
S.pyogenes (n=5)	3	60	-	-	4	80	-	-	-	-	5	100	4	80	-	-	3	60	-	-	-	-	4	80
C.freundii (n=5)	2	40	3	60	-	-	5	100	4	80	-	-	-	-	3	60	-	-	3	60	2	40	-	-
Enterobacter (n=2)	1	50	1	50	-	-	2	100	2	100	-	-	-	-	1	50	-	-	2	100	1	50	-	-
Enterococcus (n=3)	2	66	-	-	-	-	-	-	-	-	3	100	2	66	3	100	1	50	-	-	-	-	-	-
Acinetobacter (n=2)	-	-	1	50	-	-	2	100	2	100	-	-	-	-	1	50	1	50	2	100	1	50	-	-

E-Erythromycin, G-Gentamicin, Cf-Ciprofloxacin, CAZ- Ceftazidime, CO-Co-trimaxozole, CD- Clindamycin, P- Penicillin, AMC- Amoxacillin-clavulanate, CFX- Cefoxitin, IMP-Imipenem, PIT- Piparacillin/Tazobactam, Va-Vancomycin

Table.5 Showing antibiotic sensitivity pattern of anaerobic organisms

Name Of Organism	P		N		MT		Va		Cf		CFX		R		K	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Peptostreptococcus spp. (n=24)	23	95	16	66	24	100	24	100	10	41	17	70	12	50	18	75
Bacteroides spp. (n=15)	5	33	9	60	15	100	-	-	8	53	10	66	13	86	6	40
Prevotella spp. (n=12)	4	33	10	83	12	100	-	-	6	50	8	66	11	91	4	33
Fusobacterium spp. (n=5)	3	60	4	80	5	100	-	-	2	40	3	60	3	60	4	80
Clostridium spp. (n=5)	2	40	4	80	5	100	-	-	2	40	4	80	3	60	4	80

P-Penicillin, N- Neomycin, MT-Metronidazole, Va- Vancomycin, Cf- Ciprofloxacin, CFX- Cefoxitin, R- Rifampicin, K-Kanamycin

Diabetic foot is considered one of the most threatening and disabling complications for a diabetic patient as the lesions of the extremities can become so severe that the patient may risk the amputation of the toe, foot or leg[7]. Because of serious or recurrent infections and impaired healing processes, initially trivial lesion may progress to chronic non healing wounds, gangrene, or untreatable infections that can lead to limb amputation[13].

In the present study, a total of 227 organisms were isolated from 120 patients, amongst that, 166 (73.1%) were aerobes and 61 (26.9%) were anaerobes. The present study, showed aerobic predominance which is in concurrence with various studies like Chincholikar *et al.,* [7], Anandi *et al.,* [1], Gadepalli *et al.,* [9], Banoo *et al.,* [3]. Anaerobes were isolated less commonly in this study may be because of maximum number of patients had grade 2 or 3 lesion which are usually uncomplicated. As grading of lesion increases number of anaerobic organism increases because they are deep seated organism[8].

In the present study, *Pseudomonas aeruginosa* was the most commonly isolated organism and accounting for 24.7% of the total aerobic organisms. In the study of Bansal *et al.,* [4], Paul *et al.,* [16], Pappu *et al.,* [14] and Priyadarshini *et al.,* [11], reported that *Pseudomonas aeruginosa* was most common organism, accounting for 21%, 26%, 23% and 16% respectively. Our results are in near agreement with them. In contradiction, *S. aureus* is the most common organism in most of the studies like Chincholikar *et al.,* [7], Anandi *et al.,* [1]. While the prevalence of other aerobic organisms like *Staphylococcus aureus, E. coli, Klebsiella pneumoniae* was 19.8%, 18% and 12.6% respectively. Out of 61(26.9%) anaerobic organisms isolated, the most predominant anaerobic organism was *Peptostreptococcus magnus* (27.8%),

followed by *Bacteroides* sp. (24.5%). Our results are in correlation with the studies of Vishwanathan *et al.,* [20] and Zubair *et al.,* [22], where they isolated *Peptostreptococcus spp.* as 36% and 35% correspondingly. In the present study, polymicrobial etiology was seen in 65% of the cases. Mixed aerobes were seen in 52.1% cases and aerobes along with anaerobes were seen in 16.6% cases this is in concordance with Chincholikar *et al.,* [7], Banoo *et al.,* [3], where polymicrobial etiology seen as 69.5% and 64% respectively.

With regards to antibiotic susceptibility pattern of aerobic isolates, all Gram-negative bacteria showed maximum sensitivity pattern to Imipenem (96.6%), piparacillin/ tazobactam (87.28%) and least sensitivity to co-trimoxazole (33.8%), penicillin (32.4%), ciprofloxacin (31.8%). Similar findings seen in Banoo *et al.,* [3]. While all Gram positive aerobic cocci 100% sensitive to vancomycin followed by clindamycin (84.4%). MRSA was seen in 33.3% while Anandi *et al.,* [1] and Banoo *et al.,* [3] showed 20% and 66% MRSA pattern in their studies. In the present study, all the anaerobic isolated organisms were sensitive to metronidazole which is in correlation with Chincholikar *et al.,* [7], and Banoo *et al.,* [3].

In conclusion, diabetic foot ulcer is considered one of the most threatening and disabling complication for a diabetic patient. As diabetic foot infections are polymicrobial in nature. It should be recognized early and treated rigorously, then the incidence of amputation of limb will decrease drastically. The selection of the antibiotic treatment should be based on the predominant organisms which are isolated and their antimicrobial susceptibility patterns. This will improve the overall antibiotic utilization and reduce the emergence of multidrug resistant organisms.

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