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Common Bacterial Isolates of Diabetic Foot Ulcer in An Indian Tertiary Care Hospital

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I. Introduction

The world is facing a major epidemic of diabetes mellitus (DM). There are an estimated 171 million diabetic patients worldwide and this number is expected to double by the year 2030 (1). Diabetic foot ulcer is one of its most serious and complications (2). Foot ulceration is one of the leading cause of mortality and morbidity in DM especially in developing countries and frequently leads to amputation of the leg and accounts for up to 20% diabetic related hospital admission (3). Diabetic foot ulcers result from a complex interaction of two major factors: neuropathy and peripheral vascular disease. Ulceration of the diabetic foot usually follows some form of extrinsic or intrinsic trauma. Once the protective layer of skin is broken, the deep tissues are exposed to bacterial colonisation (1).The wound infection begins superficially but with delay in treatment can spread to other subcutaneous tissues. Ultimately leading to dreaded complication such as gangrene and amputation(4). These infections are polymicrobial in nature. Escherichia coli, Proteus spp, Staphylococcus aureus, Enterococcus spp are reported as frequent organism isolated from cases of diabetic foot infections(5). Early recognition of lesions and prompt initiation of appropriate antibiotic therapy is important for successful outcome(6). Initial therapy of diabetic foot infection is frequently empiric because culture data is lacking(3). Since in wound microbiology both quantitative and qualitative aspects are critical determinants of wound outcome, the aim of this study is to investigate causative pathogens and sensitivity profile in recently developed diabetic foot ulcer patients, so that the clinical outcome is good and antibiotic resistance is avoided.

II. Materials And Methodology

This prospective study was conducted in diabetic patients attending diabetic outpatient department and general surgery outpatient department of Madha Medical College & Hospital after obtaining permission from institutional ethical committee. The study period was from June 2015 to October 2016. This study included 62 diabetic subjects, of which 47 were male subjects and 14 were female subjects. Written informed consent was obtained from them. Diabetic patients with history of foot ulcer for the period of one month or less with no antibiotic usage for the previous six months were included in the study(7). Patients with history diabetic foot ulcer for more than one month and with previous six months antibiotic usage were excluded from the study. Complete history was taken from the patient and recorded in already prepared proforma that included detailed history of diabetes and duration of foot infection. The diabetic foot ulcers were classified and grouped asperWagner grading system (3). Specimens from pus, wound exudate were obtained from ulcer region for microbiological study. The surface of the ulcer was rinsed with sterile normal saline and pus/wound exudate was collected with sterile cotton swab. The specimen was cultured on Nutrient agar, Mac Conkey agar and Blood agar. The plates were then aerobically incubated at 37^0 for overnight (8). All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedure (9).Antibiotic sensitivity test was carried out by Kirby Bauer Disc Diffusion technique on Mueller Hinton agar plates. The zone of inhibition were measured and interpreted using standard zone of inhibition charts. The results were tabulated and statistical analysis done.

III. Results

This study included 62 diabetic patients, that included 47 male and 14 female diabetic patients with diabetic foot ulcer. The general clinical features of 62 diabetic patients with infected foot ulcer are tabulated in table 1.The age group ranged from 30 to 60 years. Of which, 36 belonged to 30-40 years group 18 belonged to 40-50 years and 8 belonged to 50-60 years group. 42 diabetic subjects were on oral hypoglycaemic agents, 11 subjects were on Insulin and 9 subjects were on OHA and insulin. All diabetic subjects had diabetic foot ulcer for the duration of one week to one month. All diabetic foots were classified and grouped according to Wagner grading system. According to Wagner grading system, foot lesion are divided into six grades based on depth of the wound and extent of tissue necrosis. The commonest diabetic foot ulcer in our study was grade II ulcer as perWagner grading system. 21 patients belonged to grade I, 38 patients belonged to grade II, 3 belonged to grade III, presented in table 2.All the swabs taken in this study were positive for the culture. Out of 62 culture positive specimen, 78 isolates were recovered. 46 patients had single organism infection and 16 patients had two organism infection. From 62 culture positive specimen, 46 gram positive organism isolated and 32 gram

negative organism were isolated. The organisms isolated were, 32 staphylococcus aureus, 14 streptococci, 17 pseudomonas, 10 proteus and 5 E.coli, presented in table 3.The antimicrobial susceptibility pattern of gram positive cocci is presented in table 4. Among the 32 staphylococcus aureus, 30 were resistant to ampicillin, 10 to cephalexin,8 to ciprofloxacin, 10 to cloxacillin, 6 to erythromycin, 1 to gentamicin. All 14 streptococci isolates were resistant to ampicillin, 4 to cephalexin.The antimicrobial susceptibility pattern of gram negative is shown in table 5.Among the 17 pseudomonas aeruginosa, 17 were resistant to amoxyclav, 17 resistant to cephalexin, 15 to ciprofloxacin, 5 to cefotaxim, 3 to gentamicin, and one to amikacin. Among the 10 proteus, 8 were resistant to amoxyclav, 8 to cephalexin. Among the 5 E.coli, 4 were resistant to amoxyclav, all 5 were resistant to cephalexin, ciprofloxacin,cefotaxim, gentamycin and only one to amikacin.

IV. Discussion

Since the prevalence of diabetes in India is increasing and diabetic foot ulcer management is still a challenge to the medical community, we took up this study to investigate the culture and sensitivity pattern in newly developed diabetic foot ulcer patients. The study group included both male and female subjects with age ranging from 30-60 years. The majority of foot ulcer were in the age group 30-40 yrs, since many were excluded for ulcer more than one month and previous six months antibiotic usage in other age groups. The prevalence of foot ulcer was more in males[47] than in females[14]. In this study, a total of 78 isolates were recovered from 62 culture positive specimens, in which patients had monomicrobial infection, which suggests that during initial period of infection, wound is colonised by single organism.Among the 78 microorganism isolated, 46 were gram positive organism and 32 were gram negative organism, which again suggests that during initial period of infection, gram positive organism predominantly colonizes the wound. The diabetic foot ulcer in our study predominantly belonged to grade II, according to Wagner grading system because patients had avoided medical management of ulcer. In gram positive organism, staphylococcus aureus with count of 32 was the predominant isolate followed by 14 streptococci. In gram negative organism, pseudomonas aeruginosa with the count of 17 was the predominant isolate followed by 10 proteus. Gram positive organism accounted to higher number than gram negative organism. This finding in our study correlates with study of Sharma V.K et al 3.The antimicrobial susceptibility pattern of staphylococcus aureus showed high resistance to ampicillin. The most sensitive drug was found to be gentamicin having a resistance of only 3%. Cloxacillin and Ciprofloxacin were second most in sensitivity having a resistance of only 25%. 100% of streptococci were resistant to ampicillin. Among the gram negative bacilli, the antimicrobial susceptibility pattern showed E.coli were resistant to almost all the drugs studied. Amikacin had a slightly better sensitivity to E.coli. proteus and pseudomonas also are highly sensitive to amikacin.

V. Conclusion

Our study shows that in initial period of diabetic foot ulcer, less than one month duration, the most common organism present in the foot ulcer were gram positive aerobes. Most of our patients were grade II ulcers according to Wagner grading system. In this study, gram positive cocci were sensitive to ciprofloxacin, cloxacillin and gentamicin. Gram negative bacilli were sensitive to cefotaxim and amikacin. In this study we can see that many organisms showed multidrug resistance posing a potential risk factor in management of diabetic foot infection. This can be overcome by choosing the antimicrobial therapy based on the result of culture sensitivity pattern and combination therapy. the limitations of this study includes anaerobes and fungi not being isolated because of lack of culture media facilities. By choosing the right rules for management of diabetic foot ulcer, limbs can be saved for the betterment of the society.

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Table 1 General clinical features of 62 diabetic patients with infected foot ulcer

Clinical feature	No.of Patients	Percentage
Age in years		
30-40	36	58
41-50	18	29
51-60	8	13
Sex		
Male	47	76
Female	15	24
Duration of foot ulcer		
Less than 1 week	23	37
2 weeks	15	24
3 weeks	9	15
4 weeks	15	24
Diabetic medication		
OHA	42	68
Insulin	11	18
OHA + Insulin	9	15

Table 2 Wagner grading of Diabetic foot ulcer in our study

Wagner grade	No. of Patients	Percentage
Grade 0	0	0
Grade 1	21	34
Grade 2	38	61
Grade 3	3	5
Grade 4	-	-
Grade 5	-	-

Table 3 Characteristics of diabetic foot ulcer specimen

Features	No. of Positive culture	Percentage
No.of patients with Positive culture	62	100
Samples with one organism	46	74
Samples with two organism	16	26
Gram positive cocci		
Staphylococci	32	52
Streptococci	14	23
Gram negative bacilli		
Pseudomonas	17	27
Proteus	10	16
E.coli	5	8

Table 4 Antimicrobial resistance pattern of gram positive organism

Antimicrobiological agent	Staphylococcus aureus N=32	Percentage	Streptococci N=14	Percentage
Ampicillin	30	94	14	
Cephalexin	10	31	4	
Ciprofloxacin	8	25	2	
Cloxacillin	10		1	
Erythromycin	6		2	
Gentamicin	1		0	

Table 5 Antimicrobial resistance pattern of gram negative organism

Antimicrobiological agent	Pseudomonas N=17	%	Proteus N=10	%	E.coli N=5	%
Amoxyclav	17		8		5	
Cephalexin	17		8		5	
Ciprofloxacin	15		0		5	
Cefotaxim	5		1		5	
Gentamicin	3		0		5	
Amikacin	1		0		1	