ORIGINAL ARTICLE

Clinical presentation and microorganisms sensitivity profile for diabetic foot ulcers: a pilot study

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SUMMARY

Introduction: Patients suffering from diabetes mellitus (DM) frequently present with infected diabetic foot ulcers (DFU). This study was done to record the anatomical site and the grade of ulcers according to Wagner's classification and to culture the microorganisms from the ulcers and determine their antibiotic sensitivity.

Materials and methods: Prospective study was conducted on 77 diabetic patients who were admitted with DFU from June until December 2011. Patients with end stage renal failure, those who had previous vascular surgery on the involved limb, or hyperbaric oxygen or maggot therapy for the ulcers, or had unrelated skin diseases around the involved foot were excluded from the study. Specimens for culture were obtained by a sterile swab stick or tissue sample was taken from the wound with sterile surgical instruments.

Results: Wagner's grade III and IV ulcers were most common. Majority of the ulcers involved toes (48%). Gram negative microorganisms were predominantly isolated (71.1%). Gram positive microorganisms were less frequently cultured (27.7%). Fungus was cultured from one sample (1.2%). Gram negative microorganisms were sensitive to aminoglycosides, cephalosporins or β-lactamase inhibitors. More than 40% were resistant to ampicillin. Gram positive microorganisms were sensitive to cloxacillin. MRSA were sensitive to vancomycin.

Conclusion: Empirical use of antibiotics should be curtailed to prevent development of drug resistant strains of microorganisms and MRSA. We suggest use of antiseptic solutions to clean the ulcers until antibiotic sensitivity report is available. Results of our altered treatment regimen we plan to publish in a later study.

KEY WORDS:
Diabetic foot ulcer, microbial culture, antibiotic sensitivity

INTRODUCTION

Diabetes mellitus (DM) is a universal health problem. It was reported that 171 million people suffered from DM globally in year 2000 and this is projected to increase to 366 million by the year 2030.[1] In Malaysia, the Fourth National Health and Morbidity Survey (NHMS) reported about three million Malaysians were suffering from diabetes compared with about 1.5 million people when the survey was last conducted in 2006. This statistics has doubled within period of 5 years.[2]

It is estimated that between 15% and 25% of patients suffering from DM develop foot ulcers and are responsible for the majority of hospital admissions among the diabetics.[3] In Malaysia, foot complications account for approximately 12% of all diabetic hospital admissions. In Hospital Kuala Lumpur which is the main public tertiary medical centre in Malaysia, around 17% of diabetic patients were admitted because of diabetic foot ulcer (DFU).[4]

DFU is defined as a non- or poorly healing, partial or full thickness wound, located distal to the ankle in an individual with DM. The common sites involved are the sole of the foot or the toes.[5] Non-healing infected DFU is a common reason for amputation of the involved limb in patients with DM.[6] One study, involving 223 patients with DFU, reported that nearly 50% of the patients underwent amputation for non-healing DFU.[7]

There is limited information in the local context on DFU infection particularly on the pattern of clinical presentations, causative pathogens isolated, and their antibiotic sensitivity. Diabetic foot ulcer treatment needs improvement and to achieve that we first would like to study the spectrum of causative micro-organisms of DFU. The previous published study from Malaysia by Raja *et al.* 2007 is almost a decade old. This study was undertaken to record the site of ulcers on the foot, the grade of ulcers according to Wagner's classification, to isolate the causative microorganism and to determine their antibiotic susceptibility to commonly used antibiotics.

MATERIALS AND METHODS

A prospective study was conducted among 77 patients admitted with DFU to the orthopaedics wards of a local hospital from June 2011 to December 2011. Diabetic patients aged 18 years and above who presented with foot ulcers were included in the study. Patients in end stage renal failure requiring regular haemodialysis, those with history of

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previous vascular surgery on the involved limb or hyperbaric oxygen therapy or maggot therapy were excluded from this study. Patients with unrelated skin diseases around the involved foot were also excluded.

On admission, patient's characteristics, clinical examination and details of the DFU were recorded. Wound swab for microbiological culture and antibiotic sensitivity was done for all 77 patients. Tissue sample was obtained from 11 patients with foul smelling ulcers and blood culture was done on eight patients admitted with septicaemia. Plain radiographs of the involved foot were taken for all patients to exclude any bone involvement. All patients' data was recorded by the resident medical doctors at the hospital.

Written informed consent was obtained from all participating patients. This study had been approved by the Ethics Committee of the National Medical Research Registry (NMRR-12-287-11316), Ministry of Health, and the Ethics Committee of Faculty of Medicine and Health Sciences of the local university.

Clinical Examination
After complete general examination attention was focused on the foot ulcer. Its anatomical site, the aspect of the foot predominantly involved whether dorsal or plantar, the Wagner's grade of ulcer, peripheral pulses and any sensory deficits were noted. Ulcers involving the toes were recorded according to the individual toes involved. Ulcers were graded into six grades (grade 0 –– grade V) based on Wagner's classification System.[8] All patients were followed up until discharge from the hospital.

Microbiologic Sample Collection
Specimens for swab culture were obtained from the ulcer after washing the ulcer with saline and then applying a sterile cotton tipped swab stick to the base of the ulcer for 5 to 10 seconds. The swab was put into a transport medium and sent to the microbiology laboratory as soon as possible. Tissue samples were obtained after washing and debridement of the ulcer by scrapping the ulcer base or the edges of the wound with a sterile curette and stored in sterile containers before being transported to the microbiology laboratory. Blood culture was done only for patients suspected with septicaemia. Specimens were then transported to the microbiology laboratory for further processing. The samples were inoculated on blood agar, MacConkey agar or chocolate agar and Sabouraud dextrose agar. The inoculated plates were then incubated aerobically at 37°C for 24-48 hours. Isolated organisms were then identified by conventional microbiological methods.[9]

Antibiotic Sensitivity Test (AST)
AST was performed by using disk diffusion method using Mueller-Hinton agar as described by the Clinical and Laboratory Standards Institute (CLSI) 2011.[10] The panel antibiotics used were amoxicillin (10µg/ml), cefuroxime (30µg/ml), gentamicin (10µg/ml), cotrimoxazole (30µg/ml), cefoperazone (75µg/ml), amoxicillin/clavulanic acid (30µg/ml), ceftazidime (30µg/ml), imipenem (10µg/ml), ciprofloxacin (5µg/ml), amikacin (30µg/ml), cefotaxime (30µg/ml), meropenem (10µg/ml), cefoxitin (1µg/ml), penicillin G (10µg/ml), erythromycin (15µg/ml), fucidic acid (5µg/ml), vancomycin (30µg/ml), clindamycin (2µg/ml), piperacillin (100µg/ml), piperacillin/tazobactam (10/100µg/ml), cefepime (30µg/ml), oxacillin (1µg/ml), tetracycline (30µg/ml), ceftriazone (30µg/ml), cephalexin (30µg/ml), ampicillin/sulbactam (10µg/ml), sulperazone (30/75µg/ml), netilmicin (30µg/ml), polymyxin B (30µg/ml), and linezolid (30µg/ml).

Statistical analysis
Data was analysed by using SPSS version 20.0 for descriptive statistics. Quantitative variables were expressed as means ± SD while qualitative variables were expressed as percentage (%). Association was determined by using Chi-Square Test.

RESULTS

Clinical characteristics
There were 77 known diabetic patients included in the study. Of them 47 were males (61%) with male to female ratio of 1.6:1. Their age ranged between 26 and 83 years with mean age of 56.7 years. Most patients were ethnic Malays 72.7%, followed by Indians 15.6% and Chinese 11.7%. Wagner's grade III ulcer was the commonest grade of ulcer seen in 27 patients (35.1%) as shown in Table I. Toes were the common anatomical site of ulcers (48%). Anatomical distribution of the ulcers is summarised in Table II. Although 27 patients (35.1%) had Grade III ulcers but only 21 had radiological evidence of osteomyelitis at the time of presentation to the hospital. But clinically the bone was visible in the depths of the ulcer in all 27 patients.

Microbiological Investigations
A total of 96 samples were collected from 77 patients. Samples consisted of 77 wound swabs (80.2%), 11 debrided tissues (11.4%), and eight blood (8.3%). These samples yielded 83 isolates. There was no growth from 14 samples which included all the eight blood samples, five wound swabs and one debrided tissue.

Samples from 6 (7.8%) patients had no growth, 61 (79.2%) had mono-microbial infection and ten patients (13%) had poly-microbial infection (eight patients, each had two microorganisms isolated and two patients, each had three microorganisms isolated).

Out of 83 isolates, 59 isolates were Gram negative microorganisms (71.1%) and 23 Gram positive microorganisms (27.7%). Only one fungus was isolated (1.2%) from a swab sample. No microorganisms were isolated from blood cultures. Details of types of microorganisms isolated from various samples are summarised in Table III.
The types of microorganisms isolated are correlated with the Wagner's grade of ulcer in Table IV. Both Gram positive as well as Gram negative microorganisms were almost equally cultured from Wagner grade 0, I and II ulcers (14 and 16 isolates, respectively). However, Gram negative microorganisms were more frequently cultured from Wagner grade III and IV ulcers, 42 isolates compared to nine isolates which were Gram positive. However, it was not statistically significant. Fungus was cultured from one swab sample of grade II ulcer.

Table I: Number of patients in relation to Wagner's grade of ulcer

Grade	O	I	II	III	IV	V	Total
Patients (%)	12 (15.6)	5 (6.5)	9 (11.7)	27 (35.0)	23 (29.9)	1 (1.3)	77

Table II: Anatomical site of ulcers

Site of ulcer	Number of patients	(%)
Plantar aspect	20	(26.0)
Dorsal aspect	13	(16.9)
Entire foot	7	(9.0)
Great toe	10	(13.0)
Second toe	3	(3.9)
Third toe	3	(3.9)
Fourth toe	4	(5.2)
Little toe	9	(11.7)
Multiple toes	8	(10.4)

Table III: Distributions of microorganism isolated from different types of samples in 77 patients

Types of Microorganism	Swab n (%)	Tissue n (%)	Total n (%)
Gram negative bacteria			
Proteus mirabilis	16 (22.2)	1 (9.1)	17 (20.5)
Pseudomonas aeruginosa	14 (19.4)	2 (18.2)	16 (19.3)
Klebsiella pneumoniae	9 (12.5)	2 (18.2)	11 (13.3)
Enterobacter clocae	3 (4.2)	-	3 (3.6)
Acinetobacter baumanii	1 (1.4)	-	1 (1.2)
Morganella morganii	2 (2.8)	1 (9.1)	3 (3.6)
Escherichia coli	6 (8.3)	-	6 (7.2)
Providencia stuartii	1 (1.4)	1 (9.1)	2 (2.4)
Gram positive bacteria			
Staphylococcus aureus	10 (13.9)	1 (9.1)	11 (13.3)
Streptococcus Group A	1 (1.4)	-	1 (1.2)
Streptococcus Group B	4 (5.5)	3 (27.2)	7 (8.4)
Streptococcus Group G	1 (1.4)	-	1 (1.2)
Enterococcus sp.	2 (2.8)	-	2 (2.4)
Archanobacteria haemolyticum	1 (1.4)	-	1 (1.2)
Fungi			
Candida sp.	1 (1.4)	-	1 (1.2)
Total	**72 (100)**	**11 (100)**	**83 (100)**

Antibiotic susceptibility patterns

Details of the antibiotic resistance patterns for Gram negative and Gram positive micro-organisms are shown in Table V and Table VI, respectively. Of the cultured Proteus mirabilis isolates, 43.8% were resistant to ampicillin, and 29.4% were resistant to cotrimoxazole. However, P. mirabilis showed 100% sensitivity towards amikacin, imipenem, meropenem, and ceftazidime.

Klebsiella pneumoniae, *Escherichia coli,* and *Enterobacter clocae* showed 100% sensitivity towards amikacin, ciprofloxacin, imipenem, and meropenem. Enterococcus sp. was 100% sensitive to gentamicin, vancomycin, and ampicillin.

Of the cultured *Staphylococcus aureus* 72.7% were resistant to penicillin G; and 36.4% were resistant to both clindamycin and cloxacillin. MRSA were 100% sensitive to vancomycin.

DISCUSSION

DFU are prone to microbial infections. Antibiotic therapy is a part of the management of the infected wound in addition to local wound care, control of blood glucose level and general treatment of the patient.[11] Late presentation to the hospital for treatment is common, as majority of the patients in present study had Wagner grade III (35.1%) and grade IV (29.9%). A previous study from Nigeria[12] reported Wagner's grade II ulcer as the most common presentation involving 37% patients, this suggests that our patients did seek treatment only in the late stages.

In the present study, 79% patients had mono-microbial infection, 13% had poly microbial infection and 8% had sterile cultures. Earlier study from Malaysia reported 57.2% of patients with mono-microbial infection.[13] However, poly-microbial growth has been reported as more common in

Table IV: Types of microorganisms isolated in relation to the Wagner's grade of ulcer

Types of Microorganism	Wagner 0	Wagner I	Wagner II	Wagner III	Wagner IV	Wagner V	Isolates (%)
Gram negative bacteria							
Proteus mirabilis	0	0	0	7 (28)	9 (34.6)	1 (100)	17 (20.5)
Pseudomonas aeruginosa	3 (25.0)	1 (12.5)	2 (18.2)	5 (20)	5 (19.2)	0	16 (19.3)
Klebsiella pneumoniae	1 (8.3)	1 (12.5)	3 (27.3)	2 (8.0)	4 (15.2)	0	11 (13.3)
Enterobacter clocae	1 (8.3)	1 (12.5)	0	1 (4.0)	0	0	3 (3.6)
Morganella morganii	0	0	1 (9.1)	0	2 (7.7)	0	3 (3.6)
Escherichia coli	1 (8.3)	1 (12.5)	0	2 (8.0)	2 (7.7)	0	6 (7.2)
Providencia stuartii	0	0	0	2 (8.0)	0	0	2 (2.4)
Acinetobacter baumanii	0	0	0	0	1 (3.8)	0	1 (1.2)
Gram positive bacteria							
Staphylococcus aureus	2 (16.7)	1 (12.5)	2 (18.2)	5 (20.0)	1 (3.8)	0	11 (13.3)
Streptococcus Group A	0	1 (12.5)	0	0	0	0	1 (1.2)
Streptococcus Group B	2 (16.7)	2 (25.0)	2 (18.2)	0	1 (3.8)	0	7 (8.4)
Streptococcus Group G	1 (8.3)	0	0	0	0	0	1 (1.2)
Enterococcus sp.	0	0	0	1 (4.0)	1 (3.8)	0	2 (2.4)
Archanobacterium haemolyticum	1 (8.3)	0	0	0	0	0	1 (1.2)
Fungi							
Candida sp.	0	0	1 (9.1)	0	0	0	1 (1.2)
Total isolates	**12**	**8**	**11**	**25**	**26**	**1**	**83**

Table V: Antibiotic resistance patterns of Gram negative microorganisms

Antibiotic	Proteus mirabilis (%)	Klebsiella pneumonia (%)	Escherichia coli (%)	Enterobacter clocae (%)	Pseudomonas aeruginosa (%)
Amikacin	0	0	0	0	6.2
Cefoperazone	6.2	10.0	0	100	0
Cefuroxime sodium	18.8	10.0	0	0	-
Cotrimoxazole	35.7	14.3	50.0	0	-
Ampicillin	43.8	88.9	50.0	66.7	-
Gentamicin	12.5	10.0	0	0	13.3
Ciprofloxacin	10.0	0	0	0	14.3
Imipenem	0	0	0	0	0
Meropenem	0	0	0	0	14.3
Amoxicillin/clavulanic acid	8.3	-	0	100	-
Ceftazidime	0	12.5	0	0	6.2
Ampicillin/sulbactam	-	-	-	0	100
Piperacillin	-	-	-	-	14.3
Piperacillin/tazobactam	-	-	-	-	15.4
Amoxicillin	-	20.0	0	-	-

Table VI: Antibiotic resistance patterns of Gram positive microorganisms

Antibiotic	Staphylococcus aureus (%)	Streptococcus Group B (%)	Enterococcus sp. (%)
Clindamycin	36.4	0	-
Gentamicin	18.2	-	0
Penicillin G	72.7	0	-
Erythromycin	27.3	20.0	-
Cotrimoxazole	18.2	60.0	-
Oxacillin	36.4	-	-
Fucidic acid	27.3	-	-
Rifampin	9.1	-	-
Vancomycin	0	-	0
Tetracycline	-	60.0	-
Cephalexin	-	0	-
Linezolid	-	-	50.0
Ampicillin	-	-	0

Table VII. Comparison data from previously published studies.

No.	Study	No. of patients	Wagner's Grade	No. of specimens	No. of isolates	Gram +ve %	Gram -ve %	Poly-Microbial %	Mono-microbial %	MRSA %
1.	El-Tahawy[17] (1999)	111	-	111	161	40	54	39	61	30
2.	Ako-Nai *et al*[12] (2006)	27	II and above	152	152	34.5	66.2	88.8	10	-
3.	Gadepalli *et al*[14] (2006)	80	III - V	80	183	41	59	57.5	42.5	56
4.	Raja[13] (2007)	194	-	200	287	45	52	43	57	5
5.	Citron *et al*[11] (2007)	433	-	454	1607	80.3	19.7	84	16.2	4.4
6.	Bansal *et al*[18] (2008)	103	-	118	157	24	76	35	65	55
7.	Present study (2011)	77	I - V	96	83	27.7	71.1	13	79.2	6

other studies; 82.5% from India[14] and 90.8% from Nigeria.[12] Poly-microbial infections were commonly reported in Grade V ulcers.[15] In our study, out of 77 patients only one patient had Grade V ulcer thus that may be the explanation for the fewer number of poly-microbial infection.

In the present study, Gram negative microorganisms were predominantly cultured (71.1%). This result concurs with several other studies as summarised in table VII. We realise there are shortcomings in our method of swab collection which requires further standardisation. This we hope to achieve in future with the full study.

P. mirabilis was the most frequently isolated gram negative microorganism in this study, (n=17, 20.5%). Whereas, a study from India reported *P. aeruginosa* as predominant isolated microorganism (n=31, 19.7%).[18] Different geographical area could have contributed to different types of microorganisms isolated.[14]

Fungal infection was not common. Fungus was isolated from only one wound swab in this study. Similarly, another study from Malaysia[13] reported only two out of 287 isolates (0.7%) cultured were fungus.

Antibiotic sensitivity tests showed that Gram negative microorganisms were mostly sensitive to second and third generation of cephalosporin and combination of β-lactam and β-lactamase inhibitors antibiotic. Out of 17 isolates of *P. mirabilis* seven were resistant to ampicillin in this study (43.8%). Other studies, from Malaysia[13] and Egypt[17] reported isolated *P. mirabilis* to have higher percentage of resistance towards ampicillin as 62% and 67% respectively. *P. mirabilis* was found to be 100% sensitive to amikacin and ceftazidime in this study. However, in Egypt, it was 94% sensitive.[17]

Out of total of 83 isolates in this study, 11 isolates were of *S. aureus,* out of these 5 were methicillin-resistant Staphylococcus aureus (MRSA) which is about 6% of total isolates. This contrast with the previous study from Malaysia which isolated MRSA from 5% of the total isolates.[13] However, other studies have reported even higher percentage of MRSA between 30 to 50%, which is very worrying.[14,16-18] In our study, the MRSA were all sensitive to vancomycin which is the preferred antibiotic recommended by the Malaysian National Antibiotic Guidelines.[19]

There is a distinct group of patients with DFU who had poor renal function. This precludes the administration of antibiotics specially those which are metabolised in the kidneys. Further, the empirical use of antibiotics, before the culture and sensitivity is available, has increased the risk of MRSA and drug resistant microorganism infections.[14,18] We have somewhat modified the treatment regimen for the DFU. Following are our suggestions.

For Wagner's Grade I and II ulcers to give footbaths either with aqueous 1 in 2000 chlorhexidine solution or 1 in 100 povidone solution for 10 to 15 minutes once or twice a day, depending on the status of the ulcer, followed by application of newer de-sloughing/ wound cleaning agents if indicated. No empirical antibiotics are prescribed. Antibiotics are withheld till the culture report is available.
For Wagner Grade III and higher ulcers to start metronidazole (flagyl) and gentamycin in patients provided the serum creatinine is below 90 micromols/l. Closely monitor the renal function till the culture results are available and change the antibiotics as indicated. Also use footbaths and wound de-sloughing/cleaning agents. The empirical use of antibiotics in this group, where patients come with foul smelling ulcers, is to cover for the anaerobic and increased frequency of Gram negative micro-organisms as reported by several authors.[12-14,17,18]

For patients with poor renal function we clean the wound twice or thrice a day by giving footbath with 1 in 2000 aqueous chlorhexidine solution or 1 in 100 povidone solution for 10 to 15 minutes. De-sloughing of the wound as required. Empirical antibiotics are prescribed only when the patient is septicaemic. Indicated antibiotics are prescribed after the culture report is available.

Surgical debridement for all patients is done when indicated as per the opinion of the surgeon.

We hope to report the effects of changes in our treatment regimen in the future study.

CONCLUSIONS

Wagner grade III and IV ulcers were the most common presentation of DFU. Gram negative microorganisms were commonly isolated. Proteus mirabilis was the common Gram negative microorganisms cultured. Staphylococcus aureus was the common Gram positive microorganism cultured. Contrary to the trends majority of patients had mono-microbial infection. Worryingly, MRSA were more frequently isolated as compared to the previous study from Malaysia.[13] More than 40% of Gram negative micro-organisms cultured were resistant to ampicillin. Empirical use of antibiotics prior

to availability of culture sensitivity should be curtailed to prevent development of MRSA and drug resistant strains of micro-organisms.

We advocate more use of antiseptic solutions to control the infection rather than empirical use of antibiotics. Results of our altered treatment regimen we plan to publish in a later study.

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