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Original article

Microbiology of Diabetic Foot Ulcer

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Abstract

The global prevalence of diabetes mellitus has been projected to nearly double from a baseline of 2.8% in 2000 to 4.4% by 2030 affecting over 350 million individuals. Diabetic patients have a life time risk of 25% for developing foot ulceration. Diabetic ulcers have 15-46 times higher risk of limb amputation compared with foot ulcers due to other causes. Every 30 seconds one limb is amputated globally because of diabetic foot ulcers. Peripheral neuropathy & peripheral vascular disease is present in more than 10% of cases at the time of diagnosis. Diabetic foot infections often present with subtle clinical features because of impaired leukocyte function, ischemia & peripheral neuropathy. A high degree of suspicion for infection has to be maintained especially in the case of patients with the greater risk. Serious limb threatening infection may result in systemic toxicity. This study includes 84 patients with diabetic foot ulcer infection admitted to Al Wahda Hospital, Derna, Libya from September 2018 to August 2019. Pus discharge or infected tissue specimens obtained from the patients sent to laboratory for culture and sensitivity test. The cultures revealed the presence of single organisms in 77 patients (91.67%) either gram positive or gram negative while polymicrobial growth was seen in 7 patients (8.3%). The most common cultured organism was Staphylococcus aureus (17 cases) which was sensitive to penicillin and vancomycin. Escherichia coli was the second common organism (16 cases) and was sensitive to amikacin, cefetrizole and gentamicin.

Keywords. *Diabetic Foot Infection, Culture and Sensitivity.*

Introduction

The global prevalence of diabetes mellitus has been projected to nearly double from a baseline of 2.8% in 2000 to 4.4 % by 2030 affecting over 350 million individuals [1]. Diabetic patients have a lifetime risk as high as 25% for developing foot ulceration [2]. Peripheral neuropathy and peripheral vascular disease are present in more than 10% of cases at the time of diagnosis [4]. Diabetic foot infection often presents with subtle clinical features because of impaired leukocyte function, ischaemia and peripheral neuropathy. A high degree of suspicion for infection has to be maintained.

Association of two or more features such as pus, erythema, induration swelling, pain, tenderness or warmth is indicative of infection. Serious limb threatening infection may result in systemic toxicity, only in 12-35% of these cases the fever is significant [7]. High grade fever may be indicative of deep-seated infection in tissue space, tissue necrosis and undrained pus with the potential of hematogenous spread of infection. Erythrocyte sedimentation rate (ESR) and C-Reactive Protein concentration may be normal [9,10]. Elevated concentration of C reactive protein and procalcitonin can help distinguish mild or moderately infected ulcers from those that are not infected [11]. In addition to the presence of classic pathogens foot ulcers are often contaminated or colonized by commensal organisms that on occasion become pathogens.

Clinicians should routinely use a validated classification system when assessing the severity of diabetic foot infection [12]. Wagner system of classification is widely used [13]. Classification of the severity of infection have been developed by Infectious Diseases Society of America (IDSA) and the International Working Group on Diabetic Foot (IWGDF) [14,15,16,17]. IWGDF uses the acronym PEDIS to classify diabetic foot wounds. PEDIS stands for PErfusion, Depth, Infection and Sensation [17]. Increased severity in the IDSA classification into moderate and severe correlates with the need for hospitalization and amputation [18]. Besides wound assessment the affected limb and foot should be assessed for signs of arterial ischaemia, venous insufficiency, neuropathy or biomechanical factors which promote infection [19]. Systemic signs and symptoms of infection include fever, chills, alteration in mental status, hemodynamic instability, hyperglycaemia, acidosis or renal failure. A thorough and systematic evaluation and categorization of foot ulcers helps to guide appropriate treatment [20].

Methods

84 patients with diabetic foot ulcer infection were hospitalized from September 2018 to August 2019 at Al Wahda Hospital, Derna. Patients below the age of 18 years and those with post-operative infections and multiple septic foci were excluded from the study. Pus discharge or infected tissue specimens obtained from the patient were at once sent to the laboratory and processed. Besides performing Gram stain samples were inoculated on suitable culture media such as blood agar and MacConkey's agar for isolating aerobic bacteria. After incubating for 24 hours at 37^0C, bacterial isolates were identified based on standard bacteriological methods. Antibiotic susceptibility testing was performed using

Kirby Bauers disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines. The Gram-negative colonies further identified using API system. Staphylococcal isolates were tested for coagulase enzyme production to confirm the presence of *Staphylococcus aureus*. Streptococci were grouped into A, B, C, D and G. The diabetic foot ulcers were graded using Wagner's classification system.

Wagner Classification of Diabetic Foot Ulcers

- Grade 0- No ulcers in a high-risk foot
- Grade 1- Superficial ulcer; Full thickness skin involved underlying tissues not involved
- Grade 2-Deep ulcer; Penetrates upto muscles and ligaments, bones not involved
- Grade3- Ulcer with cellulitis or abscess with osteomyelitis
- Grade 4- Localized gangrene
- Grade5- Extensive gangrene involving the whole foot.

Chronic ulcers referred to those with no improvement after 4 weeks of treatments or those not cured within 8 weeks. Demographic details were obtained. A through clinical examination was performed. Peripheral neuropathy assessed using monofilament and vibration sense was tested using tuning fork (128 Hz). Peripheral vasculature evaluated with ABI. Baseline laboratory investigations, fundoscopy and ECG done.

Results

Out of the 84 patients, 55 patients were male and 29 patients were female. The age ranged from 35 to 70 years; 5 patients were below 40 years of age, 16 between 41-50 years, 40 patients between 51-60 and 23 patients were between 61-70 years. 35 patients were known to have ishaemic heart disease (41.7%), hypertension was found in 51 patients (60.7%), for glycaemic control 52 patients were taking oral hypoglycaemics (61.9%) and 32 patients had been on insulin therapy (38.1%).

Table 1. Demographical and Clinical Data of Patients.

Varibales	**Number**
Sex of patients	
Male	55
Female	29
Age distribution	
Below 40 years	5 patients
Between 41-50 years	16 patients
Between 51-60 years	40 patients
Between 61-70 years	23 patients
Comorbidities	
Ischaemic Heart disease	35 patients (41.7%)
Hypertension	51 patients (61.9%)
Complications	
Neuropathy	66 patients (78.57%)
Nephropathy	37 patients (44%)
Glycaemic control	
Minimum FBS	80 mg
Maximum PPBS	360 mg
Oral Hypoglycaemics	52 patients (61.90%)
Patients on Insulin therapy	32 patients (38.09%)
HbA1c	

Good control	5 patients (5.95%)
Fair control	18 patients (21.42%)
Poor control	39 patients (46.43%)
Very poor control	22 patients (26.20%)
Wagner grading	
Grade I	22 patients (26.20%Z
Grade II	59 patients (70.20%)
Grade III	2 patients (3.60%)

Diabetic complications were searched for by consulting different specialties. The degree & extension of diabetic foot wound were classified in all patients.
In 77 patients (91.68%) the cultures revealed the presence of single organisms, either gram positive or gram negative.
Polymicrobial growth was seen in 7 patients (8.3%)
Out of the 28 patients with gram positive cultures showed the following organisms.

- 17 Patients with *Staphylococcus Aureus.*
- 5 Patients with Beta hemolytic streptococcus.
- 6 Patients with *Enterococcus faecalis.*

The other 49 patients of gram-negative culture had growth are as follows:

- *Pseudomonas aeruginosa* (n=10)
- *E. coli* (n=16)
- *Klebsiella pneumoniae* (n=12)
- Enterobacter spp (n=3)
- *Proteus vulgaris* (n=3)
- *Pseudomonas fluorescence* (n=2)
- *Morganella morgonii* (n=2)
- *Citrobacter freundii* (n=1)

Among Gram positive organisms a majority of *Staphylococcus aureus* were sensitive to vancomycin, oxacillin, & penicillin. MRSA was not grown in any of the cultures. *E. coli* was sensitive to netilmicin, imipenem & piperacillin/tazobactam. Klebsiella was sensitive to ceftazidime, imipenem, piperacillin tazobactam.

Table 2. Antibiotic Sensitivity of Gram-Negative Bacteria

Bacteria (no. of Isolates)	A/C	P/T	TE	CI	TS	GM	AK	NC	CFX	CTR	CAZ	IP
Escherichia coli (16)	70	88	35	32	28	30	72	100	16	32	-----	100
Klebsiella pneumoniae (12)	50	72	55	46	10	35	70	----	17	20	100	97
Proteus mirabilis (7)	30	100	100	80	50	80	100	----	40	100	100	100
Proteus vulgaris (3)	20	100	65	65	15	33	50	----	17	80	100	100
Citrobacter freundii (1)	40	100	14	30	14	43	87	----	30	71	----	100
Morganella morganii (2)	----	96	----	65	40	53	53	----	----	76.9	88	----
Pseudomonas aeruginosa (10)	100	80	----	59	---	50	65	45	----	----	29	90
Pseudomonas Fluorescens(2	----	100	----	0	---	0	100	50	----	----	0	50

Table 3. Antibiotic Sensitivity of Gram-Positive Bacteria.

Bacteria (No. of Isolates)	**P**	**A/C**	**E**	**TS**	**TE**	**CI**	**GM**	**CTR**	**OX**	**VA**
Staphylococcus Aureus (17)	10	100	70	99	70	78	90	90	100	100
Beta Haemolytic strep (5)	100	----	50	21	25	100	60	50	95	60
Enterococcus faecalis (6)	90	----	58	----	20	76	61	95	50	100

Discussion

Diabetic ulcers have 15-46 times higher risk of limb amputation compared with foot ulcers due to other causes [3]. A minimally inflamed deep ulceration may be associated with osteomyelitis [8]. Clinicians should evaluate any foot wound for the possibility of infection [5,6]. Our finding showed a relatively fewer number of patients 8.3% were infected by two or more pathogens (polymicrobial)

compared with 91.68% of patients had monomicrobial growth. A possible reason for the low incidence of polymicrobial infection in our study could be because of superficial subcutaneous infections.
As the gram-negative microbes are predominant pathogens isolated, it is essential to select antibiotics that are more effective against Gram negative bacteria in contrast to gram positive organisms,
E. coli, Klebsiella pneumoniae & Pseudomonas aeruginosa were the majority of causative gram-negative organisms. Among the Gram-positive microorganisms *Staphylococcus aureus, Enterococcus faecalis*, Beta hemolytic streptococcus were more predominant.
With regard to the susceptibility patterns, imipenem, ceftazidime, piperacillin & amikacin appeared to be the best antibiotics for therapy against Gram positive and Gram-negative organisms respectively.
Amikacin is associated with nephrotoxicity, which can deteriorate patients who already have pre-existing diabetic nephropathy & in the present study there were 37 patients who had nephropathy constituting 44% of the total number of patients, thus limiting its use in those cases.
In the present study, the severity of infection was proportionate with the depth of infection and the majority of infections were categorized as being superficial, subcutaneous.
Staphylococcus aureus is sensitive to vancomycin, oxacillin, & ceftriaxone. If the infection involves deeper tissue, then it could be polymicrobial in nature and more likely due to Gram negative microorganisms in different combination. If the infection involves deeper tissue or bone, ceftazidime, imipenem, and piperacin and tazobactam are more appropriate with sensitivity of 98-100%.
The decision on proper management of diabetic foot infection is difficult and is still matter of debate. The main stay of management of diabetic foot ulcer with infection is isolation and identification of microbial cause and treatment with appropriate & sensitive antibiotics.

Conclusion

Our study has showed that 8.3% of diabetic foot infections were polymicrobial. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most commonly identified Gram negative and Gram-positive microorganisms. Amikacin, vancomycin & ceftazidime were the most effective antimicrobial therapy. Levofloxacin and imipenem are also very effective in empiric treatment. Choosing empiric antibiotic therapy should depend upon the clinical features of the infections and the local pattern of bacterial etiology. In the management of diabetic foot ulceration, besides antibiotic therapy what is more needed is to reduce the bio-burden of the wound, this we do with sharp surgical debridement. By far repeated debridement are helpful in reducing the bio burden as is well known the number of bacteria per gram of tissue is a criterion for establishing the diagnosis of an infection, we are mechanically getting the wound cleaned. It has to be meticulously done & is rarely complete in one sitting.
It cannot be over emphasized that the problem of managing diabetic foot infection is multifaceted & antibiotic therapy is one of the factors involved so also is the glycemic control which goes a long way in controlling infections. Non enzymatic glycosylation is known to affect muscles tendons & joint mobility which are contributory to high plantar pressure, the root cause of tissue destruction.

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Conflicts of Interest

The authors declare no conflict of interest.

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