Original Research Article　　　　http://dx.doi.org/10.20546/ijcmas.2016.512.001

Microbiological Profile of Diabetic Foot Ulcer and Use of IL6 as a Predictor for Diabetic Foot Infection

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A B S T R A C T

To describe the microbiological profile of diabetic foot infections and what is the most common microorganism and to determine if the IL 6 can be used as a predictor of the diabetic foot infection. The study included 80 patients with diabetic history attended to the outpatient clinic of Diabetic foot, Internal specialized hospital, Mansoura University. 60 patients were the study group and 20 were the control group over a period from February 2015 to January 2016. Samples were cultured and identified microbiologically. The samples were measured fo the IL6 by ELISA. The antibiotic sensitivity test was done. Data for clinical presentation, bacteriology and management were collected. Statistical analysis was done. 80 patients were included. 60 patients were with infected foot lesions, 7 with uninfected lesions, 7 have diabetes only with lesion and 6 were normal person. Gran negative bacteria were more common than Gram negative bacteria. *Klebsiella* species were the commonest. Meronem was the most effective antibiotic for the Gram negative and Gram positive bacteria. There was a significant relation between the measured IL6 and the study group than the control group but not with the different microorganism pattern. Gram negative bacteria were the most common isolated bacteria from the diabetic foot infection. IL6 can be used as a predictor of the diabetic foot infection.

Introduction

People with DM have an increased risk of developing a number of serious health problems. Consistently high blood glucose levels can lead to serious diseases affecting the heart and blood vessels, eyes, kidneys, nerves and teeth. In addition, people with DM also have a higher risk of developing infections (International Diabetes Federation, 2015). Fifteen per cent of people with DM will develop a foot ulcer at some time during their life, and 85% of major leg amputations begin with a foot ulcer.

Poorly controlled DM is prone to skin infections because elevated blood sugar

reduces the effectiveness of bacteria fighting cells. Carbuncles, boils, and other skin infections may be hazardous if not properly treated (Hena *et al*., 2010).

Diabetic foot is one of the most feared complications of diabetes mellitus. The diabetic foot disease is associated with significant morbidity and mortality even to the endpoint amputation. Diabetes and its complications as foot ulcers may develop as a result of poly neuropathy, ischemia or subclinical inflammation causes or as a result of all. The term "diabetic foot" may consist of a mix of pathologies including diabetic neuropathy, peripheral vascular disease, Charcot's neuroarthropathy, osteomyelitis and foot ulceration. Diabetes is associated with 2-3 fold-increased risk of accelerated atherosclerosis. Hence those subjects with peripheral vascular disease are predisposed to poor wound healing (Sallam *et al*., 2012).

The alarming fact is that Egypt has more diabetic individuals than any other country and the incidence of foot problems and amputations remains very high, accounting for up to 20% of diabetes-related hospital admissions. This can be easily attributed to several practices prevalent in Egypt, such as barefoot walking, inadequate facilities for diabetes care, low socioeconomic status, and illiteracy (Hefni *et al*., 2012).

All diabetic foot ulcers are contaminated with a variety of organisms. Foot infection is the most common infectious cause of hospitalization in patients with DM (El-Tahawy, 2000).

The finding of an altered immune status in patients with foot ulceration is interesting in several ways. Only some markers of inflammation were up regulated (C-reactive protein (CRP), fibrinogen and interleukin 6 (IL-6) and others were not (interleukin 8

(IL-8) and interleukin 18 (IL-18) (Weigelt *et al*., 2009).

In this study, we aimed to detect the different microorganisms from the diabetic foot infections and to determine the most susceptible antibiotic for these microorganisms. Also, we tried to find the correlation of the IL 6 and the diabetic foot infections and if it can be a predictor of the diabetic foot infection.

Methods and subjects

This study was performed in the Medical Microbiology and Infection Control Unit (MMICU) from patients attended the outpatient clinic of Diabetic foot, Internal specialized hospital, Mansoura University. It was conducted in the period from February 2015 to January 2016. Eighty patients were included in this study. Out of them, 60 patients were the study group and 20 were the control group. In the study group, the leg ulcer was on the right side in 34 (56.7%) cases and on the left side in 26 (43.3%) cases. It was grade 2 in 54 (90%) cases and grade 3 in 6 (10%) cases. The median ulcer size was 3.33cm in the control group, the leg ulcer was on the right side in 4 (57.1%) cases and on the left side in 3 (42.9%) cases. All the control group cases were grade 2. The median ulcer size was 1.8cm.

History was taken from all patients to study the risk factors; obesity, smoking, DM, hypertention, neuropathy, retinopathy, nephropathy, ischemic heart disease and osteomyelitis. All patients were subjected to general clinical examination and diabetic ulcer examination. Vascular insufficiency and peripheral neuropathy were assessed. Diabetic foot was characterized according to the International Working Group on the Diabetic Foot Classifications of Diabetic Foot Infection (table 1)**.**

Complete blood picture, glycosylated hemoglobin, fasting and post prandial blood sugar, serum creatinine, liver function tests, blood lipid profile and urine examination for presence of albumin and glucose were done. Pus and blood samples were taken from patients with diabetic foot ulcer and with no antibiotic history for two days. The samples were transported immediately to the Medical Microbiology and Infection Control Unit (MMICU) lab. The samples were first examined by Gram staining. The pus samples were cultured aerobically, anaerobically and for mycological culture. For aerobic cultures, the pus was cultured on the ordinary media (blood, chocolate and MacConkey's agars). The plates were incubated at 37°C for 48 hours. For anaerobic culture, the pus aspirates were cultured on blood agar plate and incubated at 37°C for 48 hours. Mycological analysis was performed by direct examination and culture in Sabouraud Dextrose medium with chloramphenicol and cyclohexamide.

The cultures were incubated at room temperature for at least three weeks. For positive bacterial isolates, routine biochemical reactions were done using the API system. The bacterial isolates were subjected to susceptibility testing by standard Kirby Bauer disc diffusion methods (5). The susceptibility patterns of the bacterial isolates were detected following the antimicrobial agents panel recommended by Clinical Laboratory Standard Institute (CLSI), 2010. The diameter of the inhibition zone was measured in millimeters and interpreted as per CLSI guidelines. The antimicrobial discs included in this study were amoxicillin, ceftriaxone, ceftazidime, cefepime, ampicillin, amoxicillin, amoxicillin + clavulanate, cefoperazone, imipenem, meropenem, gentamicin, amikacin, vancomycin, trimethoprim-sulfamethoxazole, clindamycin, tazocin,

cefotaxime, cefuroxime, piperacillin, aztreonam, doxycycline and erythromycin. 5ml of venous blood were taken by sterile syringe and put in sterile dray test tube. Serum interleukin 6 (IL 6) was measured by enzyme-linked immunosorbent assay (ELISA) in the serum samples according to the manufacturer's instructions.

Patients with foot infections due to any other causes such as non-diabetics - post traumatic, arterial disorder alone, venous disorder alone, non-diabetic peripheral neuropathy and secondary to implant infection, surgical debridement, gangrenous wounds, those with a dry Escher, and antibiotic use before hospitalization were excluded.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel 2010 for windows (Microsoft Cor., Redmond, WA, USA). Continuous data are expressed as the mean ± SD & median (range), and the categorical data are expressed as a number (percentage).

Continuous variables were checked for normality by using Kolmogorov-Smirnov test. Mann-Whitney U test was used to compare two groups of non-normally distributed data and independent t test for parametric data. Percent of categorical variables were compared using the Chi-square ($\chi2$) test and Fischer exact test for >50% of cell count < 5 whenever needed

Comparing studied groups, one way ANOVA test for of parametric data and Kruskal Wallis test for non parametric data. Spearman correlation was done for non parametric correlation between IL 6, ulcer size and duration of disease . All tests were

two tailed. $p \le 0.05$ was considered statistically significant, $p < 0.01$ was considered highly statistically significant and $p > 0.05$ was considered non statistically significant).

Results and Discussion

Eighty patients with diabetic history attended to the outpatient clinic of Diabetic foot, internal specialized hospital, Mansoura University were included in this study in the period from September 2011 to July 2014. 60 patients were with infected foot lesions, 7 with uninfected lesions, 7 have diabetes only with lesion and 6 were normal person. There were significant correlation between patients with diabetic foot lesion and obesity, neuropathy, nephropathy and osteomyelitis (table 2). There were no significant correlation between the case or the control group and the age (t=5.18 P=0.133) or the gender ($\chi2$=0.07 P=0.79). Out of the 60 control group, 11 (18.3%) patients were subjected to amputation.

Gram-negative bacteria were isolated in 40 () cases, Gram-positive bacteria in 28 () cases and the anaerobic bacteria in 18 () cases. The different bacterial isolates were declared in table3.

The antibiotic sensitivity testing was done for all the bacterial isolates (table 4). Meronem was the most effective antibiotic for the Gram negative bacteria while the most resistant antibiotic was carbencillin. As regards the Gram negative bacteria, meronem was the most sensitive antibiotic and cefazolin was the most resistant antibiotic (table 5). The antibiotics sensitivity pattern of the anaerobic bacteria revealed that meronem was the most sensitive antibiotic while cefeperazone was the most resistant antibiotic (table 6).

There was a significant relation between the measured IL6 and the study group than the control group (table 7). There were no significant correlation between IL6 level and the different bacterial isolates (P=0.49), ulcer size (r=-0.02 p=0.9) or the duration of the disease (r=0.07 p=0.59). Median level of IL6 was 16.67 in anaerobic bacteria, 12.63 in Gram-positive bacteria and 11.7 in Gram-negative bacteria.

Diabetic foot infections are a rising problem worldwide. Patients with Diabetes regularly have constant foot ulcers. The constant non healing ulcers are more susceptible to infection that leads to decrease the healing procedure.

All patients had neuropathy100%, about 85% were under insulin treatment of diabetes, and around 70% were obese, 68.3% had hypertension, 66% with retinopathy 46.7%with ischemic heart diseases, 36% had nephropathy and 35% were tobacco smoker. As in Ahmed *et al*., (2012) the prevalence of hypertension was 57%, that of neuropathy was 62%, that of nephropathy was 17%, and that of retinopathy was 30%.

As regarded to ulcer; there was equal distribution between right and left side of the cases. The main grade of ulcer was grade 2 according to International Working Group on the Diabetic Foot which accounting (90%). The mean size of ulcer was 5.75cm. The duration of ulcer was less than one month in 70% of cases. Banashankari *et al.,* (2015) research revealed that right side foot ulcer accounted 49% of cases,46% of them had grade 2 ulcer and size of ulcer around 5 cm in 66%of cases. Zubair *et al*., showed that duration of ulcer was less than one month in 51% of his cases, grade 2 was in 35% of cases and ulcer size was more than 4 cm in 78% of patient.

About 11.7% of ulcer associated with

osteomyelitis different. In Banashankari *et al*., research, osteomyelitis was in 66% of cases. 13.8% of our study cases had previous ulcer and with amputation. Which like one reported by Ahmed *et al*., (2012) 12% of our diabetic patients had previous amputations.

Around 45% of infections were poly microbial in nature while the remaining 55% were mono microbial. Gram negative bacteria were the commonest bacteria isolated (46%) followed by Gram positive bacteria (33%) and anaerobic bacteria in 21% of the isolated organisms. These results were in concordance with Banashankari *et al*., and Sugandhi *et al*., (2014) Abdul kadir *et al*., (2012) demonstrated only 40% of the patients had two or more pathogens contrasted with 52% with mono microbial in nature. Chopdekar *et al*., (2005) revealed that 113 patients with diabetic foot ulcers showed poly-microbial in nature in 85% and single organism in 14% of patients.

The most commonly isolated Gram negative bacteria was *Klebsiella* species (19.8%) followed by *Proteus species, Pseudomonas species, Escherichia coli, Citrobacter species, Morganella morganii and Enterobacter species* respectively.

Table.1 International Working Group on the Diabetic Foot Classifications of Diabetic Foot Infection

Clinical Manifestation of Infection	PEDIS Grade
No symptoms or signs of infection	1
Local infection involving only the skin and the subcutaneous tissue (without involvement of deeper tissues and without systemic signs as described below). If erythema present, it must be >0.5 cm to ≤2 cm around the ulcer. Exclude other causes of an inflammatory response of the skin (e.g., trauma, gout, acute charcotneuro-osteoarthropathy, fracture, thrombosis, venous stasis).	2
Local infection (as described above) with erythema > 2 cm, or involving structures deeper than skin and subcutaneous tissues (e.g., abscess, osteomyelitis, septic arthritis, fasciitis), and no systemic inflammatory response signs as described down.	3
Local infection (as described above) with the signs of SIRS (systemic inflammatory response syndrome), as manifested by ≥2 of the following: • *Temperature >38°C or <36°C* • *Heart rate >90 beats/min* • *Respiratory rate >20 breaths/min or PaCO2 <32 mm Hg* • *White blood cell count >12 000 or <4000 cells/ml or ≥10% immature (band) forms.*	4

Table.2 Risk factors for studied case and control groups

Risk factor	Cases N=60	Normal person N=6	DM Patient N=7	DM with uninfected ulcer N=7	Significance
	N(%)	N(%)	N(%)	N(%)	
Obesity	42(70)	0	4(57.1)	4(57.1)	χ^2=11.61 P=0.009*
Smoking	21(35)	…	2(28.6)	0	χ^2=3.61 P=0.16
DM	51(85)	1(16.7)	4(57.1)	……..	χ^2=15.92 P<0.001*
Hypertension	41(68.3)	….	2(28.6)	4(57.1)	χ^2=4.41 P=0.11
Neuropathy	60(100)	……..	3(42.9)	7(100)	χ^2=40.47 P<0.001*
Retinopathy	40(66.7)	……	3(42.9)	5(71.4)	χ^2=1.7 P=0.43
Nephropathy	22(36.7)	……..	7(100)	7(100)	χ^2=7.31 P=0.026*
Ischemic heart disease	28(46.7)	…..	2(28.6)	2(28.6)	χ^2=1.51 P=0.47
Osteomyelitis	7(11.7)	……..	…..	7(100)	FE, P<0.001*
Duration of DM (YS) Median (Min-Max)	20 (8-38)	NA	12 (8-20)	12 (8-20)	KWχ^2=0.12 P=0.1

Table.3 Isolated bacteria from diabetic foot infections

	Bacterial isolates	No	%
Gram-negative bacteria N=40	*Klebsiella species*	17	19.8
	Proteus species	7	8.14
	Pseudomonas species	6	6.9
	Escherichia coli	5	5.8
	Cetrobacter species	2	2.3
	Morganella morganii	2	2.3
	Enterobacter species	1	1.2
Gram-positive bacteria N=28	*Staphylococcus aureus*	22	25.6
	Streptococcus pneumonia	5	5.8
	Micrococcus	1	1.2
Anaerobic bacteria N=18	*Peptostreptococcus asaccharolyticus*	8	9.3
	Peptostreptococcus anaerobius	4	4.7
	Bacteroidesfragilis	4	4.7
	Bacteroidesovatus	2	2.4
	Total	86	100

Table.4 The antibiotic sensitivity pattern of the Gram negative bacteria

Gram-negative bacteria(n)	Antibiotics Resistance n (%)											
	AMC	MEM	TPZ	LEV	OFX	CEP	CRO	FEB	BY	CTX	AK	SXT
Klebsiella species n=17	11 (64.7)	-	**12 (70.5)**	2 **(11.8)**	**10** (58.8)	**6 (35.3)**	**13 (76.5)**	**14** 82.4)	**17 (100)**	13 (76.5)	5 (29.4)	8 (47.06)
Proteus species n=7	6 (85.7)	-	2 (28.57)	3 (42.8)	5 (71.4)	5 (71.4)	3 (42.8)	5 (71.4)	7 (100)	3 (42.8)	1 (14.3)	7 (100)
Pseudomonas species n=6	6 (100)	-	2 (33.33)	2 (33.33)	2 (33.33)	5 (83.33)	6 (100)	6 (100)	6 (100)	6 (100)	1 (16.67)	6 (100)
Escherichia coli n=5	4 (80)	-	4 (80)	1 (20)	2 (40)	3 (60)	3 (60)	5 (100)	3 (60)	5 (100)	2 (40)	3 (60)
Cetrobacter species n=2	2 (100)	-	1 (50)	-	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	-	-
MorganellaMorganii n=2	2 (100)	-	1 (50)	1 (50)	1 (50)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)
Enterobacter species n=1	-	-	1 (50)	-	-	-	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)

Table.5 The antibiotic sensitivity pattern of the Gram positive bacteria

Gram-positive bacteria	Antibiotics Resistance n (%)												
	AMC	MEM	TPZ	LEV	OFX	CEP	SXT	CXM	E	CZ	OX	VA	CL
Staphylococcus aureus n=22	15 (68.2)	2 (9.1)	7 (31.8)	10 (45.5)	9 (40.9)	17 (77.3)	14 (63.6)	14 (63.6)	8 (36.4)	17 (77.3)	15 (68.2)	7 (31.8)	15 (68.2)
Streptococcus pneumoniae n=5	2 (40)	1 (20)	3 (60)	1 (20)	3 (60)	3 (60)	2 (40)	3 (60)	3 (60)	5 (100)	3 (60)	-	2 (40)
Micrococcus n=1	1 (100)	-	-	-	-	1 (100)	-	-	1 (100)	1 (100)	1 (100)	-	1 (100)

Table.6 The antibiotic sensitivity pattern of the anaerobic bacteria

Anaerobic bacteria *Peptostreptococcus*	AMC	MEM	TPZ	LEV	OFX	CEP	RIF	MT	ND	E	CN	VA
						Antibiotics Resistance n (%)						
Peptostreptococcusas accharolyticus n=8	3 (37.5)	0	2 (25)	4 (50)	4 (50)	6 (75)	7 (87.5)	2 (25)	6 (75)	4 (50)	5 (62.5)	3 (37.5)
Peptostreptococcusan aerobius n=4	1 (25)	-	2 (50)	-	1 (25)	3 (75)	2 (50)	2 (50)	-	1 (25)	2 (50)	-
Bacteroides species												
Bacteroides fragilis n=4	3 (75)	1 (25)	1 (25)	-	1 (25)	3 (75)	1 (25)	3 (75)	4 (100)	3 (75)	3 (75)	2 (50)
Bacteroidesovatus n=2	1 (50)	-	1 (50)	-	-	-	-	1 (50)	2 (100)	2 (100)	1 (50)	-

Table.7 Measured IL6 in both the study and the control groups.

IL 6	Cases N=60	Control N=20	Significance
Median (Min-Max)	12.73 (5.14-671.25)	4.55 (0.8-5.95)	Z=6.46 P<0.001*

The most commonly isolated Gram positive bacteria was *Staphylococcus aureus (25.6%) followed by Streptococcus pneumonia and Micrococcus* accounting (5.8%, 1.2%) respectively. The commonly isolated anaerobic bacteria was *Peptostreptococcus asaccharolyticus (9.3%)* followed *by Peptostreptococcus anaerobius, Bacteroides fragilis and Bacteroides ovatus*. This is in accordance to Shankar *et al*., (2012) and Sugandhi *et al*., (2011).

In contrast, Hena and Grother showed that the most common isolated pathogens were *S. aureus* (43.2%), *Pseudomonas aeruginosa* (24.3%), *E. coli* (15.3%), *C. koseri* (2.7%), *P. vulgaris* (6.3%) and *Klebsiella pneumoniae* (9%). Similar results were reported by Chopdekar *et al*., stated that *Staphylococcus aureus* was the commonest Gram-positive isolated and *Pseudomonas aeruginosa* was the commonest Gram negative isolated organisms.

In the present study antibiotic sensitivity testing demonstrated that Meronem was found to be the most sensitive antibiotic used for treatment of Gram negative bacteria followed by Levofloxacin and Amikacin while Carbenicillin was the most resistant antibiotic. As regard to Gram positive, Meronem was found to be the most sensitive antibiotic for treatment followed by vancomycin, while Cefazolin was the most resistant one. These results were in accordance Esmat and Saif Al Islam Meronem, Levofloxacin or Amikin might be proper single agents for exact scope (except for MRSA). In Hena and Grother, all the Gram negative isolates were susceptible to Meronem. Meronem should therefore be used as a monotherapy against polymicrobial infections in difficult Gram negative infections.

Among 22 *Staphylococcus* species, 68% were MRSA. Chopdekar *et al*., (2005) and Sugandhi *et al*., (2011) found that MRSA

were accounting for 51%, 50% respectively. When we measured level of interleukin6 in serum of cases and control groups we found that Median level of IL6 was found 12.73 pg/ml in cases which is higher than diabetic patient with uninfected ulcer, only diabetic Patient and Normal person which accounting (5.19, 4.55, 2.79 pg/ml) respectively. This was approved previously by Weigelt *et al*., (2009).

IL6 level was higher in patients under insulin therapy than others who did not use insulin. Also, Sallam and El-Sharaw revealed that patient under insulin therapy had higher blood levels of markers of the acute-phase response, including: sialic acid, serum amyloid A, cortisol, CRP and the main cytokine mediator of the response, interleukin-6.

In conclusion, the most common bacteria isolated from the diabetic foot infections were Gram negative bacteria. *Klebsiella* species were the most common. Meropenem was the most sensitive antibiotic in all types of bacteria isolated from the diabetic foot infections. there was significant correlation between the IL6 and the cases of diabetic foot infections but no significance between it and the individual bacteria. Thus, it can be used as a predictor for the diabetic foot infections.

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