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Bacteriological study of diabetic foot ulcers and pattern of antibiotic susceptibility in Al-Kindy teaching hospital _ 2017 (Baghdad-Iraq)

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Abstract
Background: Diabetes is a chronic endocrine disorder affecting the body‘s metabolism and resulting in structural changes affecting the organs of the vascular system. Serious complications resulting from diabetes include coronary heart disease, stroke, retinopathy, renal failure, peripheral artery disease, and neuropathy. Diabetic foot ulcer is one of the complications associated with diabetes mellitus characterized by the triad of neuropathy, infection and ischaemia.
Aim of the study: To detect the most common type of bacteria accompanied with diabetic foot ulcer and to study the antibiotic susceptibility on these bacteria *in vitro*.
Patients and method: A cross sectional descriptive study was conducted at Al-Kindy Teaching hospital in the period from the first of May, 2017 to the end of April 2018.Convenient sample of 100 diabetic foot patients their ages ranges from (<40 _ >70) years old, who were willing to participate in this study and were available at the time of data collection both sex were included.
Results: Mean age of the respondents were (54.9±10.2) years, (58%) with primary level of education, (83%) of them were unemployed. Most (97%) diabetic foot patients were type 2 DM, and (76%) of them had high HbA1c level. The main culture sensitivity results were; Proteous (38.0%), Pseudomonaus aerogenousa (19.0%), Staphylococcus aureus (11.0%), the Antibiotic sensitivity test was done for (87) diabetic foot patient and results revealed that Meroponim sensitivity was (97.7%) of diabetic patients, Azethromycin sensitivity was (67.8%) of patients, Gentamycin sensitivity was (65.5%) of patients, Amikacin sensitivity was (90.8%) of patients, Ceftriaxone sensitivity was (47.1%) of patients and Vancomycine sensitivity in (48.3%) of patients.
Conclusion: Gram negative bacteria were more predominant and the Proteous is the main microorganism then, Pseudomonaus aerogenousa, Staphy aureus, Klebsila, E. coli., High levels of resistance to ceftriaxone and vancomycin were found.

Keywords: diabetes mellitus, diabetic foot, antibiotic

Introduction

Diabetes is a chronic endocrine disorder affecting the body‘s metabolism and resulting in structural changes affecting the organs of the vascular system. Serious complications resulting from diabetes include coronary heart disease, stroke, retinopathy, renal failure, peripheral artery disease, and neuropathy [1].
The two main forms of diabetes are type I diabetes and type 2 diabetes. Type 1 diabetes is a result of pancreatic islet beta-cell destruction usually due to an autoimmune response which results in insulin deficiency requiring exogenous insulin to prevent serious complications. Type 2 diabetes is characterized by insulin resistance and/or abnormal insulin secretion [2]. In people with type 2 diabetes, blood sugar must be controlled either through diet, with oral hypoglycemic drugs or in severe cases with exogenous insulin [3]. Type 2 diabetes accounts for over 90% of all diabetes cases worldwide [4].
Diabetic foot ulcer (DFU) is one of the complications associated with diabetes mellitus characterized by the triad of neuropathy, infection and ischaemia. The ulcer results from complex physiological processes of the triad with neuropathy playing the central role in addition to disturbances of sensory, motor, and autonomic functions. More than 60% of diabetic feet ulcers are as a result of underlying neuropathy caused by hyperglycaemic induced metabolic disorders [5].
Diabetic foot infections are one of the major long-term complications of type 2 diabetes mellitus which can result in gangrene and lower extremity amputation. Every year 4 million people around the world develop foot ulcers. Patients with diabetes are 25 times more likely to lose a leg than those without the condition, and up to (70%) of all leg amputations occur in people with diabetes. The result is that a leg is lost to diabetes every 30 seconds somewhere in the world [6].
The prevalence of diabetic foot ulcers is described to be between (4%) and (10%) of the diabetic population, with a lifetime risk of up to (25%). The incidence of foot ulcers in diabetic patients varies between (2 - 6%) in both of Western Europe and North America; and between (19% - 29%) in the Middle East [7].

The prevalence of amputation in patients with diabetes varies globally (1/1,000 inhabitants in Madrid and Japan to up to 20/1,000 in some Indian tribes in North America) [8]. In Iraq, the prevalence of Diabetic foot is about (2.3%), and the prevalence of amputation is about (0.7%) [9].

Common Microbial agent in patient with infected diabetic ulcer
Gram-negative bacteria were more commonly isolated compared with Gram-positive bacteria [12].

Patients and method
Study design and duration
A cross sectional descriptive study was conducted at Al-Kindy Teaching hospital in the period from the first of May, 2017 till April 2018.

Study Subjects
Convenient sample of 100 diabetic foot patients, who were willing to participate in this study and available at the time of data collection included both sex.

Tool for data collections
Data were collected using a structured and pre-test questionnaire from (which was prepared in English and then translated to Arabic language) and via face to face interview using morisky medication adherence scale.
The Morisky scale is a validated scale designed to estimate the risk of medication non-adherence. It has been cited in over 70 articles since its publication in 1986. It's used for many different diseases such as hypertension, hyperlipidemia, asthma, and HIV. Scores are based on patient responses to four, Yes or No questions. as shown in (table 1).

Table 1: (Morisky Medication adherence scale MMAS-4)

Question	Yes	No
Do you ever forget to take your medication?		
Are you careless at times about taking your medication?		
Sometimes if you feel worse when you take the medication, do you stop taking medication?		
When you feel better do you sometimes stop taking your medication?		

0=high adherenc, 1-2=Medium, 3-4=low adherence

Inclusion criteria
All patients were complained from active diabetic foot ulcer who attended to the hospital during the study period were included in the current study.

Exclusion criteria
1. Diabetic patients with healed ulcer
2. Non-diabetic patients with foot ulcer

Method
All patients were subjected to physical examination.
A swab was taken from the infected ulcers after debridment with a sterile scalpel and rinsed with sterile normal saline (done by trained person), and then sample cultured on blood and MacConkey agar, incubated for 24 hours to identify the type of bacteria, and by using biochemical tests in Al-kindy central labratory, genus and species of bacteria were confirmed. After that AB- susceptibility test was done by kirby -Bauer disc diffusion method, the antimicrobial discs which were used are those of Meroponim, Amikacin, Azithromycin, Gentamycin, Vancomycin and ceftriaxone. then left for another 24 hrs. and the results were interpreted using the suggested grouping of Anti-microbial agents prepared by USFDA-2018. As shown in below table 2.

Table 2

Group A	Enterobacteriacae	Pseudomonas aerugenosa	Staphylococcus spp.	Enterococcus spp.
Primary test and report	Ampicillin	Ceftazidime	Azithromycin	Ampicillin
	cefazolin	Gentamycin	Clarithromycin	Pencillin
		Tobramycin	Erythromycin	
	Gentamycin	Piperacillin-tazobactam	Clindamycin	
	Tobramycin		Oxacillin	
			Cefoxitin	
			Pencillin	
			Trimethoprim-	

			sulfamethoxazole	

Group B	Amikacin	Amikacin	Ceftaroline	Daptomycin
Optional	Amoxicillin-	Aztreonam	Daptomycin	Linezolid
Primary	Clavulanate	Cefepime	Linezolid	Tedizolid
Test	Ampicillin-	Ceftazidime-	Tedizolid	vancomycin
Report	Sulbactam	avibactam		
selectivity	Ceftazidime-	Ceftoionzane-		
	Avibactam	tazobactam		
	Ceftolozane-			
	Tazobactam			
	Piperacillin-			
	tazobactam			
	cefuroxime	Ciprofloxacin	Doxycycline	
		levofloxacin	Minocycline	
	cefepime	Doripenem	Tetracycline	
		imipenem	Vancomycin	
	Cefotetan			
	cefoxitin	Meropenem		
	Cefotaxime			
	ceftriaxone			
	Ciprofloxacine		Rifampicin	
	Levofloxacin			
	Doripenem			
	Ertapenem			
	Imipenem			
	Meropenem			
	Trimethoprim-Sulfamethoxazol			

Results

The result of the study shown in the following tables

Table 3: Distribution of sample according to Demographic characteristics of diabetic foot patients

Variable	No.	Percentage %
Age mean ± SD (54.9±10.2 years)		
<40 years	5	5.0
40-49 years	24	24.0
50-59 years	34	34.0
60-69 years	33	33.0
≥70 years	4	4.0
Total	100	100.0
Gender		
Male	52	52.0
Female	48	48.0
Total	100	100.0

Table 4: Distribution of sample according to DM characteristics of diabetic foot patients

Variable	No.	Percentage
DM Type		
Type 1	3	3.0
Type 2	97	97.0
Total	100	100.0
DM duration mean ± SD (10.7±6.9 years)		
≤5 years	29	29.0
>5 years	71	71.0
Total	100	100.0
Treatment Type		
Insulin	48	48.0

Oral agents	50	50.0
No treatment	2	2.0
Total	100	100.0

Table 5: Distribution of sample according to Clinical characteristics of diabetic foot patients

Variable	No.	Percentage
HbA1c level mean ± SD (8.2±1.9 %)		
Good control<7	24	24.0
Poor control>7	76	76.0
Total	100	100.0
History of previous admission to the hospital for diabetic foot ulcer		
Yes	6	6.0
No	94	94.0
Total	100	100.0

Table 6: Distribution of patients according to Morisky scale on adherence of patients to anti-diabetic medications

Morisky questions	No.	Percentage
Q 1: Do you ever forget to take your medicine?		
Yes	40	40.0
No	60	60.0
Total	100	100.0
Q2: Are you careless at times about taking your medication?		
Yes	43	43.0
No	57	57.0
Total	100	100.0
Q3: Sometimes if feel worse when take the medication, do you stop taking medication?		
Yes	21	21.0
No	79	79.0
Total	100	100.0
Q4: When you feel better, do you sometimes stop taking your medication		
Yes	31	31.0
No	69	69.0
Total	100	100.0
Morisky scale mean ± SD (0.34±0.3)		
Poor adherence	23	23.0
Medium adherence	29	29.0
High adherence	48	48.0
Total	100	100.0

Table 7: Types of bacteria in the culture

Variable	No.	Percentage
Culture sensitivity		
Proteous	38	38.0
Pseudomonaus aerogenousa	19	19.0
Staphylococcus aureus	11	11.0
Klebsila	8	8.0
E. coli	4	4.0
Enterobacter	2	2.0
Staph epidermidis	2	2.0
Staphylococcus & E. coli	2	2.0
Staphylococcus & Enterobacter	1	1.0
No growth	13	13.0
Total	100	100.0

Table 8: Antibiotics sensitivity results of diabetic foot patients

Variable	No.	Percentage
Meroponim sensitivity		
Yes	85	97.7

No	2	2.3
Total	87	100.0
Amikacin sensitivity		
Yes	79	90.8
No	8	9.2
Total	87	100.0
Azithromycin sensitivity		
Yes	59	67.8
No	28	32.2
Total	87	100.0
Gentamycin sensitivity		
Yes	57	65.5
No	31	34.5
Total	87	100.0
Vancomycin sensitivity		
Yes	42	48.3
No	45	51.7
Total	87	100.0
Cefriaxone sensitivity		
Yes	41	47.1
No	46	52.9
Total	87	100.0

Table 9: Association of DM characteristics to Morisky scale

Variable	Morisky scale						P
	Poor		Medium		High		
	No.	%	No.	%	No.	%	
DM Type							
Type 1	0	-	3	10.3	0	-	0.02* *Significant*
Type 2	23	100.0	26	89.7	48	100.0	
DM Duration							
≤5 years	13	56.5	12	41.4	4	8.3	<0.001** *Highly significant*
>5 years	10	43.5	17	58.6	44	91.7	
Treatment Type							
Insulin	5	21.7	17	58.6	26	54.2	0.006* *Significant*
Oral agents	18	78.3	10	34.5	22	45.8	
No treatment	0	-	2	6.9	0	-	

* Fishers exact test, ** Chi-square test

Table 10: distribution of bacteria according to the sensitivity and resistance to antibiotics

Type of bact.	Sensitive	Resistance
1. Staph aureus	Meroponem, Amikacin, ceftriaxone, Vancomycin,	Azethromycin, gentamycin
2. Enterobacter	Meroponem, Azethromycin, gentamycin, Amikacin	ceftriaxone, Vancomycin
3. Staph aureus + Enterobacter	Meroponem, Azethromycin, gentamycin, Amikacin, Vancomycin,	ceftriaxone
4. Klebseila	Meroponim, Azethromycin, gentamycin, Amikacin	ceftriaxone, Vancomycin,
5. pseudomonaus aerogenousa	Meroponem, Azethromycin, Amikacin, Vancomycin,	gentamycin, ceftriaxone
6. Proteous	Meroponem, Azethromycin, gentamycin,Amikacin, ceftriaxone,	Vancomycin,
7. Staph epidermidis	Amikacin, Vancomycin,	Meroponem, Azethromycin, gentamycin, ceftriaxone
8. E. coli	Meropoeim	Azethromycin,gentamycin, Amikacin, ceftriaxone, Vancomycin

Discussion

Diabetic foot is one of the most common devastating complications among other chronic complications of diabetes mellitus. It is the leading cause of non-traumatic amputation throughout the world. There are multiple factors which lead to development of foot ulcer in diabetic patients which may even result in amputation if not treated [13].

Old age was considered one of the independent risk factors for the development of diabetic foot ulcer and risk of ulceration increases two to four-fold with age in diabetes [14, 15]. In the present study, the majority of patients were above 50 years with a percentage (71%) which is in accord with Shabaki *et al* [16]. In which (63%) where above 50 years, this may be an indication of higher level of physical activities undertaken by aged persons with diabetes to run their family and increased prevalence of comorbidities such as neuropathy, periphral vascular disease, and kidney disease in this age group.

Regarding to the gender distribution in this study, (52%) were male. Higher males prevalence has been reported by Harrison and Lederberg [17]. this may be due to the higher level of outdoor physical activities in hot humid enviroment with inadequate and improper feet care among males in comparison to females. In addition to that, males are more exposed to trauma and tend to wear improper footwear, especially in our culture. [18, 19] Hefni *et al*. revealed that female was little bit more than male and this may be due to difference in samples collection, [20].

Al-Rubeaan, *et al*. (2015) [13, 36] mentioned that as expected, and reported by others, the percentage of type 2 diabetic patients was more among diabetic foot cases in many studies (42) [21, 22]. Most diabetic foot patients in this study were type 2 DM with a percentage of (97%) while type 1 represented (3%) only.

Another important common risk factor identified for the development of foot ulcer in diabetic patients is longer duration of diabetes mellitus and poor glycemic control. In our study mean DM duration of patients was 10.7±6.9 years, 71% of them had duration of more than 5 years, while Kateel, *et al* [21]. found that mean duration of this disease was 15 years.

In this study, HBA1c value was used because it is the gold standarad test for glycemic control. In diabetic patients, good glycemic control is defined as having HBA1c value <7% and poor glycemic control has >7% [24, 25].

Half of patients with type 2 Diabetes fail to achieve good glycemic control (HBA1C < 7). Poor medication adherence is the major cause [26]. Most critical factors for poor adherence are, perceived treatment efficacy [27]. hypoglycemia [28]. treatment complexity and convenience [29]. cost [30] and physician trust [31, 32].

In this study Morisky Medication Adherence Scale was used to measure adherence. there was highly significant association observed between increased age and being a female with poor adherence. Sanja-Geisel reported that there is NO gender pattern detectable because it does not exist [33]. However, as medication adherence is influenced by many factors, it could be a gender pattern that is overlain by other influences on health.

Delay in obtaining needed medical care and poor diabetic self-care behavior make rural residency [34] and low education [35] of significant association with poor adherence.

Currently, studies reported adherence rate as low as 67% for oral glucose-lowering agents (cramer, 2004) [34]. Delamater reported that among factors associated with medication adherence is simple prescription that consist of 1 diabetes drug per day was higher than more than 2 drugs.

Type of treatment is another important factor affecting glycemic control: The American Diabetes association and the European Association for the study of Diabetes recently issued a consensus algorithm for management of type 2 diabetes identifying insulin as the most effective glucose-lowering agent [37].

The majority of patients with diabetic foot in this study were on oral hypoglycemic agents (50%) and insulin (48%), while 2% of diabetic patients were not receiving treatment. This is in agreement with that mentioned by Al-Rubeaan, *et al* [13, 36].

Bacterial growth was detected in (87%) of the samples which is an approximated to results which are found by Paul *et al*. (2009), [37] who cultivate d bacteria with a percentage of (92%).

Regarding the type of bacteria in infected diabetic foot ulcer in this study, it was found that Gram negative bacteria were more predominant and Proteus is the main microorganism followed by, *Pseudomonas aruginosa, Staphylococcus aureus, Klebsiella, E. coli*, and less on is *Staph aureus & E. coli*. These results agreed with results of Gadepalli *et al*., (2006) [38, 40] and results of Turhan *et al*, (2013) [39].

Also results were in tune with other studies done in India which also showed that Gram negative bacteria were the most predominant organisms in Diabetic foot ulcers [42, 43].

In contrast to these results, a study reported by Anvarinejad, *et al*., who found the most common isolated bacteria were *Staphylococcus* spp, and *E. coli* respectively [44].

source of infection, use of antibiotics during the study for treatment, sample collection method, geographical variation and type and severity of the infection can influence the pathogen diversity in different geographical areas.

The antibiotic sensitivity test was done for 87 diabetic foot patient and showed that Gram-negative were sensitive to Meropenem followed by Azithromycin while Meropenem and Amikacin showed good activity against Gram-positive.

Meropenem showed highly activity against both Gram negative and positive bacteria, with a percentage of (97%) and this result disagreed with results of Raja, who found that Gram negative are sensitive to Imipenem and Gram positive are sensitive to Vancomycin [45].

Conclusion

1. Gram negative bacteria were more predominant and *Proteous* is the main microorganism followed by, *Pseudomonaus aerogenousa*, *Staphy aureus*, *Klebsila* and *E. coli*.
2. Alarmingly high levels of resistance to Ceftriaxone was found.

Recommendations

1. Increase the awareness of the diabetic patients about the diabetic foot care especialy adherence to medication.
2. For correct management of diabetic foot infection we recommend culture speciemen which help to identify susceptibility of antimicrobial drugs for maximum efficacy.

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