

CorrelationShip Between Complication of Diabetes And Biofilm Form By Bacterial Isolate Isolated From Diabetic Foot Ulcer (DFU)Among Iraqi Diabetic Patients

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ABSTRACT

Background:Diabetic Foot ulcer (DFU)are the most common economicproblems in diabetic patients (both type I and II), Sothat's more severe and longer cure than non-diabetic patients, that has many degree of ulcer as mild, moderate, or severewhich maybe leading to development of gangreneand it is characterized by many important pathological complications as Hypertension; Retinopathy; Nephropathy and Neuropathy.

Objectives –Biofilm form by Bacterial isolate isolated from DFUandstudy their correlated with many important factors as gender; age; type of Diabetic; Duration of ulcer and complication of diabetes such as Hypertension; Retinopathy; Nephropathy and Neuropathy.

Material and methods –From 72 diabetic patients with DFUwho attended the canter forEndocrinology and Diabetes were collected swab samples ,used classical conventional techniques to culture all sample and Gram staining followed diagnostic by Vitek system, so biofilm detection by microtiter plate (Mtp), as well as the complication of diabetic were diagnosis by specialized physician.

Results –Total of diabetic foot patients were males dominant over (73.3%) than female (26.3%) , second age group of 41- 60 years were most prevalence (54.4%) whilst (21%) in third age group (>61),So produced biofilm high percentage (54.4%) in male ,but (15.8%) in female as well as the second age group (41-60) have high form biofilm (55%) whereas both the first and third age group (< 41 and > 61) have low percentage (22.5%), andtype II more than type I in both duration (>10 year and < 10 year) as (49 , 24.6 %) respectively in type II ,and (8.8 , 17.5 %) respectively in type I , and biofilm formation was (15.8 %) of the bacteria isolated DFUof patients with diabetes type I whilst (54.1%) in type II ,so biofilm were formed predominantly by diabetic patients with Nephropathy (30 %) followed diabetic patients with Hypertension (28 %) and Neuropathy (24.5%) , whilst only (17.5 %) with retinopathy , Soboth *E.col* and *Klebsiella pneumoniae* to be equally responsible for diabetic foot ulcer as percentage (20%) successive followed by *Staphylococcus aureus* (14%) and *Pseudomonas aeruginosa*(10%).

Conclusion –Diabetic foot ulcer (DFU) in males dominant over females also biofilm produced in male more than female and maximum number of DFU belonged to second age group of 41- 60 years,so second age group have high form biofilm than others, as well as type II more than type I in both duration (>10 year and < 10 year),as well as Biofilm form by bacteria isolated from DFU of patients with diabetes type I more than type II.

Biofilms formed predominantly by diabetic patients with Nephropathy followed diabetic patients with Hypertension and Neuropathy as well as both *E.col* and *Klebsiella pneumoniae* to be equally responsible for diabetic foot ulcer.

Keywords: Diabetic foot ulcer (DFU), Biofilm, type of Bacterial isolate

INTRODUCTION:

Diabetes mellitus (DM) is a common disease in Iraq with a prevalence of 1.2 million cases According to global diabetes scorecard in 2015, one of the most Complication of DM was Diabetic foot ulcer (DFU).

DFU and infection arises from improper healthcare and uncontrolled diabetes (American Diabetes Association, 2011 ; Mendes and Neves, 2012) (Figure - 1), and affect about 15% of diabetic patients during their life as well as (40-80 %) of complication representing a major causing of morbidity and mortality (Prompers *et al.*, 2008 and Kumari *et al.*, 2016) , that's differences in diabetic patients according to many factors as the differences of the life styles, jobs and professional activities that may be caused tolerate the foot to high pressure and other different conditions (Gangania and Singh ,2016), as well as common complications among Iraqi diabetic patients were DFU associated with significant mortality and morbidity (Tappet *et al.*, 2003), and more than 15% of diabetic patients have DFU which lead to amputations more than 80,000 per year in united states (Boulton *et al.*, 2005).

Abnormal secretion of insulin in diabetic patients causing release high levels of glucose in blood as well as variety of complications such as retinopathy; arteriosclerosis; nephropathy; neuropathy (Nihad Khalawe, 2005 and Hosseinie *et al.*, 2008) and Diabetic foot ulcer which is one of most common complications among the diabetic patients (Tappet *et al.*, 2003)



Figure (1): diabetic foot ulcer (DFU)

long history of DM; older patients; used many antidiabetic medications and physical activity with less the frequent inspection of feet were some of the major risk factors for developing DFU among Iraqi diabetic patients, whilst frequency of glucose monitoring is inversely related to recurrence of DFU (Mohammed *et al.*, 2016).

MATERIALS AND METHODS:

1) All Swabs collected which collected from 62 DFU patients who attended the Center for Endocrinology and Diabetes, and cultured on both media (MacConkey and blood) agar plates and incubated at 37°C overnight, then identified the colonies by standard techniques (Collee *et al.*, 2006.) and VITEK System.

2) Determination of biofilm by microtiter plate (M.t.p) or tissue culture plate method as the following: Adding 230µl of Trypticase Soya Broth (TSB) on each 96 well flat-bottomed microtitre plate, and 20µl of overnight bacterial culture, the wells which contained broth only consider as negative control, and incubated plates aerobically (at 35°C for 24 hours), then by 300µl of sterile distilled water, washing three times, and fixing the bacteria adhering to wells by using 250µl of the methanol for 15 Minutes, then staining all the wells with 250µl of crystal violet (1% solution) for 5 minutes, after then removing the excess stain by washing and dried all wells after then adding 250µl of 33 % (v/v) glacial acetic acid and measured the optical density (O.D) by ELISA auto reader for each well at 490nm. This tests are carried out in triplicate and calculated the averaged, The cut-off optical density (O.D c) are determine as 3 Standard Deviations (S.D) above the mean O.D of the negative control, bacterial Strains can be classified as producer and non-producer biofilm (Stepanovic *et al.*, 2000).

3) Nephropathy, Neuropathy and Retinopathy were reported based on both the clinical and physical examination which documented in the patient's file.

Statistical Analysis: For the analysis of studies data, Microsoft Office Excel 2010 and SPSS (Statistical Package for Social Sciences) were used as software program.

RESULTS

Table –1: Distribution of Diabetic patients and control according to gender and sex.

Gender	Diabetic patients (%)	Control
Male	42 (73.7)	7(46.6)
Female	15(26.3)	8(53.4)
Total	57(100)	15(100)
Age (years)		
< 40	14(24.6)	8(53.3)
41- 60	31(54.4)	6(40)
>61	12 (21)	1(6.7)
Total	57(100)	15(100)

In table -1 showed the total of diabetic foot patients were 57 studied, males were dominant over (73.3%) than female (26.3%), the maximum number of diabetic foot patients belonged to the second age group of 41-60 years were 54.4% while low percentage (21%) in third age group (>61).

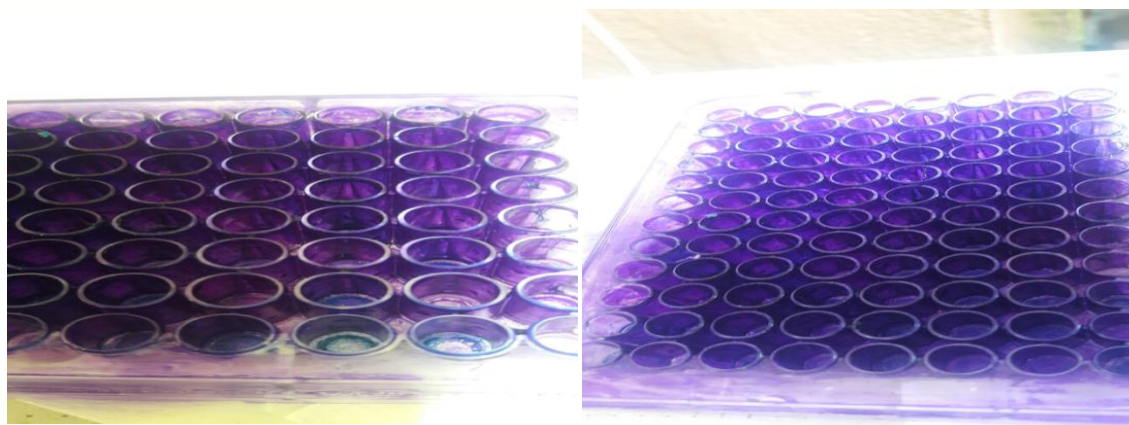


Figure (2): Biofilm form assay by microtiter plate (M.t.p)

Table- 2: Distribution of biofilm form according to gender and age.

Gender	Biofilm Form		Total	Control
	+	-		
Male	31(54.4)	11(19)	42(73.6)	7(46.7)
Female	9(15.8)	6(10.6)	15(26.4)	8(53.3)
Total	40(70)	17(30)	57(100)	15(100)
Age (years)				
< 40	9(22.5)	5(29.4)	14(25)	8(53.3)
41- 60	22(55)	9(53)	31(54.4)	6(40)
>61	9(22.5)	3(17.6)	12(21)	1(6.7)
Total	40(100)	17(100)	57(100)	15(100)

In table -2 appearance produced biofilm high percentage (54.4%) in bacterial isolated from male diabetic foot ulcer, whilst of biofilm formed by bacterial isolated from female diabetic foot ulcer as (15.8%) but the percentage (10.9 %) as non produced, as well as the second age group (41 – 60) have high form biofilm (55%) whereas both first and third age group (<41 and > 61) have low percentage (22.5%).

Age factor play important role in causing diabetic foot ulcer, similar with Reiber and Ledoux; 2003 who showed prevalence of DFU varied between (1.7-3.3) % in the younger whilst (5–10)% in older patients (Reiber and Ledoux; 2003), current study correspond with results of Katsilambros *et al.*, 2003 who showed highest present (3.6%) in patients older than 65 years, and (3.4%) in the age 45–64 years, whilst (1.6%) in 18–44 years.

Table- 3: Distribution of bacterial biofilm according to type of Diabetic and Duration of ulcer.

Type of diabetes	Duration of ulcer		bacterial Biofilm		Total	Control
	< 10 year	>10 year	+	-		
Type I	10 (17.5)	5 (8.8)	9 (15.8)	6 (10.6)	15 (26.3)	6 (40)
Type II	14 (24.6)	28 (49)	31 (54.1)	11 (19.3)	42 (73.7)	9 (60)
Total	24 (42.2)	33 (57.8)	40 (70.1)	17 (29.9)	57 (100)	15 (100)

In table -3 according to duration of ulcer, type II more than type I in both duration (>10 year and < 10 year) as (49, 24.6) % and (88, 17.5)% respectively in type II and I, as well as Biofilm formation was seen in (15.8 %) of the bacteria isolated for diabetic foot ulcer of patients with diabetes type I whilst (54.1%) in type II.

This also contradicted Kumar *et al.*, who found that diabetic foot ulcer were most common in type I (86.6% to 91.06)% than type II (Kumar *et al.*, 2016). As well as many study as Katsilambros *et al.*, 2003 explained the risk of ulcer in diabetic foot patients were increased two - four folds with increased age and duration of diabetes . Result of [Al-Rubeaan *et al.*, 2015](#) showed foot complications were increased with the duration of diabetes and age amongst male patients, as well as DFU were more prevalence in type 2 diabetic patients than type 1 diabetic patients.

Table- 4: Distribution of bacterial biofilm according to complication

Type of complication	No. of diabetic patients with complication	Biofilm form		No. of diabetic patients without complication
		+	-	
Hypertension	16 (28)	9 (15.8)	7 (12.2)	15
Retinopathy	10 (17.5)	7 (12.2)	3 (5.3)	
Nephropathy	17 (30)	14 (24.5)	3 (5.3)	
Neuropathy	14 (24.5)	10 (17.5)	4 (7.2)	
Total	57 (100)	40 (70)	17 (30)	

In table (4) showed out of 57 patient have complication diabetic, as Nephropathy was more frequents (30%) in DFU followed both Neuropathy and Hypertension as (24.5 and 28) % respectively, so (70 %) form biofilm, so Biofilm were formed predominantly by diabetic patients with Nephropathy (24.5%) followed diabetic patients with Neuropathy (17.5%) and Hypertension as (15.8%), but low produced biofilm in patients with retinopathy (12.2%).

[Al-Rubeaan *et al.*, 2015](#) showed in his study the neuropathy were more frequent in DFU, followed by retinopathy and nephropathy respectively, also Cheng *et al.*, 2006 showed prevalence of DFU were higher in males than females who explained male has higher foot pressure and limited joint mobility ,As well as males are more exposed to trauma (Al-Wahbi, 2006) added to the fact that women are more interested and care from men.

Table- 5 : Single and Mixed bacterial isolates from DFU.

Mixed bacterial culture	Number	Single bacterial culture	Number
E.coli+ <i>Pseudomonas. aeruginosa</i>	1(2)	E.coli	10(20)
<i>Pseudomonas . aeruginosa</i> + <i>Klebsiella pneumoniae</i>	1(2)	<i>Klebsiella pneumoniae</i>	10(20)
E.coli + <i>Staph. Epidermidis</i>	1(2)	<i>Staph.aureus</i>	7(14)
E.coli + <i>Klebsiella pneumoniae</i>	1(2)	<i>Pseudomonas. Aeruginosa</i>	5(10)
<i>Staph.aureus</i> + E.coli	1(2)	<i>Staph. Epidermidis</i>	4(8)
<i>Klebsiella pneumoniae</i> + <i>Staph. epidermidis</i>	1(2)	<i>Proteus .mirabilis</i>	2(4)
<i>Proteus .mirabilis</i> + <i>Strep. pyogenes</i> Group A	1(2)	<i>Strep.pyogenes</i> Group A	1(2)
<i>Strep. pyogenes</i> Group A + <i>Staph.aureus</i>	1(2)	<i>Acinetobacter sp.</i>	1(2)
<i>Staph.aureus</i> + <i>Klebsiella pneumoniae</i>	1(2)	-	-
<i>Proteus .mirabilis</i> + <i>Klebsiella pneumoniae</i>	1(2)	-	-
Total	10(2)	Total	40(2)

Table 5 were founded both *E.col* and *Klebsiella pneumoniae* to be equally responsible for diabetic foot ulcer as percentage (20%) successive followed by *Staphylococcus aureus* (14%) and *Pseudomonas aeruginosa*(10%).

Many previous studies such as Akhi *etal.*, 2015 elucidate the gram-positive bacteria were more isolate from diabetic foot ulcer than gram- negative bacteria, that is fully compatible with Daniel *etal.*,2013 study which appeared 7 isolate of gram-positive bacteria as 1 strain of *Enterococcus* and 6 strain of *Staphylococcus*, In contrast to these studies, Gadepalli *et al.*,2006 was founded gram- negative bacteria as (*Proteus sp.* , *E. coli*, and *Pseudomonas aeruginosa*)were predominant more than gram- positives bacterial isolate.

The Skin problems or skin infections are common in diabetic patients so, high level of glucose in blood provide suitable culture for pathogenic bacteria and other microorganisms as well as reduce ability of body to heal itself (Ceriello, 2005)

CONCLUSION:

- 1) Diabetic foot ulcer in males dominant over females also biofilm produced in male more than female and maximum number of DFU belonged to second age group of 41- 60 years, so second age group have high form biofilm than others.
- 2) Type II more than type I in both duration (>10 year and < 10 year), as well as Biofilm form by bacteria isolated from DFU of patients with diabetes type I more than type II.
- 3) complication in diabetic foot ulcer of Iraqi patients are Nephropathy Hypertension
- 4) Foot complications were increased with duration of diabetes and age amongst male patients, as well as D.F were more prevalence in type 2 diabetic patients than type 1.
- 5) Biofilms formed predominantly by diabetic patients with Nephropathy followed with Hypertension and Neuropathy.
- 6) Both *E.col* and *Klebsiella pneumoniae* to be equally responsible for diabetic foot ulcer followed *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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