Serum C - Reactive Protein Level in Diabetic Foot Patients and Their Relation with Bacterial Isolates

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Abstract

Background: Foot infections are one of the major complications of diabetes mellitus and a significant risk factor for lower extremity amputation. C-reactive protein is an acute-phase reactant, rises dramatically in response to infection.

Objective: To determine the microbial isolates of patients with diabetic foot infections and their relation with C-reactive protein level in their sera.

Materials and Methods: A prospective study of 90 patients with diabetic foot infections admitted to different public and private hospitals in Erbil city center-Iraq between June 2011 and May 2012 was undertaken. Bacteriological specimens were obtained and processed using standard procedure. The patients serum had been tested for C-reactive protein by high sensitive Enzyme linked Immunosorbent Assay (ELISA).

Results: A total of 130 pathogens were isolated from 90 diabetic foot patients 46 (51%) of the patients had polymicrobial infection, 37 (41%) had single organism and 7 (8%) had no growth. Gram positive (G+ve) bacteria 60(53%) were more commonly isolated than Gram negative (G-ve) bacteria 53(47%). *Staphylococcus aureus* and *Escherichia coli* were the most frequently among G+ve and G-ve isolates respectively. No significant difference was found between mean serum levels of C-reactive protein in patients infected with G+ve bacteria versus G-ve bacteria, although their concentration was more in the later. However, highly significant differences ($P<0.01$) were observed between both G+ve and G-ve bacteria versus no bacterial isolate in patients.

Conclusion: C-reactive protein serum level was higher in patient with diabetic foot infected by G-ve bacteria, although G+ve bacteria represented a major bacterial isolates.

Key words: Diabetic Foot; Infection; Microorganism; C-reactive protein.

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الخلاصة

خلفية الدراسة: يعدالتهاب القدم واحدة من مضاعفات مرض السكري ومن المخاطر الفعلية لبترالاطراف.اذ يعد البروتين الفعالC واحدة من اهم تفاعلات الطور الحاد حيث اذ بشدة كاستجابة للاخماج.

هدف الدراسة: التحري عن العلاقة بين البكتريا المعزوله من مرضى السكر المصابين بجرح القدم مع مستوى البروتين الفعال C في مصول هؤلاء المرضى.

المواد وطرائق العمل: اجريت الدراسة على تسعون من مرضى السكر المصابين بجرح القدم ادخلو العديد من المستشفيات العامة والخاصة في مدينة اربيل ما بين شهر حزيران ٢٠١١ولغاية شهر مايس ٢٠١٢. جمعت العينات البكتيرية وعوملت بالطرق المعيارية للزرع الجرثومي والتشخيصي. مصول المرضى كذلك استخدمت للتحري عن البروتين الفعال Cباستخدام اختبار الخميرة للامتزاز المناعي ذو الحساسية العالية.

النتائج:اوضحت الدراسة الحالية بان ١٣٠كائن ممرض عزل من ٩٠ مريضا بالسكري والمصابين بجرح القدم نسبةالمرضى المصابين بانواع متعددة من الكائنات الممرضة كانت٤٦ (٥١%) ، الاصابة بنوع واحد كان ٣٧ (٤١%) بينما كانت النسبة ٧(٨%).للذين لم تظهر لديهم نمو بكتيري. البكتريا الموجبة لصبغة غرام٦٠(٥٣%) عزلت اكثر من البكتريا السالبة لصبغة غرام ٥٣(٤٧%) وكذلك كانتا *Staphylococcus aureus*و*Escherichia coli* من اكثر البكتريا المعزولة لدى المرضى. كما كشفت البيانات الاحصائية عن عدم وجود علاقة احصائية معتمدة بين مستوى البروتين الفعال C والمرضى المصابين بالبكتريا الموجبة لصبغة غرام والمصابين البكتريا السالبة لصبغة غرام على الرغم من ارتفاع مستواها عند السالبة بينما كانت العلاقة قوية عند المقارنة بالمرضى الذين لم تعزل منهم البكتريا.

الاستنتاجات :المستوى المصلي للبروتين الفعال C كان اكثرفي مرضى السكر المصابين بجرح القدم والذين كانت لديهم اصابة بالبكتريا السالبة لصبغة غرام الاكثرعزلا علما ان البكتريا الموجبه كانت الاكثرمن بين المرضى.

مفتاح الكلمات: مرضى السكري ذو جرح القدم، اصابة ، كائنات ممرضة، البروتين الفعالC.

Introduction

Diabetes mellitus (DM) is a serious health problem that is rapidly expanding worldwide. One of the more frequent diabetic complications is diabetic foot (DF)[1]. Foot infections are among the most common bacterial infections encountered in patients with DM in clinical practice. These infections and their squeal are also the most common cause of disability and the reason for lower-limb amputation [2].

Once the skin is broken, the underlying tissues are exposed to colonization by pathogenic organisms [3]. The resulting wound infection may begin superficially, but with delay in treatment and impaired body defense mechanisms, it can spread to the subcutaneous tissues and to even deeper structures [4][5].

Because microorganisms are always present on skin wounds, diagnosis of infection must be based on microbiological findings but not on clinical criteria [6]. One of the earliest discovered biomarkers used to diagnose infection is C-reactive protein (CRP) [7]. Which is an acute-phase reactant, and its level measurements are frequently used to aid in the diagnosis of bacterial infections. It Synthesized by the liver and triggered by cytokines (IL-1, IL-6 and TNF-α) and its levels increase within 4-6 hours of an inflammatory stimulus [8]. C-reactive protein produced not only during infection but also in many types of inflammation, it binds to polysaccharides in pathogens, activating the classical complement pathway [9].

Diabetic foot infections are predominantly polymicrobial and *Staphylococcus aureus* (*S. aureus*) is the most prevalent isolate together with other aerobes and anaerobes [1]. Anaerobes are rarely the sole pathogen, but they often participate in a mixed infection with aerobes, especially in cases of deep tissue infection [4].

This study was design to isolated different microorganism from diabetic foot ulcer and related with levels of serum CRP.

Subjects, Materials and Methods

This prospective study comprised of 90 DF patients admitted to different public and

private hospitals in center of Erbil city-Iraq during the period between June 2011 and May 2012. The patients were clinically assessed and full information had been taken directly from the patients or their relatives and the information was arranged in an informative formula sheet which includes: Age, gender, other variable and type of diabetes. Diabetes foot patients were classified according to Wagner's classification and they had been tested for both bacteriologic and serologic investigations. Soft tissue, pus, aspirates, biopsies or swabs were collected and cultured for aerobic and anaerobic bacteria with the identification of causative microorganism by using the analytic profile index(API) system. Also the patient serum had been tested for CRP quantitatively by using Enzyme linked Immunosorbent Assay (Human CRP ELISA kit, DRG, USA). The study was approved by Ethics Committee-college of medicine. SPSS was used for statistical analyses in the present study.

Results

Out of the 90 patients with DF, the frequency of DFI was found to be more common among males than the females. Male: female ratio was (1.3:1). The age of DF patients ranged between 35 years to 85 years. Causative bacteria were isolated in 83 of 90 patients, and 130 isolates were obtained with an average of 1.44 isolates per patient Table (1).

Table (1): Demographic profile for diabetic foot patients.

General characteristics of diabetic foot patients, number	
Age (years)	58.5 (35-85)
Sex (Male/Female)	51 (43) / 39 (57)
Diabetes mellitus Type 2/type 1	85 (94.4) /5 (5.6)
No. of isolates (130)	
Aerobes	113 (87)
Anaerobes	17 (13)

Out of 130 isolates, *S. aureus* was the predominant isolates (20%). In contrast, *Cedecea davisae* was least predominant isolates (0.76%). Among anaerobes, *Peptostreptococcus spp* was the predominant isolates (6.15%) (Table 2).

Table (2): Frequency of aerobic and anaerobic bacteria isolated from 83 DF patients.

Type of bacteria Aerobes	No.	Percentage
Gram positive		
Staphylococcus aureus	26	20
Coagulase Negative Staphylococcus	17	13.07
Enterococcus spp.	11	8.46
Streptococcus spp	6	4.61
Total	60	46
Gram negative		
Escherichia coli	20	15.38
Proteus spp.	8	6.15
Pseudomonas spp	7	5.38
Klebsiella oxytoca	4	3.07
Acinetobacter baumani	4	3.07
Enterobacter clocaea.	3	2.3
Morganella morgani	2	1.53
Aeromonas hydrophila	2	1.53
Citrobacter frundi	2	1.53
Cedecea davisae	1	0.76
Total	53	41
Anaerobes		
Peptostreptococcus spp.	8	6.15
Bacteroides fragilis	5	3.84
Fusobacterium spp.	2	1.53
Clostridium clostridioforme	2	1.53
Total	17	13

Highly significant difference was present between the mean of serum CRP level in patient with sterile growth compare with each of G+ve, G -ve and mixed (P>0.01). Mean CRP level in patients infected with mixed was significantly higher than those with G+ve (P>0.05). In contrast no significant differences were present between mean CRP levels in patient with G-ve compared with both G+ve and mixed (P<0.05) using T test (Table 3).

Table (3): Gender CRP means serum concentration in types of isolate.

Type of isolate	No.90	CRP serum concentration	p-value F- test
		Mean± SE	
Gram +ve	25	4.41±0.61	0.007 P<0.01 HS
Gram -ve	12	5.74±0.86	
Mixed	46	6.15±0.47	
No growth	7	2.27±0.19	
Gram +ve Vs Gram -ve		0.22 NS	T test
Gram +ve Vs Mixed		0.03 S	
Gram +ve Vs no growth		0.002 HS	
Gram -ve Vs Mixed		0.68 NS	
Gram -ve Vs no growth		0.002 HS	
Mixed Vs no growth		0.003 HS	
P<0.05: Significant; P<0.01: highly significant; P>0.05: No significant Mixed: Mix isolates; No growth: Sterile			

Out of 90 patients, the positive cultures were either pure or mixed, and negative cultures were observed in 7 patients. No statistical difference was present between the mean of serum CRP level between the pure culture and mix culture. However, highly significant difference was present between pure and mixed culture with sterile culture P>0.01 using T test (Table 4).

Table (4): CRP means serum concentration according to type of culture.

Type of culture	No.90 (%)	CRP serum concentration No. Mean+ SE	p-value F- test
Pure culture	37 (41%)	4.84± 0.61	0.005 P<0.01 HS
Mix culture	46 (51%)	6.15±0.47	
No growth	7 (8%)	2.27±0.19	
Pure Vs Mixed		0.64 NS	T test
Pure Vs no growth		0.001 HS	
Mixed Vs no growth		0.001 HS	
P<0.01: highly significant; P>0.05: No significant Pure: Pure isolates; Mixed: Mixed isolates			

There was a highly significant difference showed between type of isolates and gender. Diabetes foot patients infected with G+ve isolates were higher among male than female, while frequency of G-ve isolates were higher in female than male. Also mixed isolates were higher in male than female. However, sterile growth was higher in female. Out of 83 patients with positive culture, numbers of male 50 (60.2%) were higher than female 33 (39.8) with highly significant differences (P<0.01) using Chi square test (Table 5).

Table (5): Gender frequency of different isolates.

Type of isolate	Total No.	Gender		Chi square	
		Female No. (%)	Male No. (%)	P value	Probability
Gram +ve	25	10 40	15 60	42.70	P<0.01 HS
Gram -ve	12	7 58.3	5 41.7		
Mixed	46	16 34.8	30 65.2		
Total	83	33 39.8	50 60.2		
No growth	7	6 85.7	1 14.3		

Discussion

Diabetic foot ulcers are common and serious complications of chronic DM. In parallel with increased prevalence of this disease, the prevalence of foot infections are increasing worldwide [10][11]. In this study, more males presented with diabetic foot infection, which is consistent with findings of other studies [12][13][14]. This may be due to higher level of outdoor activity among males compared to females [15].

In our prospective study, 130 species of bacteria isolated from specimens taken from 90 patients. Bacteriological analysis revealed that 83 of patients (92%) had positive culture while only 7 patients (8%) had negative culture. This is consistent with the finding of Al-tahawy *et al* [16]. Because Patients with diabetes are particularly susceptible to foot infection primarily because of neuropathy, vascular insufficiency, and diminished neutrophil function [2][5].

In the present study *S.aureus* was the most frequent species among the aerobic and anaerobic bacteria that was isolated from the diabetic foot infection. This is consistent with finding of many researchers [1][11][16][19]. This predominance due to *S. aureus* is the most important true pathogen of skin infections in general and probably in uncomplicated diabetic ulcer infection as well [20].

Though previous studies Zubair *et al.*, Abdulkadir *et al.*,[12][19] showed G-ve aerobes as predominant agents in diabetic foot infections, we frequently isolated G+ve bacteria (46%) compared to G-ve bacteria (41%). Similar to our findings, Kandemir *et al* and Abdulrazak *et al.*, [11][18]. Showed predominant involvement of G +ve isolates.

There was a highly significant elevated CRP level in DF patients infected with G-ve bacteria compared to those infected with G+ve. Abe *et al.*, [21]. Sharing us the same result. Our finding suggests that different types of pathogen-associated molecular patterns may induce different and magnitudes of inflammatory response.

Anaerobes were isolated in less than one-third of the patients and almost always in mixed culture. This is in contrast to the findings of several other studies that failed to isolate anaerobes, possibly because of sub-optimal study protocols [22]. The anaerobes isolated from our study are consistent with other reported studies, in which Peptostreptococcus spp. were the predominant isolates [23].

Most of our patients are of mild to moderate degree of severity. Grades 1 and 2 ulcers, which represent the majority of wounds treated at non-surgical clinics, usually do not develop deep pockets or undermined edges that lead to the proliferation of anaerobic bacteria. Anaerobic infections develop in ulcers of higher grades (Pathare *et al.*, [24]. Sapico *et al.*, [25]. This can explain the low isolation rate of anaerobics compared with others.

Our findings showed a relatively higher number of patients (51%) grew two or more pathogens compared to monomicrobial etiology, 41%. Raja found 42% of patients developed mixed growth and Renina *et al.*, revealed 58.9% were of polymicrobial organisms [26][27]. In contrast, other literature documents that the prevalence of polymicrobial infection could be as high as 80%- 87.2% [28][29]. A possible reason for the low incidence of polymicrobial infection in the present study may be due to the role of severity of infection [30].

Regarding the number of bacterial isolates and genders, in DF patients number of infected male was higher than female. Also mixed isolates were higher in male than female. Male diabetic foot patients with mixed isolates may have poor glycemic control and hence they have higher bacterial

isolates compared to their female counterparts [31].

In conclusion, our study has showed that 51% of diabetic foot infections were polymicrobial. *Staphylococcus aureus* and *Escherichia coli* were the most commonly identified gram positive and gram negative microorganisms respectively. Regarding to C-reactive protein our study showed that gram-negative bacteria are the most commonly related with serum CRP elevation in diabetic foot patients.

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