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# Procalcitonin and Proinflammatory Parameters in Diabetic Foot Infection as New Predictive Factor

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**Abstract.** Diabetic foot is a common complication of diabetes due to changes in blood vessels and nerves, often leads to ulceration and subsequent limb amputation if not treated early. A new diagnostic marker of bacterial infections is procalcitonin. C-reactive protein, Interleukin1β, Interleukin-6 and tumor necrosis factor-α as proinflammatory parameters increased in Diabetic foot infection. We evaluated above parameters in patients with diabetic foot infections in different grades. A total of 130 diabetic patients were enrolled in this case control study between June 2011 and March 2012 in Rizgary, Emergency and Hawler Teaching Hospitals, 90 of them with diabetic foot lesion as a patient group. 40 without foot lesion, as a patient control and 20 individuals as healthy control. Assessment of above parameters in sera of study groups and also bacteriological tests (bacterial isolation and identification) were done. Serum procalcitonin levels significantly increased in patients with diabetic foot with higher Wagner grades (III, IV and V) (0.28±0.04, 0.30±0.07 and 0.60 ±0.11) respectively (P<0.01), indication for amputation (0.45±0.06) (P<0.01), and polymicrobial infection (0.345±0.043) (P<0.05). The severity of foot ulcer based on Wagner classification system was also associated with circulating levels of C-reactive protein, Interleukin1β, Interleukin-6 and tumor necrosis factor-α (G III, IV and V) (5.36±0.70, 6.38±0.65, and 9.13 ±0.88), (1.21±0.08, 1.56±0.16 and 2.02±0.07), (23.02± 2.98, 36.32± 5.75 and 43.36± 6.16), and (215.39± 16.8, 259.21± 40.7 and 398.45±33.4) respectively (P<0.01). A new useful diagnostic parameter in infected diabetic foot patients may be a procalcitonin especially in those with higher Wagner grades and with polymicrobial infection.



## INTRODUCTION

Diabetic foot infection (DFI) is one of the most feared complications of Diabetes mellitus (DM) [1, 2]. Diabetic foot disease presents in various ways such as ulcer, infection/abscess, and gangrene [3]. About 15% of people with diabetes will develop a foot ulcer at some time during their life, and 85% of major leg amputations begin with a foot ulcer [4, 5]. Most of DFIs are polymicrobial [6], gram-positive bacteria, such as *Staphylococcus aureus* (*S. aureus*) and coagulase negative staphylococci are the most common pathogens [7]. A mixture of aerobic gram positive, aerobic gram-negative (e.g., *Pseudomonas spp*., *Escherichia coli*, *Klebsiella spp*. and *Proteus spp*.) and anaerobic organisms causes infection in deep limb-threatening infection or chronic wounds [8, 9].

Multiple classification systems exist for diabetic foot ulceration and foot syndrome. The most widely recognized classification is the Wagner system, which grades ulcers from 0 to 5 based largely on ulcer depth and severity [10, 11]. The severity of diabetic foot infections ranges from mild and self-limited to limb- and even life threatening [12, 13]. Infection characterizes the presence of an inflammatory response and tissue injury as a result of host interaction with multiplying bacteria [11]. The early recognition of infection is paramount in the management of diabetic foot disease, thus a rapid and reliable test to rule out bacterial infection would be helpful in decision making [14].

Procalcitonin (PCT) has recently emerged as a powerful biomarker for an early and accurate diagnosis of bacterial infection [15, 16]. PCT is a precursor of hormone calcitonin (CT), secreted by C- cells of thyroid glands; it

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is thoroughly transformed into calcitonin [17, 18]. PCT levels in the circulation are very low while in bacterial infections, proinflammatory mediators induce CT mRNA. In contrast to thyroidal cells, adipocytes and other parenchymal cells lack secretory granules, and hence, unprocessed PCT is released in a nonregulated, constitutive manner [19, 20].

C-reactive protein (CRP) is an acute-phase reactant found in the blood that is produced by hepatocytes in the setting of infection or tissue injury. Rapid, marked increases in CRP occur with inflammation, infection, trauma, malignancies, and autoimmune disorders [21]. Diabetic foot wounds are marked by a persistent and dysregulated inflammatory phase, an enhanced production and release of proinflammatory cytokines such as (IL-1β, IL-6 and TNF α) causes disturbance the balance between proinflammatory and anti-inflammatory macrophages [22]. The study aimed to evaluate the level of PCT, CRP, IL-1β, IL-6, and TNF-α in diabetic patients with and without foot lesions, and to demonstrate the possible relationship with the nature of a causal microbial agent**.**

## PATIENTS AND METHODS

A prospective case control study was conducted on 90 diabetic patients with foot lesion referred to Rizgary, Emergency and Hawler Teaching Hospitals in Erbil city-Iraq in a period between June 2011 and May 2012.The patients were clinically assessed by the treating medical team, and all wounds were graded when they were admitted to the hospital according to the Wagner classification system into five grades (grade I - grade V) [11, 23]. Forty diabetic patients without foot lesion attended Leila Qasim center for diabetes, they were free from foot wounds and acute or chronic disease. Their age, sex and type of diabetes were matched with patients group, and 20 apparently healthy individuals chosen from hospital staff, who have no history of clinical evidence of any acute or chronic disease.

Blood sample was drawn from each individual of study groups.Immunomicrobiologic investigations were done to estimate level of different immunological biomarkers in the serum of study groups by quantitative determination of procalcitonin VIDAS® B·R·A·H·M·SPCT, CRP (DRG-USA), IL-1β, IL-6 and TNF-α (RayBiotech-USA) by enzyme linked immune sorbent assay (ELISA). Isolation and identification of the causative agents in the DFI were also done by using different types of API system such as API staph, API 20 E, API strep and API 20 A (Biomerex). Statistical analysis was performed by SPSS (Statistical Package for Social Sciences) version 18. The study was undertaken after gaining approve of the ethics committee of the college of medicine/ Hawler medical university.



## RESULTS AND DISCUSSION

Diabetes mellitus is a serious health problem that is rapidly expanding worldwide. One of the more frequent diabetic complications is diabetic foot which results from a complex interaction between a numbers of risk factor such as neuropathy, peripheral vascular disease, foot deformity and trauma [9]. Diabetic patients have also functional changes in microcirculation, cellular activity and growth factor activation processes, which increasingly disrupt wound healing [22]. The study was designed to investigate some immunological biomarkers (PCT, CRP, IL-1β, IL-6, and TNF-α) in 90 diabetic patients with foot lesion, 40 diabetic patient controls and 20 healthy controls.

"TABLE 1," lists the demographic characteristics of the patients with DFL, including the association of different variables with the disease. The age of the 90 patients with DFL in this study, ranged from 35-85 years with a mean age of (58.5) years, in which 51 (57%) were male and 39 (43%) patients were female. Male to female ratio was 1.3:1. Bacteriological analysis revealed that 83 of patients (92.22%) had positive culture while only 7 patients (7.78%) had negative culture. Almost similar results were obtained by other researchers [26, 27]. Patients with diabetes are particularly susceptible to foot infection primarily because of neuropathy, vascular insufficiency, and diminished neutrophil function [25].

**TABLE 1.** *Demographic profile of 90 consecutive patients with DFL*

| Patient characteristics | Value |
|---|---|
| **Age (years): Mean ±SE** | 58.50±1.10 |
| **Sex: No (%)** | |
| Male/female | 51 (56.7) / 39 (43.3) |
| **Type of DM: No. (%)** | |
| Type I / Type II | 5 (5.6)/ 85 (94.4) |
| **RBS on admission (mg/dl): Mean ±SE** | 328.3±12.24 |
| < 200: No (%) | 13 (14.4) |
| ≥ 200: No (%) | 77 (85.6) |
| **Duration of DM: No. (%)** | |
| <10 years | 35 (38.9) |
| ≥10 years | 55 (61.1) |
| **Wagner's grade: No. (%)** | |
| Grade I | 14 (15.5) |
| Grade II | 24 (26.6) |
| Grade III | 25 (27.7) |
| Grade IV | 15 (16.6) |
| Grade V | 12 (13.3) |
| **History of ulceration: No. (%)** | |
| With previous ulceration/ Without previous ulceration | 62 (68.9) / 28 (31.1) |
| **Culture result** | |
| Positive culture/Negative culture | 83 (92.22) /7 (7.78) |

DM: Diabetes mellitus; RBS: Random blood sugar

The microbiological profile was also done on patients group, and the association between circulating levels of the studied biomarkers and the microbial isolates were investigated. The total number of aerobic isolated bacteria was 113 (83.7%), among these isolates, *Staphylococcus spp.* was the predominant isolates, appearing in 43 (38 %), representing by *S. aureus* (26 (23 %) and coagulase negative staphylococci 17 (15.04%) "TABLE 2," 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 [28].

Out of 113 aerobic bacteria, gram-positive bacteria (Gram+ve) 60 (53.1%) were seen to be more commonly isolated than gram-negative bacteria (Gram-ve) 53 (46.9%), and among 135 isolates of 90 patients, only one pathogen (monomicrobic) isolated from 37 (41%) patients. More than one pathogen (polymicrobic) was present in 46 (51%) patients. Almost similar result was obtained by other studies [26, 29].

**TABLE 2.** *Types and profiles of organisms isolated in 83 patients with DFL*

| Type of isolate | No. (%) | Type of isolate | No. (%) |
|---|---|---|---|
| **Aerobes** | **113 (83.7)** | **Anaerobes** | **17 (12.6)** |
| **Gram positive aerobes** | | ***Peptostreptococcus* spp.** | **8 (47.05)** |
| *Staphylococcus aureus* | 26 (23) | *Bacteroides fragilis* | 5 (29.41) |
| Coagulase negative staphylococci | 17 (15.04) | *Clostridium clostridioforme* | 2 (11.76) |
| *Enterococcus* spp. | 11 (9.7) | *Fusobacterium* spp. | 2 (11.76) |
| *Streptococcus* spp. | 6 (5.3) | Total bacterial isolates | 130 |
| **Total** | **60 (53.1)** | | |
| **Gram negative aerobes** | | **Fungi** | **5 (3.7)** |
| *Escherichia coli* | 20 (17.7) | *Candida albicans* | 5 (100) |
| *Proteus* spp. | 8 (7.07) | **Total microbial isolates** | **135** |
| *Pseudomonas* spp. | 7 (6.19) | | |
| *Klebsiella oxytoca* | 4 (3.53) | | |
| *Acinetobacter baumani* | 4 (3.53) | | |
| *Enterobacter clocaea* | 3 (2.65) | | |
| *Morganella morgana* | 2 (1.76) | | |
| *Aeromonas hydrophila* | 2 (1.76) | | |
| *Citrobacter frundi* | 2 (1.76) | | |
| *Cedecea davisae* | 1 (0.88) | | |
| **Total** | **53 (46.9)** | | |

The mean of serum PCT concentration in patient with DFL was (0.26±0.02), however in both PC and HC, their concentrations were the same **(**0.04± 0.00**)** with statistically highly significant difference (P<0.01) (Figure 1). However, means of CRP, IL-1β, IL-6 and TNF-α, were (5.31±3.20), (1.18±0.06), (23.04±2.04) and (208.24±13.71)) respectively, which was significantly higher than those in both control groups (P<0.001) "TABLE 3," PCT concentration in the plasma of healthy subjects is very low (0.01-0.05 ng/ml). It is induced by bacterial endotoxin or inflammatory cytokines. PCT is preferentially induced during severe generalized bacterial, parasitic or fungal infections with systemic manifestations rather than inflammatory reactions of noninfectious origin [17, 30].

Indeed, CRP, IL-1β, IL-6 and TNF-α levels were higher in diabetic patient without foot lesion (PC) when compared to HC, and its level was higher in patient with DFL when compared to PC. This result can be explained in term of Weigelt *et al*., [31] suggestion that acute foot ulcers may lead to a rise in the level of acute-phase proteins, cytokines and chemokines even without concomitant infection and positively correlated with measures of insulin resistance [32]. Hyperglycemia can lead to enhanced glycolysis and mitochondrial overproduction of reactive oxygen species, which directly induce the activation of nuclear factor-κβ, which cause induce the release of IL-1β and IL-6 by human monocytes cultured under high glucose conditions [33].

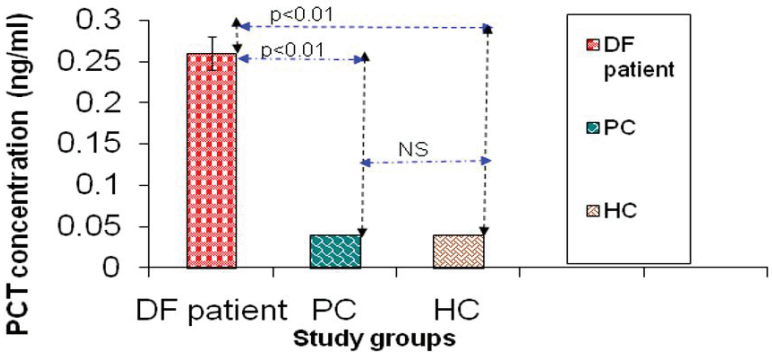


**FIGURE 1.** Serum level of PCT (ng/ml) in patients with DFL and control groups

**TABLE 3.** *Serum levels of PCT, CRP, IL-1β, IL-6 and TNF-α in patients with DFL and control groups*

| Parameters | DF patient No.90 | PC No.40 | HC No.20 | P value (F-test) |
|---|---|---|---|---|
| | Mean ±SE | Mean ±SE | Mean ±SE | |
| PCT (ng/ml) | 0.26 ± 0.02 | 0.04 ± 0.00 | 0.04 ± 0.00 | P<0.001 |
| CRP (mg/l) | 5.31±3.20 | 3.51±1.67 | 1.10±1.04 | P<0.001 |
| IL-1β (pg/ml) | 1.18±0.06 | 0.51±0.08 | 0.18±0.01 | P<0.001 |
| IL-6 (pg/ml) | 23.04±2.04 | 6.14±1.18 | 2.60±0.11 | P<0.001 |
| TNF-α (pg/ml) | 208.24±13.71 | 99.38±6.81 | 40.13±2.32 | P<0.001 |

HC: Healthy control; PC: Patient control; P≤0.01: Highly significant

There was a linear increase in the PCT and others studied biomarkers levels with increase in Wagner's grade, such as PCT mean level was 0.04± 0.00 in G1 and 0.60 ±0.11 in G V "TABLE 4,". There are a close correlation between the PCT and other studied parameters concentrations with the severity of systemic inflammation, which reflect different grades of Wagner system. Almost similar result was obtained by Zubair *et al* [34] who stated that the severity of ulcers based on university of Texas classification was also associated with circulating levels IL-6, hsCRP, and TNF-α3. Our result showed that the specific combination of Wagner grade with serum inflammatory markers appears to be a particularly sensitive strategy that may allow for greater detection of diabetic foot grading.

**TABLE 4.** *Levels of PCT, CRP, IL-1β, IL-6 and TNF-α in patients with DFL according to Wagner grades*

| Parameter | G I No.14 | G II No. 24 | G III No. 25 | G IV No. 15 | G V No. 12 | P value F- test |
|---|---|---|---|---|---|---|
| | Mean ±SE | Mean± SE | Mean ±SE | Mean ±SE | Mean ±SE | |
| PCT ng/ml | 0.04± 0.00 | 0.16± 0.02 | 0.29±0.04 | 0.30 ±0.07 | 0.60 ±0.11 | P<0.01 |
| CRP mg/l | 3.14± 0.29 | 3.94± 0.43 | 5.36±0.70 | 6.38 ±0.65 | 9.13 ±0.88 | p<0.01 |
| IL-1β (pg/ml) | 0.52±0.04 | 0.88±0.08 | 1.21±0.08 | 1.56 ±0.16 | 2.02±0.07 | P<0.01 |
| IL-6 (pg/ml) | 6.71± 1.09 | 14.12± 2.29 | 23.02±2.98 | 36.32 ±5.75 | 43.36 ±6.16 | P<0.01 |
| TNF-α (pg/ml) | 98.15± 3.8 | 138.06± 11.6 | 215.39±16.8 | 259.21 ±40.7 | 398.45±33.4 | P<0.01 |

P<0.05: Significant; P<0.01: Highly significant; P>0.05: No significant; G: Grade

Diabetic foot patients with indication for amputation (Group A) had highly significant serum PCT, CRP, IL-1β, IL-6 and TNF-α levels (0.45± 0.06, 7.71 ± 0.54, 1.67±0.10, 38.8±3.8 and 316.7±29.2) compared to (0.17 ± 0.02, 4.11 ± 0.33, 0.94±0.06, 15.1±1.6 and 157.3±11.6) in patient without indication for amputation (Group B) respectively, with highly significant difference (P<0.01) "TABLE 5,", this result agree with other researchers result [35, 36]. Elevated baseline levels of acute phase reactants were associated with clinical treatment failure in DFIs [37].A study demonstrated the elevated expression of IL-1β and excessive TNF-α in non-healing wounds [38]. This may be the case, as high TNF-α has been described not only as inducing IL-1β and its own synthesis but also their synergistic effects resulting in the suppression of extracellular matrix synthesis, thus contributing to persistent inflammatory activity and tissue destruction in diabetes.



**TABLE 5.** *Serum levels of PCT, CRP, IL-1β, IL-6 and TNF-αaccording to indication for amputation*

| Parameters | Group A No. 30 | Group B No. 60 | P value |
|---|---|---|---|
| | Mean± SE | Mean± SE | t-test |
| PCT (ng/ml) | 0.45 ± 0.06 | 0.17 ± 0.02 | P<0.001 |
| CRP (mg/l) | 7.71 ± 0.54 | 4.11 ± 0.33 | P<0.001 |
| IL-1β (pg/ml) | 1.67 ± 0.10 | 0.94 ± 0.06 | P<0.001 |
| IL-6 (pg/ml) | 38.8 ± 3.8 | 15.1 ± 1.6 | P<0.001 |
| TNF-α (pg/ml) | 316.7±29.2 | 157.3±11.6 | P<0.001 |

Group A: Patients with indication for amputation; Group B: Patients without indication for amputation<0.01: Highly significant.

patients with DFL infected with poly-microbial infections (mixed) had a significantly higher serum PCT, IL-1β, IL-6 and TNF-α level compared to those infected with mono-microbial infections (pure), *S aureus* as the most common isolate (P<0.05). CRP had a comparatively higher level in patients with DFL infected with poly-

microbial infections such as *S aureus*, *E coli* and *Enterococcus spp* isolates but without any statistically significant difference (P>0.05) "TABLE 6,". The reason may be due to infection with multiple microorganisms not only contributed to deterioration of the wound condition locally, but also led to systemic involvement [39].

Also Brodska *et al* [40] stated that plasma PCT levels can be influenced by multiple factors such as individual genetically determined immune alert as well as the degree and type of microbial aggression or the extent of inflammation. The result of researcher, demonstrated that PCT has emerged as a biomarker for bacterial infections because it correlates with the extent and severity of microbial invasion in different infections [41].

**TABLE 6.** *Serum levels of PCT, CRP, IL-1β, IL-6 and TNF-αaccording to type of culture*

| Parameters | Pure No. 37 | Mixed No. 46 | P value |
|---|---|---|---|
| | Mean± SE | Mean± SE | t-test |
| PCT (ng/ml) | 0.206 ± 0.038 | 0.345 ± 0.043 | P<0.05 |
| CRP (mg/l) | 4.843± 0.504 | 6.152±0.475 | NS |
| IL-1β (pg/ml) | 1.051 ± 0.088 | 1.387 ± 0.092 | P<0.05 |
| IL-6 (pg/ml) | 17.62 ± 2.397 | 29.89 ± 3.171 | P<0.01 |
| TNF-α (pg/ml) | 182.7 ± 18.74 | 244.7 ± 20.54 | P<0.05 |

Pure: Patients with monomicrobial infection; Mixed: Patients with polymicrobial infection;P<0.05: Significant; P>0.05: No significant.



Patients infected with gram-negative bacteria also had higher serum PCT, CRP, IL-1β, IL-6 and TNF-α levels (0.238 ± 0.030, 5.741 ± 0.861, 0.957± 0.113,15.89± 3.033 and 172.5±25.1versus 0.191 ± 0.055, 4.412 ± 0.641,1.245 ± 0.124, 21.22±3.79 and 203.8±24.8) than those infected with gram-positive bacteria, without a significant difference, (P>0.05) "TABLE 7,". Our finding is partly consistent with [41] who demonstrated significantly higher in vitro production of IL-6, IL-8, TNF-α, and IL-1β in *E. coli* infections compared to the production of these proinflammatory cytokines in staphylococci. Abe *et al*., [42] affirmed that gram-negative bacteremia induces greater magnitude of inflammatory response than gram-positive bacteremia, and he suggests that different types of pathogen-associated molecular patterns may induce different type and magnitudes of inflammatory response. PCT detection should be included in the diagnosis strategy of patients with DFL.

**TABLE 7.** *Serum levels of PCT, CRP, IL-1β, IL-6 and TNF-α levels according to type of bacteria*

| Parameters | Gram +ve No. 25 | Gram –ve No. 12 | P value |
|---|---|---|---|
| | Mean± SE | Mean± SE | t-test |
| PCT (ng/ml) | 0.191 ± 0.055 | 0.238 ± 0.030 | NS |
| CRP (mg/l) | 4.412 ± 0.614 | 5. 741 ±0.861 | NS |
| IL-1β (pg/ml) | 0.957± 0.113 | 1.245 ± 0.124 | NS |
| IL-6 (pg/ml) | 15.89± 3.033 | 21.22±3.79 | NS |
| TNF-α (pg/ml) | 172.5±25.1 | 203.8±24.8 | NS |

Gram +ve: Patients with pure gram positive infection; Gram -ve: Patients with pure gram negative infection;P>0.05: No significant.

## CONCLUSIONS

Most of the DFI been found to be of a polymicrobial etiology, prevalence of gram-positive cocci was higher than gram-negative bacilli, and *Staphylococcus aureus* was the most common isolate. Procalcitonin assay is a new and valuable serological marker to detect bacterial infection in DFI compared to CRP and other inflammatory markers.

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