Procalcitonin levels and other biochemical parameters in patients with or without diabetic foot complications

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Background: Diagnosis of infection in diabetic foot ulcer (DFU) is not always simple. The analytic precision of procalcitonin (PCT) was evaluated to clarify the use of PCT for distinguish the presence of infection in DFU in comparison to other inflammatory markers. **Materials and Methods:** This study comprised 88 subjects distributed into four groups: 16 nondiabetic healthy subjects (group control), 17 patients with type 2 diabetes mellitus without foot Complication (group DM), 25 patients with noninfected diabetic foot (group NIDF), and 30 patients with infected diabetic foot (group IDF). Fasting blood samples were taken for measurement of glucose, hemoglobin A1C, lipid profile, renal function, erythrocyte sedimentation rate (ESR), and white blood cell (WBC) and its derivatives. Plasma PCT was determined using an enzyme-linked immunosorbent assay. **Results:** PCT, WBC, ESR, and neutrophils (NEU) were found significantly higher in IDF group than other groups. The receiver operating characteristic analysis showed that sensitivity, specificity, the best cutoff value, and the area under the curve were for ESR (100%, 93%, 31.5 mm/h, 1; $P < 0.001$), for PCT (87.5%, 86.7%, 66.55 pg/dl, 0.977; $P < 0.001$), for NEU (93.8%, 93.3%, 5.35, 0.957; $P < 0.001$) and for WBC (93.8%, 90%, 9.29×10^9/L, 0.942; $P < 0.001$), respectively. **Conclusion:** The outcomes of this study recommend that PCT can be an asymptomatic marker in the diagnosis of infection in DFU with higher Wagner grades in combination with different inflammatory markers.

Key words: Diabetes mellitus, diabetic foot ulcer, inflammatory markers, procalcitonin

INTRODUCTION

Diabetic foot infection is most essentially characterized as any bizarre infection in a person's foot with diabetes mellitus (DM).[1] Vascular deficiency, infection, and inability to actualize powerful treatment of diabetic foot ulcers (DFUs) are connected with secondary therapeutic complexities, for example, osteomyelitis, and amputations.[2] A study in the United States reported that 38% of all the amputations were correlated with DFU; this can prompt extreme morbidity and mortality,[3] they are in more serious danger of sudden death, myocardial infarction, and lethal stroke than those without a past filled with DFU.[4] DFU puts colossal money burden on the patient and the health care administrations, although it is preventable.[5] Diagnosing an infection in DFUs is defiance, and particularly genuine when the clinical appraisal is not decisive.[6] The traditional markers in this setting incorporate leukocyte count and C-reactive protein (CRP) and also the erythrocyte sedimentation rate (ESR), but the diagnosis not specific for DFU infection. Although, isolation of the pathogenic organism from DFU has high specificity but lack to sensitivity and time consume. Other systemic markers specific to bacterial infection, for example, procalcitonin (PCT), orosomucoid, and haptoglobin have been assessed for the determination of infection.[7]

In this study, the analytic precision of PCT level was evaluated in comparison with other inflammatory

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markers in DFU as a pointer to make the difference between infected and noninfected DFU.

MATERIALS AND METHODS

Study population

This case-control study has been conducted from October 2015 to end of February 2016. Seventy-two patients with type 2 DM (T2DM) were recruited among registered patients of National Diabetes Center and National Center of Hematology. T2DM was diagnosed using American Diabetes Association criteria (Expert Committee on the Diagnosis and Classification of DM, 2015); fasting serum glucose (FSG) ≥126 mg/dl (normal value 70–110 mg/dl) or 2 h postprandial glucose ≥200 mg/dl (normal value <140 mg/dl).[8]

Consultant physicians in the Diabetes Centers took a detailed history and did complete clinical examinations. Patients had been divided into 4 groups. Fifty-five patients with DFU were split into two groups according to Infectious Diseases Society of America and the International Working Group on the Diabetic Foot criteria (the Wagner classification system assesses ulcer depth and the presence of osteomyelitis or gangrene using the following grades: Grade 0 [pre- or post-ulcerative lesion], Grade 1 [partial/full thickness ulcer], Grade 2 [probing to tendon or capsule], Grade 3 [deep with osteitis], Grade 4 [partial foot gangrene], and Grade 5 [whole foot gangrene]). Noninfected diabetic foot (NIDF) group included 25 participants with diabetic foot, without infection, with age ranged between 36 and 65 years and mean age (51.24 ± 6.82) year, and infected diabetic foot (IDF) included 30 participants with diabetic foot, complicated by infection, their age ranged between 40 and 65 years with mean age (54.32 ± 6.43) year. Clinical signs of infection that recorded in this study were redness, swelling of the wound, pus spots, or exudates from the wound, fever, and pain in the infected area.[9] DM group included 17 patients with T2DM without any foot complications; age ranged between 41 and 67 years and mean age (49.35 ± 8.70) year. Sixteen nondiabetic healthy participants with age ranged between 40 and 63 years old and mean age (47.06 ± 6.89) year, allocated for the control group. Patients, who receive antibiotic treatment for < 10 days or with any other source of infection, have been excluded from the study.

All subjects were informed of the purpose of the study and their oral consent was obtained. The study was approved by the Ethics Committee of College of Science.

Demographic and clinical data of patients and control group were collected in the form of age, gender, weight, height, waist, and hip. Waist to hip ratio (WHR), body mass index (BMI), and waist to height ratio (WHtR) were calculated. Other diabetic complications (retinopathy, neuropathy, nephropathy, and cardiomyopathy) were documented for the patients groups. All T2DM patients, treated with either oral glucose lowering agents (metformin, glibenclamide, or mix of both drugs) or with insulin had been included in the study.

Samples and laboratory analysis

A volume of 10 ml of venous blood was drawn from each subject after fasting for 8–12 h, using 10 ml disposable syringe. The blood sample was divided into three aliquots; the first aliquot was 4 ml of blood were transferred into EDTA tube for measuring hemoglobin A1C (using a commercial kit, SDA1cCareTM, SD Biosensor, Germany), ESR using Westergren tube, and white blood cell (WBC) and its derivatives using autoanalyzer device (Abbott, USA).

Serum blood was processed for the measurement of FSG using commercial kit (Biolabo, France). Lipid profile (total cholesterol, triglyceride, high-density-lipoprotein-cholesterol were measured using commercial kits [Biolabo, France]. Low-density lipoprotein-cholesterol [LDL-C] and Very Low-Density Lipoprotein [VLDL]) were calculated using Friedewald's equation.[10] Renal Function Tests (urea, creatinine, and uric acid) were measured using autoanalyzer device (Biolabo, France). Blood plasma was used for PCT estimation, using Human PCT ELISA kit (Abcam, USA) with a sensitivity limit <20 pg/ml and range (27.43–20000) pg/ml.

Statistical analysis

Statistical analysis was performed using SPSS software version 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA).

Continuous variables were expressed as the mean ± standard deviation. Kolmogorov–Smirnov test was used to check normality of the data. The comparison of quantities of all parameters between all groups was analyzed using Student's *t*-test. One-way ANOVA with *post hoc* test was used for comparison of mean between groups. The Mann–Whitney U-test was used for continuous variables, and a Chi-square test was used for categorical variables. The diagnostic accuracy of each inflammatory marker was described by the following parameters: sensitivity and specificity, which were used to calculate the optimal cutoff value. The area under the curve (AUC) was used to assess the diagnostic accuracy. The value of $P < 0.05$ was considered statistically significant.

RESULTS

The demographic and clinical data of the four groups (C, DM, IDF, and NIDF) are summarized in Table 1.

As shown in Table 1, with respect to age, gender, WHtR, and BMI; no significant difference was noted among the four groups (*P* > 0.05). However, WHR in NIDF and DM groups were significantly higher than IDF and control groups (*P* < 0.01 and *P* < 0.05, respectively). The higher WHR in patients of NIDF and DM is an indication to the presence of central obesity more than in IDF and control groups.

The mean value of ESR in IDF group was significantly higher than other groups (*P* < 0.01), whereas, no significant difference was found in relation to ESR among DM, NIDF, and control groups (*P* > 0.05). WBC and neutrophils (NEU) levels in group IDF were higher significantly than in DM, NIDF, and control groups (*P* < 0.01).

Serum PCT concentrations in patients with IDF were higher significantly than in patients with DM, NIDF, and control groups (*P* < 0.01). However, no significant differences were noted in the mean value of PCT among patients with DM, NIDF, and control groups (*P* > 0.05).

An increase in PCT levels were found as the grade of Wagner increase, as shown in Table 2. Furthermore, a significant increase was observed in PCT concentration in IDF group (2, 3, 4, and 5 Wagner grades) when compared with that of NIDF group (0 and 1 Wagner grades) (*P* < 0.0001).

Receiver operating characteristic (ROC) analysis was performed to reveal the diagnostic accuracy of using PCT

Table 1: Demographic and clinical data of patients and control groups

Demographic	C	DM	NIDF	IDF	P (*post hoc* test)
Number	16	17	25	30	–
Gender (male/female)	8/8	9/8	15/10	22/8	0.37
Age (year)	47.06±6.89	49.35±8.70	51.24±6.82	54.32±6.43	0.161
Duration of disease(year)	–	7.24±6.534	10.6±5	12.24±5.67	0.06
BMI (kg/m²)	28.06±3.71	30.24±5.67	29.72±4.7	31.2±10.68	0.585
WHR	0.87±0.07	0.96±0.09	0.97±0.11	0.93±0.15	0.009**
WHtR	0.54±0.04	0.59±0.06	0.62±0.09	0.6±0.12	0.095
FSG (mg/dL)	94.88±16.94	222.35±72.93	252.64±98.73	258.5±104.03	<0.0001**
HbA1c (%)	5.42±0.46	8.68±1.98	9.54±1.49	10.18±1.12	<0.0001**
TC (mg/dL)	166.75±26.57	188.35±24.46	197.28±43.67	199.77±37.47	0.022*
TG (mg/dL)	90.56±28.53	179.47±19.75	183.32±31.15	255.67±59.78	<0.0001**
HDL-C (mg/dL)	41.1±13	39.82±8.23	38.11±8.67	33.29±6.97	0.041*
LDL-C (mg/dL)	100.94±13.77	109.12±33.03	122.48±40.01	115.3±34.44	0.222
VLDL-C (mg/dL)	18.19±5.67	40.06±13.32	36.68±6.32	51.2±12	<0.0001**
Urea (mg/dL)	24.19±5.58	26.18±7.77	32.58±10.81	37.36±18.05	0.003**
Uric acid (mg/dL)	3.97±0.37	3.74±1.2	3.96±0.99	4.9±1.12	<0.0001**
Creatinine (mg/dL)	0.75±0.132	0.83±0.17	0.87±0.2	1.1±0.57	0.008**
Lymphocytes	2.63±0.83	2.72±0.699	2.77±0.84	2.49±0.66	0.553
Monocytes	0.53±0.162	0.517±0.136	0.559±0.196	0.857±0.297	<0.0001**
Eosinophils	0.175±0.1	0.27±0.17	0.25±0.17	0.29±0.18	0.164
Basophiles	0.067±0.02	0.079±0.032	0.084±0.035	0.09±0.033	0.02*
Neutrophil	4.04±0.85	3.98±1.19	4.96±1.44	9.63±4.14	<0.0001**
WBC (10⁹/L)	7.433±1.37	7.56±1.6	8.63±1.96	13.38±4.33	<0.0001**
ESR (mm/hr)	13.69±7.32	21.47±15.89	25.2±14.26	76.73±33.39	<0.0001**
PCT (pg/dL)	38.66±18.65	43.77±15.33	45.1±9.43	160.54±112.71	<0.0001**

Data are expressed as mean±SD, and analyzed using ANOVA. Significant *P* values are **P<0.01, *P<0.05. C = Control group; DM = Diabetic patients; BMI = Body mass index; ESR = Erythrocyte sedimentation rate; FSG = Fasting serum glucose; HbA1c = Hemoglobin A1c; HDL-C = High density lipoprotein-cholesterol; IDF = Infected diabetic foot ulcer; LDL-C = Low density lipoprotein -cholesterol; NIDF = Noninfected diabetic foot ulcer; PCT = Procalcitonin; TC = Total cholesterol; TG = Triacylglycerols; VLDL = Very low density lipoprotein; WBC = White blood cells; WHR = Waist-hip ratio; WHtR = Waist- height ratio; SD = Standard deviation

Table 2: Mean±standard deviation and percentage of procalcitonin values among different types of diabetic foot grades according to Wagner classification

DFU	Wagner Grade	Patients, *n* (%)	PCT (pg/dL)		P value using *t*-test between NIDF and IDF groups
			Mean±SD for each Wagner grade	Mean±SD of two groups	
NIDF (*n*=25)	Zero	5 (20)	35.03±4.22	57.73±6.56	P<0.0001
	One	20 (80)	45.94±16.37		
IDF (*n*=30)	Two	7 (24)	102.05±41.41	251.35±117.72	
	Three	13 (43)	103.35±58.3		
	Gangrene four and five	10 (34)	275.82±114.48		

PCT = Procalcitonin; SD = Standard deviation; NIDF = Noninfected diabetic foot; IDF = Infected diabetic foot ulcer; DFU = Diabetic foot ulcer

and other inflammatory markers (ESR, WBC, and NEU) to distinguish infection in DFU. Sensitivity, specificity, the best cutoff value, and the AUC are presented in Table 3 and Figure 1; for ESR were (100%, 93%, 31.5 mm/h, 1; *P* < 0.001), for PCT (87.5%, 86.7%, 66.55 pg/dl, 0.977; *P* < 0.001), for NEU (93.8%, 93.3%, 5.35, 0.957; *P* < 0.001) and for WBC (93.8%, 90%, 9.29 × 10⁹/L, 0.942; *P* < 0.001), respectively. Cutoff values lower than this have higher sensitivity, but low specificity and vice versa. Hence, a cutoff value at which sensitivity and specificity got balanced was taken in this study.

DISCUSSION

DFU is an important complication of diabetes, whose prevention and prompt treatment of infection with antimicrobials is imperative.[11]

This study aimed to assess using PCT in the diagnosis of infection in DFU in comparison with some inflammatory markers. Predictive factors of bacterial inflammation in those with diabetes may help us to reduce antibiotic resistance and treatment response. Consistent with other studies,[11-13] significant increase of WBC, ESR, and PCT levels in IDF compared with other groups were presented in this study.
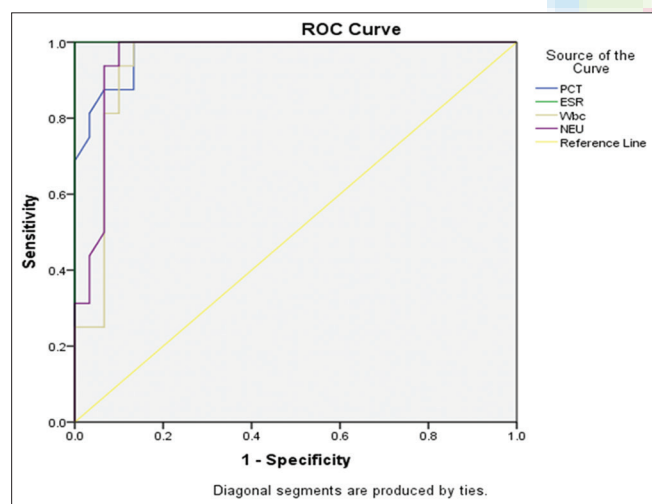

Figure 1: The receiver operating characteristic curve represents the specificity, and sensitivity of procalcitonin, erythrocyte sedimentation rate, white blood cell and neutrophils in infected diabetic foot group

Somewhat, increased PCT concentrations were seen in inflammatory response with minor systemic bacterial infections. PCT level is normally increase in patients with serious and systemic infection,[14] also PCT level is higher in acute diseases with extreme systemic reactions caused by infection; for instance, serious sepsis or septic shock. However, localized infections not always cause PCT increase.[15]

Since PCT is considered an acute phase protein,[13] a group of diabetic patients without foot complication was enrolled, to exclude the inflammatory process accompanying diabetes that may cause an increase of PCT concentration. The results here showed no significant difference in PCT concentration in patients with DM than that in control group.

Some observational studies have suggested that PCT might be a reliable marker for infection,[16-18] whereas other studies did not support these observations.[11,13,19]

In this study, higher levels of PCT were present with higher Wagner grades in IDF patients, a result consistent with other studies.[11,17,20] Considering that, it is more effective than other laboratory markers in bone infection diagnosis,[17] bone involvement,[21] in distinguishing Gram-negative sepsis from Gram-positive in DFUs.[22]

The results in this study reveal that ESR has the highest AUC and the greatest statistical significance in IDF group. Similar findings have been demonstrated by Jonaidi Jafari *et al.*, 2014 who reported that the area under the ROC curve for ESR was the greatest (0.967; *P* < 0.001), followed by CRP (0.871; *P* < 0.001), PCT (0.729; *P* < 0.001) and in the end, by WBCs (0.721; *P* = 0.001) and the specificity and sensitivity of ESR were higher than PCT or WBC. They concluded that PCT can be helpful in the diagnosis of infection, and the higher efficiency of ESR in denoting infection when compared with PCT could be explained by the mild nature of infection in diabetic foot wounds with low-grade.[11]

Li *et al.*, 2016 performed the ROC analysis to reveal the diagnostic accuracy of using PCT or CRP concentrations to distinguish Gram-negative sepsis from Gram-positive

Table 3: The specificity, sensitivity, best cutoff value, and the area under the curve of procalcitonin, erythrocyte sedimentation rate, white blood cells and neutrophils in infected diabetic foot ulcer group

Parameter	AUC	*P*	95% CI		Cutoff value	Sensitivity %	Specificity %
			Lower bound	Upper bound			
ESR mm/h	1	<0.001	1	1	31.5	100	93
PCT pg/dL	0.977	<0.001	0.944	1	66.55	87.5	86.7
NEU	0.957	<0.001	0.899	1	5.35	93.8	93.3
WBC (10⁹/L)	0.942	<0.001	0.871	1	9.29	93.8	90

AUC = The area under the curve; CI = Confidence interval; ESR = Erythrocyte sedimentation rate; NEU = Neutrophils; PCT = Procalcitonin; WBC = White blood cells

sepsis. An optimal cutoff value of 2.44 ng/ml (sensitivity of 77.1%, specificity of 68.4%), was found, and they reached a conclusion that significantly higher PCT level can serve as a marker to diagnose Gram-negative sepsis from Gram-positive sepsis, although these inflammatory markers failed to distinguish Gram-positive sepsis from fungal sepsis.[22]

Jeandrot *et al.,* 2008 compared the sensitivity and specificity of PCT with other inflammatory markers (orosomucoid, haptoglobin, albumin, CRP, WBC, and NEU count). They concluded that PCT is not superior in distinguishing diabetic foot wounds with infection from noninfection.[7]

CONCLUSIONS

PCT can be the asymptomatic marker in the diagnosis of DFU infection at higher Wagner grade in combination with different inflammatory markers such as ESR, NEU, and WBC. Further studies are needed to assess the relationship of PCT levels with different types of bacterial infections.

Conflicts of interest
There are no conflicts of interest.

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