Plasma adiponectin, IL-6, hsCRP, and TNF-α levels in subject with diabetic foot and their correlation with clinical variables in a North Indian tertiary care hospital

Mohammad Zubair1,2, Abida Malik1, Jamal Ahmad2
1Department of Microbiology, 2Rajiv Gandhi Centre for Diabetes and Endocrinology, Faculty of Medicine, J. N. Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Abstract

Aim: Pro- and anti-inflammatory processes are crucial in different phases of wound healing and their disturbances interfere with tissue homeostasis after the manifestation of ulcers, leading to chronic non-healing wounds. However, data on the association between inflammation and acute foot syndrome are scarce.

Materials and Methods: Circulating levels of acute-phase reactants and cytokines were measured in diabetic patients with ulcer (n = 162) and without ulcer (n = 162) in a case control study. Results: Of the patients, 85.1% had type 2 diabetes. Subjects with diabetic foot ulcer showed lower median plasma level of adiponectin [8.4 (7.1–9.2) ng/ml vs. 13.4 (12.1–14.2) ng/ml], and higher median plasma levels of interleukin-6 (IL-6) [32.5 (9.4–44.8) ng/ml vs. 6.7 (4.6–14.6) ng/ml], high-sensitivity C-reactive protein (hsCRP) [12.6 (11.2–13.6) mg/ml vs. 8.4 (7.1–9.2) mg/ml], and tumor necrosis factor-alpha (TNF-α) [99.4 (79.9–121.5) ng/ml vs. 4.9 (4.5–5.6) ng/ml]. A positive correlation was found between body mass index (BMI) (r = −0.088, P < 0.264) and retinopathy (r = 0.249, P < 0.001) for adiponectin. For IL-6, it was between grade of ulcer (r = 0.250, P < 0.001), BMI (r = −0.161, P < 0.04), low density lipoprotein-cholesterol (LDL-C) (r = −0.155, P < 0.049), triglycerides (r = −0.165, P < 0.035), retinopathy (r = −0.166, P < 0.035), nephropathy (r = −0.199, P < 0.011), and smoking (r = −0.164, P < 0.036). For hsCRP: grade of ulcer (r = 0.236, P < 0.002), BMI (r = −0.155, P < 0.048), LDL-C (r = −0.174, P < 0.026), triglycerides (r = −0.216, P < 0.005), retinopathy (r = −0.165, P < 0.037), nephropathy (r = −0.028, P < 0.007), and smoking (r = −0.164, P < 0.036), while total cholesterol (r = −0.209, P < 0.007) and neuropathy (r = 0.141, P < 0.072) for TNF-α. Conclusions: This study demonstrates that diabetic subjects with various grades of diabetic foot ulcer showed a higher IL-6, hsCRP, TNF-α, and lower adiponectin plasma levels in comparison with diabetes without foot ulcer, independent of the concomitant infections. It would be interesting to find out whether an activation of immune system precedes the development of foot ulcer and whether anti-inflammatory therapies might be effective in improving the outcome in such patients.

Key words: Correlation, diabetic foot ulcer, India, inflammatory marker

Introduction

The worldwide incidence of diabetes is increasing rapidly, and diabetic foot syndrome is becoming more and more important as a major diabetes complication. The lifetime risk of diabetic patients for developing a chronic foot wound has been estimated to reach about 15–20%, and despite considerable international efforts, foot ulcers continue to be responsible for a high number of lower limb amputations that are associated with decrease in quality of life and increased risk of mortality.1,2 The major risk factors for foot ulcer are diabetic polyneuropathy and peripheral arterial disease.3 Interestingly, data on the relevance of systemic inflammation are very scarce in this context, although low-grade immune activation represents an important risk factor not only for the development of type 2 diabetes4 but also for several macrovascular
Every subject with a American Diabetic Association (ADA) The status of the
foot ulcer was defined as a full-thickness skin defect that required ≥14 days for healing. The ankle–brachial index (ABI) was calculated as the ratio of the ankle systolic blood pressure (defined as the higher of the dorsalis pedis or posterial tibialis measurement) divided by the higher brachial systolic pressure. Subject was classified as having Peripheral arterial disease (PAD) when they had an ABI ≤0.9 and/or when they had undergone a peripheral arterial bypass or amputation. American Diabetic Association (ADA) criteria for hypertension were used (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg in subjects who were not taking antihypertensive medication or antihypertensive treatment, yet present on admission). Hypercholesterolemia was defined as total serum cholesterol ≥150 mg/dL and hypertriglyceridemia as total serum triglyceride ≥200 mg/dL on the basis of ADA-2010 criteria. Coronary artery disease was determined on the basis of history of physician-diagnosed angina, myocardial infarction, or any previous revascularization procedure assessed by questionnaire. Cerebrovascular disease (transient ischemic attack (TIA)/ischemic stroke) was assessed by history, specific neurological examination executed by specialists, and hospitalized or radiological (brain computed tomography or brain magnetic resonance) records of definitive TIA or stroke. All patients had blood pressure, serum glucose, creatinine, serum cholesterol levels, serum triglyceride levels, and urinary albumin excretion (UAE) values measured on first day of admission to the hospital. Duration of ulcer, site and size of ulcer, history of smoking, history of previous amputation and clinical outcome were noted of every patient. Clinical assessment for signs of infection (swelling, exudates, surrounding cellulitis, odor, tissue necrosis, crepitation, and pyrexia) was made by one researcher, classifying the ulcers and determining the presence of clinical signs of infection. Ulcer size was determined by multiplying the longest and the widest diameters and expressed in centimetres square. The wound was graded and staged at the time of hospitalization according to the University of Texas wound classification system as grade 1 (superficial wound, not involving tendon, capsule, or bone), grade 2 (wound penetrating to tendon or capsule), and grade 3 (wound penetrating bone or joint). Grade 0 patients (pre- or post-ulcerative site that has healed) were excluded from the study. Diagnosis of extension to the bone was made in majority of patients by probing with a sterile steel probe. In the absence of sinus tract or an exposed bone, a standard radiograph showing signs of osteomyelitis in the bone was considered definitive and later on magnetic resonance imaging (MRI) was done to confirm the osteomyelitis in suspected patients. Amputation was defined as the complete loss in the transverse anatomical plane of any part of the lower limb.

**Materials and Methods**

The study was a hospital-based prospective cohort study. We recruited 162 diabetic subjects with foot ulceration (group A) hospitalized between 2009 and 2011 at the Rajiv Gandhi Centre for Diabetes and Endocrinology of Jawaharlal Nehru Medical College Hospital, Aligarh Muslim University, Aligarh, India. We also recruited 162 diabetic patients without foot ulceration (group B) admitted to our department for other causes between 2009 and 2011. All patients gave informed consent to take part in this study. Foot ulcer was defined as a full-thickness skin defect that required ≥14 days for healing. Every subject with diabetic foot was matched for age (±3 years), sex, and body mass index (BMI). Patients with inflammatory or infectious diseases, autoimmune and rheumatic diseases, cancer, hematomatous diseases, and those who were under treatment with anti-inflammatory drugs, pregnant and lactating female patients were excluded. We also excluded patients with recent venous thromboembolism.

A detailed history and physical examination was carried out for every subject. Data were collected on age, sex, anthropometric measurements (BMI), duration of diabetes, glycemic control prior to and during the hospital stay, lipid profile, presence of retinopathy, nephropathy (creatinine > 1.5 mg/dL or presence of micro- or macro-albuminuria), neuropathy (absence of perception of the Semmes-Weinstein monofilament at 2 of 10 standard planter sites on either foot), and peripheral vascular disease (ischemic symptoms and intermittent claudication of rest pain, with or without pedal pulses or posterior tibial pulses).
Sample collection and determination of Adp, IL-6, hsCRP, and TNF-α levels

Plasma samples were collected in a fasting state and immediately placed on ice, clarified by centrifugation at 3000 \( \times g \) for 5 min at 4°C, and kept frozen at –80°C until assay analysis. Plasma levels of Adp, IL-6, hsCRP, and TNF-α were measured by immunoenzymatic enzyme-linked immunosorbent assay (ELISA) method (Ani Biotech Oy, Orgenium Laboratories, Helsinki region, Finland). Regarding the sensitivity of Adp, the analytical limit of detection was 0.18 ng/ml; intra- and inter-assay coefficients of variation (CVs, %) were 6.8 and 6.2, respectively. For IL-6, the analytical limit of detection was 7.89 pg/ml; intra- and inter-assay CVs (%) were 7.4 and 6.5, respectively. For hsCRP, the analytical limit of detection was 0.12 ng/ml; intra- and inter-assay CVs (%) were 5.2 and 6.2, respectively. For TNF-α, the analytical limit of detection was 0.15 ng/ml; intra- and inter-assay CVs (%) were 6.8 and 6.2, respectively.

Statistical methodology

The results were analyzed using the SigmaPlot Version 11.1 program. The Shapiro–Wilk test was used to evaluate normality of variables. The differences between the groups were calculated with Student’s \( t \) or the nonparametric U-Mann–Whitney tests. Results are expressed as median (lower quartile ↔ upper quartile) for continuous variables and percentages for categorical data, with \( P < 0.05 \) considered significant. Logistic forward regression analysis, multiple linear regression, and Chi-square were used to assess the association between all clinical variables and inflammatory parameters that independently predicted foot ulcer development with a \( P < 0.05 \). Risk for ulcer development was also estimated by odds ratio (OR) and risk ratio (RR) with 95% confidence intervals (CIs) that independently predicted the foot ulcer.

Results

Baseline characteristic of subjects with diabetic foot in comparison with subjects without diabetic foot are given in Table 1. In group A, 63.5% of subjects were males, while 62.9% of subjects in group B were males. In group A, 82.7% of subjects had diabetes mellitus type 2, while in group B type 2 diabetes was present in 90.1% of subjects. Regarding the duration of diabetes, 68.6% of subjects in group A versus 75.7% of subjects in group B could be diabetic by >10 years, whereas 31.4% versus 24.0% could be diabetic by <10 years in the respective groups. 38.2% of subjects in group A versus 57.4% of subjects in group B were treated with insulin, 53.7% versus 25.9% with oral anti-diabetics, and 33.6% versus 45.0% were under treatment with both insulin and oral anti-diabetic drugs. 87.6% of subjects in Group A versus 45.0% of subjects in group B were smokers, 56.7% versus 41.3% had hypertension, and 50.6% versus 29.0% showed neuropathy. Retinopathy was observed in 50.6% in group A as compared to 23.4% in group B, and 54.4% versus 19.1% subjects had nephropathy in groups A and B, respectively. Subjects in group A also presented, in comparison with those in group B, increased mean ± SD levels of glycated haemoglobin (HbA1c %) (9.6 ± 0.203 vs. 7.9 ± 0.86), BMI (kg/m\(^2\)) (24.84 ± 4.53 vs. 24.03 ± 4.23), serum creatinine (mg/dl) (1.24 ± 0.56 vs. 1.11 ± 0.52), low density lipoprotein-cholesterol (LDL-C; mg/dl) (75.89 ± 18.34 vs. 104.38 ± 30.1), high density lipoprotein-cholesterol (HDL-C; mg/dl) (34.6 ± 3.34 vs. 44.3 ± 7.7), total cholesterol (mg/dl) (136.93 ± 13.7 vs. 181.9 ± 32.3), and triglycerides (mg/dl) (95.6 ± 21.7 vs. 157.0 ± 83.1) [Table 1]. Finally, subjects of group A showed lower median serum level of Adp [8.4 (7.1–9.2) vs. 13.4 (12.1–14.2)] ng/ml, higher median plasma levels of IL-6 [32.5 (9.4–44.8) vs. 6.7 (4.6–14.6)] ng/ml, hsCRP [12.6 (11.2–13.6) vs. 8.4 (7.1–9.2)] mg/ml, and TNF-α [99.4 (79.9–121.5) vs. 4.9 (4.5–5.6)] ng/ml [Table 2].

Univariate analysis

On univariate analysis, the factors which showed a positive association in predicting the foot ulcer were BMI (>25 kg/m\(^2\)) (OR 20.18, RR 1.45), HbA1c (>6.9%) (OR 4.37, RR 1.77), neuropathy (OR 6.88, RR 3.12), retinopathy (OR 3.34, RR 1.91), hypertension (OR 1.64, RR 1.28), nephropathy (OR 3.12, RR 1.87), smoking (OR 4.53, RR 2.99), HDL-C (<40 mg/dl) (OR 1.16, RR 1.07), LDL-C (>100 mg/dl) (OR 1.07, RR 1.03), and triglycerides (>200 mg/dl) (OR 1.40, RR 1.19). In Chi-square test, the following were the predictive factors: BMI (>25 kg/m\(^2\)) (\( P < 0.001 \)), HbA1c (>6.9%) (\( P < 0.001 \)), total cholesterol (>150 mg/dl) (\( P < 0.0001 \)), LDL-C (>100 mg/dl) (\( P = 0.823 \)), neuropathy (\( P < 0.0001 \)), retinopathy (\( P < 0.0001 \)), hypertension (\( P = 0.03 \)), nephropathy (\( P < 0.0001 \)), and smoking (\( P < 0.0001 \)) [Table 3].

Multivariate analysis

On multivariate analysis, the factors which showed a positive association in predicting the foot ulcer by multiple linear regression and forward stepwise regression analyses were BMI (>25 kg/m\(^2\)) (\( P < 0.046 \) and \( P < 0.001 \)), total cholesterol (>150 mg/dl) (\( P < 0.001 \) and \( P < 0.001 \)), triglycerides (>200 mg/dl) (\( P < 0.014 \) and \( P < 0.038 \)), neuropathy (\( P < 0.002 \) and \( P < 0.003 \)), retinopathy (\( P < 0.013 \) and \( P < 0.008 \)), hypertension (\( P = NS \) and \( P < 0.001 \)), nephropathy (\( P < 0.007 \) and \( P < 0.003 \)), plasma Adp (ng/ml) (\( P < 0.001 \) and \( P < 0.001 \)), plasma IL-6 (ng/ml) (\( P = 0.05 \) and \( P < 0.001 \)), plasma hsCRP (mg/ml) (\( P < 0.001 \) and \( P < 0.001 \)), and plasma TNF-α (ng/ml) (\( P < 0.001 \) and \( P < 0.001 \)) [Table 4].
Correlation analysis
There was a significant positive correlation corrected for age and BMI, between Adp and different ulcer grades (r = 0.118, P = 0.035) and retinopathy (r = 0.249, P = 0.001).

There was a significant positive correlation corrected for age and BMI, between IL-6 and ulcer grades (r = 0.250, P = 0.001), BMI (>25 kg/m²) (r = 0.161, P = 0.040), triglycerides (>200 mg/dl) (r = 0.165, P = 0.035), LDL-C (>100 mg/dl) (r = 0.155, P = 0.049), retinopathy (r = 0.166, P = 0.035), nephropathy (r = 0.199, P = 0.011), and smoking (r = 0.164, P = 0.036).

There was a significant positive correlation corrected for age and BMI, between hsCRP and ulcer grades (r = 0.236, P = 0.002), BMI (>25 kg/m²) (r = 0.155, P = 0.048), triglycerides (>200 mg/dl) (r = 0.165, P = 0.037), nephropathy (r = 0.208, P = 0.007), and smoking (r = −0.165, P = 0.036).

There was a significant positive correlation corrected for age and BMI, between TNF-α and total cholesterol (>150 mg/dl) (r = 0.209, P = 0.007), and neuropathy (r = 0.141, P = 0.072) was also observed [Table 5].

DISCUSSION
In this study, we have demonstrated a higher plasma IL-6, hsCRP, and TNF-α, and low plasma Adp levels in diabetic subjects with diabetic foot in comparison with diabetics without diabetic foot. These associations were present
Table 3: Chi-square test, odds ratio, risk ratio, multiple linear regression analysis, forward stepwise regression analysis, and one-way ANOVA to study the independent variables predicting foot ulcer in diabetic patients

| Independent variable | Odds ratio (95% CI) | Risk ratio (95% CI) | YATES | P-value | Multiple linear regression analysis | Forward stepwise regression analysis | One-way ANOVA |
|----------------------|---------------------|---------------------|--------|---------|-------------------------------------|-------------------------------------|--------------|
| BMI (>25 kg/m²)      | 20.18 (1.35–3.30)   | 1.45 (1.16–1.81)    | 25.67  | <0.001  | NS                                  | NS                                  | <0.05        |
| HbA1c (>6.9%)        | 4.37 (2.33–8.19)    | 1.77 (1.46–2.15)    | 22.25  | <0.0001 | NS                                  | NS                                  | <0.05        |
| Triglycerides (>200 mg/dl) | 0.18 (0.06–0.17) | 0.28 (0.20–0.40) | 80.94  | <0.0001 | NS                                  | NS                                  | NS           |
| HDL-C (<40 mg/dl)    | 1.40 (0.86–2.28)    | 1.19 (0.91–1.54)    | 1.54   | 0.216   | NS                                  | 0.014                               | 0.038        |
| LDL-C (>100 mg/dl)   | 1.16 (0.71–1.87)    | 1.07 (0.84–1.38)    | 0.24   | 0.624   | NS                                  | NS                                  | <0.05        |
| Neuropathy           | 1.07 (0.69–1.67)    | 1.03 (0.83–1.29)    | 0.05   | 0.823   | NS                                  | NS                                  | <0.05        |
| Retinopathy          | 6.88 (3.96–11.9)    | 3.12 (2.10–4.67)    | 51.24  | <0.0001 | NS                                  | 0.002                               | 0.003        |
| Hypertension         | 3.34 (2.07–5.38)    | 1.91 (1.44–2.55)    | 24.47  | <0.0001 | <0.003                             | 0.013                               | 0.008        |
| Nephropathy          | 1.64 (1.05–2.54)    | 1.28 (1.02–1.59)    | 4.46   | 0.3     | NS                                  | <0.001                              | <0.001       |
| Smoking              | 3.12 (1.91–5.11)    | 1.87 (1.38–2.53)    | 20.35  | <0.0001 | <0.007                             | 0.003                               | <0.05        |
| Smoking              | 4.53 (2.77–7.41)    | 2.99 (1.68–3.31)    | 37.18  | <0.0001 | NS                                  | NS                                  | <0.05        |

The following independent variables were considered for the model: Plasma adiponectin (ng/ml), plasma cathepsin D (RFU/µg), plasma IL-6 (ng/ml), BMI (>25 kg/m²), plasma hsCRP (mg/ml), HbA1c (>6.9%), total cholesterol (>150 mg/dl), triglycerides (>200 mg/dl), HDL-C (<40 mg/dl), LDL-C (>100 mg/dl), neuropathy, retinopathy, hypertension, smoking. Only the variables that had a P value <0.05 were considered in the final fitted model NS, nonsignificant; DNT, do not test; *Shapiro–Wilk test; #Kolmogorov–Smirnov test; NS: Non significant

Table 4: Multiple linear regression analysis, forward stepwise regression analysis, and one-way ANOVA to study the independent variables predicting foot ulcer in diabetic patients

| Independent variable | Multiple linear regression analysis | R² | P-value 1 | Forward stepwise regression analysis | R² | P-value 2 |
|----------------------|-----------------------------------|----|-----------|-------------------------------------|----|-----------|
| Plasma adiponectin   | -0.0189                           | -0.0009 | 0.05      | -0.0009                             | -0.0009 | 0.0001 |
| Plasma IL-6          | -0.0009                           | 0.05  | <0.001    | -0.0009                             | 0.0001 |
| Plasma hsCRP         | 0.0145                            | <0.001 | <0.001    | 0.0145                              | <0.001 |
| Plasma TNF-α         | 0.0053                            | <0.001 | <0.001    | 0.0053                              | <0.001 |

The following independent variables were considered for the model: Plasma adiponectin (ng/ml), plasma IL-6 (ng/ml), plasma hsCRP (mg/ml). Only the variables that had a P value <0.05 were considered in the final fitted model NS, nonsignificant; DNT, do not test; *Shapiro–Wilk test; #Kolmogorov–Smirnov test

when age (±3 years), sex, and BMI (±2 kg/m²) were matched and comorbidities were taken into account in a univariate and multiple regression analyses. The severity of foot ulcer based on University of Texas classification was also associated with circulating levels of IL-6, hsCRP, and TNF-α and not with plasma Adp level.

Adp levels can be assessed by either of the three variables: total Adp, high molecular weight Adp (HMWA), and the serum Adp index (SA index). Recently, Almeda-Valdes et al. reported that total Adp, HMWA, and the SA index had similar utility for the identification of the metabolic abnormalities.[9] This finding may stimulate the use of Adp in clinical and epidemiological settings as the measurement of total Adp is better standardized, cheaper, and more accessible than the other two approaches. Hypoadiponectinemia is an early sign of a complex cardiovascular risk factor predisposing to the atherosclerosis process and also in accelerating its progression. Adp also exhibits anti-inflammatory and atheroprotective actions in various tissues by suppressing the expression of vascular adhesion molecules and scavenger receptors which reduced the expression of the inflammatory cytokine (TNF-α) by raising NO production which ultimately reduces the process of proliferation and migration of smooth muscle cells.[10,11] Our findings of lower median plasma levels of Adp in subjects with diabetic foot could confirm this issue. Furthermore, we also observed a significant negative correlation between Adp plasma levels and retinopathy, and these findings suggest a possible role of hypoadiponectinemia as a putative marker for both prevalent and incident retinopathy. In patients with diabetic foot, lower plasma levels of Adp could be linked to foot ulcers’ pathogenesis by microvascular and inflammatory mechanisms. Indeed, recent studies suggest that Adp may play a role in the inflammatory vascular response by inhibiting the expression of adhesion molecules on endothelial cells[10,11] and suppressing macrophage function.[12] The association between the low levels of Adp and low levels of HDL-C is independent of cardiovascular risk, whereas high levels of Adp are associated with high levels of HDL-C, indicating a protective risk profile.[13] In our study, both univariate and multivariate analyses showed the predictive role of Adp plasma levels toward predicting the diabetic foot.
The patients with diabetic foot with higher plasma levels of IL-6 could be linked to foot ulcers’ pathogenesis by microvascular and inflammatory mechanisms. There was a significant increase in the median circulating level of IL-6 levels in diabetic foot compared with diabetic without foot ulcer. A significant positive association of IL-6, metabolic markers, and some clinical and laboratory variables has also been reported in previous studies. There was a significant increase in the median circulating level of IL-6 levels in diabetic foot compared with diabetic without foot ulcer. A significant positive association of IL-6, metabolic markers, and some clinical and laboratory variables has also been reported in previous studies. The concentration was also significantly associated with different grades of ulcer, high LDL-C, nephropathy, and retinopathy. These findings further underline the importance of inflammatory and metabolic “milieu” such as cytokines in foot complications in diabetics as already reported for other vascular complications of diabetes. In a prospective study conducted by Aruna et al., IL-6 was found to be a determinant of type 2 diabetes mellitus (T2DM) risk factors. To our knowledge, no prior epidemiological evidence has been available linking baseline IL-6 to diabetes incidence and the risk of foot ulceration. However, cross-sectional studies have reported an increase in circulating plasma CRP levels in insulin-resistant and overt T2DM. Approximately 25% of systemic IL-6 originates from subcutaneous adipose tissue and is thought to modify adipocyte glucose and lipid metabolism and body weight.

Elevated level of circulating plasma hsCRP frequently clusters with well-established risk factors of T2DM such as obesity and insulin resistance. Therefore, we extensively evaluated the role of hsCRP a potential marker of systemic inflammation can work as an independent risk factor for diabetic foot ulcer and its co-morbidities related complications and this were compared with diabetic subjects without foot ulceration. In a multivariate regression analysis, different grades of ulcer, nephropathy, and smoking sensation were significantly associated with the risk of diabetic foot. The correlation of hsCRP and all markers of hyperglycemia, insulin resistance, and dyslipidemia has been reported. This relationship appears to be affected by obesity because they markedly attenuated on adjustment for BMI. Higher HbA1c levels were observed in all the North Indian subjects with an increase in hsCRP, which is also seen in multiple regression analysis with obesity and insulin resistance. There are many factors that affect the plasma CRP levels. Sesmilo et al. reported that smokers have high levels of CRP and soluble intercellular adhesion molecule in type 1 diabetes mellitus (T1DM) which decrease after the cessation of smoking. The result of the current study goes a step further, as the median hsCRP circulating levels significantly increased with the grades of diabetic foot ulcer.

TNF-α may act as a local intensification signal in pathological processes associated with chronic inflammation. This cytokine may mediate the synthesis of acute-phase proteins which are able to initiate and support inflammatory process in the vascular wall. As a consequence, an increased expression of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) on endothelial cells is induced, which serve as chemo-attractants for monocytes and other inflammatory cells. A high dose of TNF-α induces apoptosis, a mechanism of physiological cell death, in vitro. According to literature analysis, no studies are available demonstrating higher plasma TNF-α levels in diabetic foot ulcer patients.

These findings (low plasma Adp, and high plasma IL-6, hsCRP, and TNF-α) further underline the importance of inflammatory and metabolic “milieu” in foot complications in diabetes, as it was already reported for other vascular conditions.
and clinical complications of diabetes.\textsuperscript{10,14,32} The strength of our study includes a relatively large study population, comprehensive immunological study comprising different compartments of the immune system, and the availability of additional data on the potential confounders so that multiple regression analysis was possible to analyze the impact of metabolic factor or co-morbidities on immune system. The only limitation was that this study included diabetic subjects with and without diabetic foot, which means that one of the factors had already been included. Therefore, the hsCRP level could not be analyzed for this risk factor.

To summarize, in this study, our findings strongly support the hypothesis that Adp may play an important role in the pathogenesis of foot ulceration, independent of BMI, sex, and age. However, further investigations of the underlying mechanisms, focusing on Adp isomer distribution, are needed to elucidate the association of Adp with comorbid conditions of diabetes. To our knowledge, this is the first report from India on Adp levels in diabetic foot ulcer (DFU) patients. Elevated hsCRP, TNF-\(\alpha\), and IL-6 was found to be a powerful independent risk determinant. The circulating levels of plasma hsCRP, TNF-\(\alpha\), and IL-6 were elevated among diabetic foot patients, although these associations were markedly attenuated after multivariate analysis. Our observations, coupled with other workers’ experimental evidence, support a possible role of hsCRP, TNF-\(\alpha\), and IL-6 in the pathogenesis of diabetes. The patients with foot ulcers exhibit a specific and nonrandom upregulation of hsCRP, TNF-\(\alpha\), and IL-6, and downregulation of Adp in the circulation. These associations were independent of multiple potential confounders and were mainly associated with severity of ulceration (different grades of ulcer using University of Texas system). Further studies are needed to test whether this immune activation precedes the development of foot ulcer in diabetic subjects. Moreover, the characterization of beneficial and deleterious immune mediators in the process of wound healing in patients with ulcerations would be important to identify potential therapeutic targets and immunomodulating treatment options. Our data also raise the possibility that inflammatory markers might provide an adjunctive method for early detection of risk for this disease. It is possible to hypothesize on the participation of locally released markers in the development of DFU. It would be interesting to find out whether an activation of immune system precedes the development of foot ulcer and whether anti-inflammatory therapies will be effective in improving the outcome in such patients.

**Acknowledgments**

We thank Dr. Farida Hussain, USV India Pvt. Ltd., for providing the kits used in this research free of cost. We would also like to thank Dr. Idrees Mubarak, SR, and Dr. Rafat Fatima, diabetic educator, Rajiv Gandhi Centre for Diabetes and Endocrinology, for clinical evaluation of diabetic patients, and for dietary advice and monitoring the patients’ diets, respectively.

**References**

1. Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA 2005;293:217-28.
2. Zubair M, Malik A, Ahmad J. Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India. Foot (Edinb) 2011;21:6-14.
3. Boulton AJ. The diabetic foot from art to Science. The 18th Camillo Golgi Lecture. Diabetologia 2004;47:1343-54.
4. Roghmann MC, Siddiqui A, Plaisance K, Standiford H. MRSA colonization and risk of MRSA bacteremia in hospitalized patients with chronic ulcer. J Hosp Infect 2001;47:98-103.
5. Amstrong DJ, liswood PJ, Todd WF. Prevalence of mixed infection in the diabetic pedal wound: A retrospective review of 112 infections. J Am Podiatr Med Assoc 1995;85:533-7.
6. Gadeppalli R, Dhwain B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care 2006;29:1727-32.
7. Jeffcott WJ, Game F, Cavanagh PR. The role of proinflammatory cytokines in the cause of neuropathic osteoarthropathy (acute charcot foot) in diabetes. Lancet 2005;366:2058-61.
8. Beaujoulin M, baghdalgian S, Glandu-lissais M, Barmach G, Liaudet coopman E. Overexpression of both catalytically active and inactive Cathepsin D by Cancer cells enhanced apoptosis dependent chemosensitivity. Oncogene 2006;25:1967-73.
9. Jerry P, Krzystol S, Edward B. Cathepsin D inhibitors from potato reverse inhibition of collagen biosynthesis in wounded skin of rats with experimental diabetes. Acta Biochim Pol 1999;38:115-8.
10. Jager A, Kostense PJ, Ruth HG, Heine RJ, Nijpels G, Reder AT. “Cytokines in the vitreous of patients with proliferative diabetic retinopathy.” Am J Ophthalmol 1992;114:731-6.
11. Almeda-Valdes P, Cuevas-Ramos D, Mehta R, Gomez-Perez FJ, Cruz-Bautista I, Arellano-Campos O, et al. Total and high molecular weight adiponectin have similar utility for the identification of insulin resistance. Cardiovasc Diabetol 2010;9:26.
12. Almeda-Valdes P, Cuevas-Ramos D, Mehta R, Gomez-Perez FJ, Cruz-Bautista I, Arellano-Campos O, et al. Total and high molecular weight adiponectin have similar utility for the identification of insulin resistance. Cardiovasc Diabetol 2010;9:26.
13. Mahmoud A, Tabassum R, Chavali S, Dixviwi OP, Bharadwaj M, Tandon N, et al. High-sensitivity C-Reactive protein levels and type 2 diabetes in Urban North Indians. J Clin Endocrinol Metab 2009; 94:2123-7.
14. Boulton AJ. The diabetic foot: From art to science. The 18th Camillo Golgi lecture. Diabetologia 2004;47:1343-53.
15. Boyko EJ, Ahroni JH, Cohen V, Nelson KM, Heagerty PJ. Prediction of diabetic foot ulcer occurrence using commonly available clinical information: The Seattle diabetic foot study. Diabetes Care 2006;29:1202-7.
16. Chambers JC, Eda S, Bassett P, Karim Y, Thompson SG, Gallimore JR, et al. C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. Circulation 2001;104:145-50.
17. Drown DJ, Engler MM. New guidelines for blood cholesterol by the National Cholesterol Education Program (NCEP). National Cholesterol Education Program (NCEP). Prog Cardiovasc Nurs 1994;9:43-4.
18. Febbraio MA, Pedersen BK. Muscle derived interleukin-6: Mechanisms for activation and possible biological roles. FASEB J 2002;16:1335-47.

19. Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. Adults. Diabetes Care 1999;22:1971-7.

20. Frohlich M, Imhof A, Berg G, Hutchinson WL, Pepys MB, Boeing H, et al. Association between C-reactive protein and features of the metabolic syndrome: A population-based study. Diabetes Care 2000;23:1835-9.

21. Frühbeck G, Gomez-Ambrosi J, Muruzabal FJ, Burrell MA. The adipocyte: A model for integration of endocrine and metabolic signaling in energy metabolism regulation. Am J Physiol 2001;280:E827-47.

22. Sugimoto H, Shikata K, Wada J, Horiuchi S, Makino H. “Advanced glycation end products-cytokine-nitric oxide sequence pathway in the development of diabetic nephropathy: Aminoguanidine ameliorates the overexpression of tumour necrosis factor-a and inducible nitric oxide synthase in diabetic rat glomeruli.” Diabetologia 1999;42:878-86.

23. Halvatsiotis I, Tsiotra PC, Ilkonidis I, Kollias A, Mitrou P, Maratou E, et al. Genetic variation in the adiponectin receptor 2 (ADIPOR2) gene is associated with coronary artery disease and increased ADIPOR2 expression in peripheral monocytes. Cardiovasc Diabetol 2010;9:10.

24. Hartemann-Heurtier A, Robert J, Jacqueminet S, Ha Van G, Golnadd JL, Jarlier V, et al. Diabetic foot ulcer and multidrug-resistant organisms: Risk factors and impact. Diabet Med 2004;21:710-5.

25. Kolb H, Mandrup-Poulsen T. An immune origin of type 2 diabetes? Diabetologia 2005;48:1038-50.

26. Balasubramanyam M, Rema M, Premanand C. “Biochemical and molecular mechanisms of diabetic retinopathy.” Curr Sci 2002;83:1506-14.

27. Schram MT, Chaturvedi N, Schalkwijk C, Giorgino F, Ebeling P, Fuller JH, et al. “Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes: The EURODIAB Prospective Complications Study.” Diabetes Care 2003;26:2165-73.

28. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 1997;82:4196-200.

29. Mohan V, Deepa R, Velmurugan K, Premalatha G. Association of C-reactive protein with body fat, diabetes and coronary artery disease in Asian Indians: The Chennai Urban Rural Epidemiology Study (CURES-6). Diabet Med 2005;22:863-70.

30. National Cholesterol Education Program Panel: Second report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel II). Circulation 1994;89:1333-445.

31. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol 2008;19:433-42.

32. Orban Z, Remaley AT, Sampson M, Trajanoski Z, Chrousos GP. The differential effect of food intake and beta-adrenergic stimulation on adipose-derived hormones and cytokines in man. J Clin Endocrinol Metab 1999;84:2126-33.

Cite this article as: Zubair M, Malik A, Ahmad J. Plasma adiponectin, IL-6, hsCRP, and TNF-α levels in subject with diabetic foot and their correlation with clinical variables in a North Indian tertiary care hospital. Indian J Endocr Metab 2012;16:769-76.

Source of Support: Nil, Conflict of Interest: None declared.