High Serum Concentration of Interleukin-18 in Diabetic Patients with Foot Ulcers

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Background: It is well known that interleukin-18 (IL-18) plays a key role in the inflammatory process. However, there are limited data on the role IL-18 plays with diabetic foot ulcers, an acute and complex inflammatory situation. Therefore, we aimed to evaluate serum IL-18 levels of diabetic patients with foot ulcers.

Methods: Twenty diabetic patients with acute foot ulcers, 21 diabetic patients without a history of foot ulcers, and 21 healthy volunteers were enrolled in our study. Circulating levels of IL-18, and other biochemical markers are parameters of inflammation and were measured in all three groups.

Results: Diabetic patients both with and without foot ulcers had high IL-18 concentrations ($P < 0.001$ and $P = 0.020$, respectively) when compared with the nondiabetic volunteers. Those with foot ulcers had higher levels of IL-18 level ($P < 0.001$), high-sensitivity C-reactive protein (hsCRP) ($P = 0.001$), and erythrocyte sedimentation rate (ESR) ($P < 0.001$) than those without foot ulcers.

Conclusions: We found that serum IL-18 concentrations were elevated in diabetic patients with acute diabetic foot ulcers. However, these findings do not indicate whether the IL-18 elevation is a cause or a result of the diabetic foot ulceration. Further studies are needed to show the role of IL-18 in the course of these ulcers. (J Am Podiatr Med Assoc 104(3): 222-226, 2014)

Interleukin-18 (IL-18), originally identified as an interferon-γ (IFN-γ)–inducing factor, is a member of the IL-1 cytokine superfamily and plays a central role in the inflammatory cascade and in the processes of congenital and immune dysfunction. The interaction between diabetic foot ulcers and cytokines other than IL-18 has been previously researched in the literature. However, the role of IL-18 in diabetic patients both with and without foot ulcers compared with nondiabetic individuals has yet to be studied. Therefore, we aimed to evaluate the correlation between foot ulcers and IL-18 in a cross-sectional study of diabetic patients and to compare IL-18 levels in both groups of diabetic patients with the levels in healthy volunteers.

Methods

We included 20 diabetic patients with acute foot ulcers, 21 diabetic patients without a history of foot ulcers, and 21 healthy volunteers as a control group.

Diabetic foot ulcers are a major source of suffering in the diabetic population. The risk for developing a foot ulcer at some point is nearly 20%. Treatment of diabetic foot ulcers is costly, and this is associated with an impaired quality of life. Low-grade inflammation and immune activation are closely related to the pathogenesis and complications of diabetes. This activation may also increase the incidence of diabetic foot ulcers. Furthermore, the balance between pro- and anti-inflammatory processes is crucial in the progression of wound healing, and disturbances of the immune system lead to the chronic and nonhealing ulcer known as diabetic foot syndrome.
The study was approved by the Ethics Committee of Harran University, Sanliurfa, Turkey. Written informed consent was obtained from all study participants, and the study was performed in accordance with the Declaration of Helsinki.

**Study Participants**

Patients with type 1 diabetes, renal failure, class III or IV heart failure according to the New York Heart Association (NYHA) classification, cirrhosis, pregnancy, and malignancy were excluded. Diabetic foot infection was diagnosed by the presence of systemic signs of infection, purulent secretion, or at least two local findings of inflammation (eg, redness, warmth, induration, pain, and tenderness). Patients with Wagner grade 4 and 5 diabetic ulcer and with thrombosis revealed on Doppler ultrasound were also excluded. As control groups, 21 diabetic patients and 21 nondiabetic individuals were included, and the same exclusion criteria were applied for all groups.

**Measurements**

Body mass index was calculated as the weight in kilograms divided by the square of the height in meters. Blood pressure was determined as the mean of two measurements obtained in an office setting by the conventional cuff method using a mercury sphygmomanometer after at least 5 minutes of rest.

Fasting plasma glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), urea, and creatinine levels were measured in all groups. Also, the levels of glycated hemoglobin (A1c), urinary albumin, and creatinine levels needed to calculate the albumin-creatinine ratio and glomerular filtration rate (GFR) were determined in the diabetic groups. We used the extended Modification of Diet in Renal Disease study equation formula for estimating the GFR:\[^3^]\(\text{eGFR} \text{ (mL/min/1.73m}^2\) = 170 \times \text{serum creatinine (mg/dL}^{-1})^{0.996} \times \text{(BUN/2.144)}^{-0.170} \times \text{[serum albumin (g/L)/(10}^{-0.318} \times \text{age (years)}^{-0.170} \times 0.761} \text{(if female)} \times 1.21} \text{(if black)}\)\].

In addition, inflammation parameters such as high-sensitivity C-reactive protein (hsCRP), erythrocyte sedimentation rate (ESR), and leukocyte count were measured in patients with diabetes mellitus.

Venous blood samples after fasting for 12 hours were collected from all participants to measure IL-18. These were then centrifuged and stored at −80 °C until the day of analysis. Before being analyzed, serum samples were transferred to −20 °C and were dissolved at room temperature. Serum IL-18 levels were measured with an enzyme-linked immunosorbent assay kit (Biosource International Inc, Camarillo, California).

**Statistical Analyses**

For the statistical evaluation of data, the statistical software program SPSS for Windows version 15.0 (SPSS, Chicago, Illinois) was used. Data were given as proportions (%) for categorical variables and as mean ± standard deviation (SD) for continuous variables. Proportion comparisons were made with the \(\chi^2\) test. Differences were analyzed by the Student’s unpaired \(t\) test or the Mann-Whitney \(U\) test. Comparisons among multiple groups were performed by one-way analysis of variance (ANOVA) with a Scheffe post hoc test or Kruskal-Wallis test for continuous variables. For the correlation analyses, a nonparametric Spearman rank test was used.

**Results**

Creatinine levels were significantly higher only in the diabetic foot ulcer group compared with the control group \((P = 0.009)\). Levels of HDL were significantly lower in the diabetic foot ulcer group when compared with the diabetic control and healthy control groups \((P = 0.046\) and \(<0.001\), respectively). Also, LDL levels in diabetic patients with foot ulcers were significantly lower than in the healthy controls \((P = 0.049)\). The clinical and biochemical features of all groups are shown in Table 1.

The durations and treatments of diabetes were not significantly different between the two diabetic groups. There were no differences between the two diabetic groups regarding renal function, which was defined by the GFR and microalbumin-creatinine ratio in spot urine. In patients with diabetic foot ulcers, hsCRP and ESR levels were significantly higher than in diabetic patients without foot ulcers \((P = 0.001\) and \(P < 0.001\), respectively). The IL-18 level of the diabetic foot ulcer group was significantly higher than those of the other two groups \((P < 0.001)\). Furthermore, the diabetic patients without foot ulcers had a significantly high IL-18 level compared with the healthy individuals in the control group \((P = 0.020)\) (Table 2).
Correlation analyses were performed between the diabetic groups along with establishing the parameters for diabetes control (glucose and HbA1c), renal function (GFR, microalbumin-creatinine ratio), lipids (LDL, HDL, TG), and inflammation (HsCRP, ESR, WBC). The IL-18 level was positively correlated with hsCRP ($r = 0.405$, $P = 0.01$) and ESR ($r = 0.461$, $P = 0.03$). We did not find any correlation between IL-18 and the other parameters mentioned above.

### Discussion

The present study demonstrated that serum IL-18 concentrations were significantly higher in diabetic patients with foot ulcers than in age-matched diabetic patients without ulcers and in healthy individuals in the control group, and they were also significantly higher in diabetic patients without foot ulcers than in age-matched participants in the control group.
Esposito et al. first demonstrated that IL-18 levels were significantly higher in diabetic patients than in nondiabetic individuals. Elevated levels of IL-18 have been observed in diabetic patients in other studies. Moreover, Thorand et al. reported the correlation between elevated levels of IL-18 and risk of type 2 diabetes mellitus as being an independent risk factor for diabetes. Our results were compatible with the results from these two studies.

Foot ulcers are a common complication in diabetic individuals with a corresponding significant health-care burden and morbidity. Proinflammatory cytokines and various peptide growth factors are key players in the wound healing process. The role of IL-18 during cutaneous wound repair was shown in mice by Kämpfer et al. They also reported that diabetes-related wound healing revealed large amounts of IL-18 in mice. Interleukin-18 induces the release of neutrophil and monocyte chemoattractant proteins and is thought to be involved in the amplification of signals guiding increasing numbers of immune cells into the wound at the onset of wound repair.

In acute diabetic foot syndrome, the levels of IL-18 were investigated only in one research study in which Weigelt et al. showed that the serum levels of IL-18 did not change in diabetic patients with acute foot ulcers when compared with those without foot ulcers. In our study, we observed that the IL-18 levels were significantly higher in the diabetic foot ulcer group than in the diabetic patients without foot ulcers and in healthy control groups. The levels of IL-18 were elevated both with microvascular and macrovascular complications of diabetes. Furthermore, elevated levels of IL-18 have been found in the serum of patients with surgical wounds and sepsis and in the blister fluid of patients with toxic epidermal necrolysis who had no diabetes mellitus. Additionally, it has been described that diabetic patients with foot ulcers undergo acute phase reaction.

In our study, we found that IL-18 is positively correlated with hsCRP and ESR, which was the most widely used acute phase reactants. As expected, hsCRP and ESR were significantly higher in the foot ulcer group in our study, than in diabetic individuals without foot ulcers. As described by Weigelt et al., immune activation is an important risk factor for several micro- and macrovascular complications of diabetes. Additionally, IL-18 has potent immunomodulatory effects. These known facts suggest that IL-18 may play a prominent role in the course of diabetes and diabetic foot ulcers. The control group in the study by Weigelt et al. was significantly younger and had a higher A1c level compared to the diabetic foot ulcer group; these differences may have influenced their result. It has been shown that IL-18 levels were positively correlated with A1c level. In our study, we had more compatible groups in terms of age and A1c levels. We also excluded patients with Wagner grade 4 and 5 ulcers who had gangrene, thereby limiting our study to a specific population. Although Weigelt et al. had a large group, they included all patients with gangrenous lesions in all stages, according to the University of Texas classification. The IL-18 response may be reduced in the necrosis phase, and our results may be associated with this situation, unlike the results of Weigelt et al. In addition, the incompatibility observed between our study and that published by Weigelt may be attributable to sample size differences.

Our study had several limitations: the number of study participants in both the patient and control groups was limited; we could not stratify our patients with foot ulcers according to grade of ulcer; and our study was a cross-sectional study. Therefore, we were not able to observe IL-18 with regard to the prediction of wound healing or amputation.

Conclusions

In conclusion, the IL-18 level, an important cytokine in inflammation and immunity, increased in diabetic patients with acute foot ulcers, and further studies are needed to show the role of IL-18 in the progression of these ulcers. Moreover, the role of IL-18 in the process of wound healing in patients with diabetic foot ulcerations may be valuable for diagnostic and therapeutic purposes.

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Conflict of Interest: None reported.

References


