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Research paper

Assessment of Immunological Biomarkers in Diabetic Patients with and without Foot Ulcers

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Abstract

Abstract:
Ulcers of the foot caused by diabetes (DFU) are among the most severe consequences of diabetes mellitus, significantly diminishing patients' quality of life and often resulting in persistent infections and potential limb loss. This study aimed to assess the serum levels of four immunological biomarkers: interleukin-10 (IL-10), interleukin-18 (IL-18), tumor necrosis factor-alpha (TNF-α), and basic fibroblast growth factor (bFGF) in patients with diabetes and in patients without foot ulcers, as well as in healthy participants. The study enrolled 90 participants, including diabetic patients with foot ulcers, diabetic patients without ulcers, and healthy controls. Then, the Blood samples were collected and analyzed using ELISA to determine serum concentrations of IL-10, IL-18, TNF-α, and bFGF. Statistical analyses included ANOVA, Duncan's multiple range test, and ROC curve evaluation. The results indicate that IL-18 and TNF-α levels were significantly elevated in both diabetic groups compared to controls ($P < 0.001$), indicating persistent systemic inflammation. IL-10 levels were significantly higher in diabetics without ulcers than in controls ($P < 0.05$), suggesting a compensatory anti-inflammatory response. Interestingly, bFGF was significantly elevated in patients with ulcers ($P < 0.001$), potentially reflecting a local reparative response. ROC analysis revealed that bFGF had high diagnostic accuracy (AUC = 0.809) in differentiating DFU patients from healthy individuals. The finding highlighted that the observed cytokine profiles suggest that DFUs are characterized by an inflammatory–reparative imbalance. Elevated IL-18 and TNF-α indicate sustained inflammation, while reduced IL-10 and altered bFGF levels reflect impaired resolution and tissue repair mechanisms. These markers, particularly bFGF, show promise as potential diagnostic tools for identifying and monitoring DFU progression.

Keywords: *Diabetes Mellitus; Diabetic Foot Ulcer; Cytokines; IL-10; IL-18; TNF-α; Bfgf.*

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, resulting from impaired insulin secretion, insulin resistance, or a combination of both. Globally, DM continues to pose one of the most pressing public health challenges of the 21st century, with prevalence rates projected to rise dramatically in the coming decades (Cho et al., 2018). The long-term complications of uncontrolled diabetes are diverse and often debilitating, encompassing microvascular (neuropathy, nephropathy, retinopathy) and macrovascular (cardiovascular and cerebrovascular disease) outcomes. Among these, diabetic foot ulcers (DFUs) represent one of the most severe and life-altering consequences. Epidemiological studies estimate that 15–25% of individuals with diabetes will develop a foot ulcer during their lifetime, with a significant proportion progressing to infection, hospitalization, or even lower-limb amputation (Byrnes et al., 2024; Zhang et al., 2017).

DFUs are a multifactorial condition, arising from the complex interplay of peripheral neuropathy, vascular insufficiency, immune dysfunction, and poor glycemic control. Neuropathy leads to loss of protective sensation, vascular impairment reduces tissue perfusion, and immune dysfunction compromises wound healing. Together, these factors create a setting in which minor injuries can evolve into chronic, non-healing ulcers (Yousif et al., 2024). Importantly, DFUs are not only a clinical burden but also a significant socioeconomic challenge, particularly in low- and middle-income countries where the cost of care and risk of disability are disproportionately higher (Anyim et al., 2019).

A central pathophysiological mechanism in DFU development is chronic inflammation, which disrupts the normal wound healing cascade. The healing process involves three coordinated phases: hemostasis/inflammation, proliferation, and remodeling. In diabetic patients, this sequence is often disrupted, resulting in a prolonged inflammatory phase with impaired resolution. Cytokines and growth factors are pivotal in orchestrating this process, as they regulate cellular recruitment, angiogenesis, extracellular matrix deposition, and tissue repair (Mohsin et al., 2024). Dysregulation of these signaling molecules has been increasingly recognized as a major contributor to impaired healing in DFUs (Falanga, 2005).

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Recent research has emphasized the role of specific immunological biomarkers in DFU pathogenesis and progression. Interleukin-10 (IL-10) functions as a potent anti-inflammatory cytokine, counterbalancing the deleterious effects of pro-inflammatory mediators. Conversely, interleukin-18 (IL-18) and tumor necrosis factor-alpha (TNF-α) are central drivers of systemic inflammation and tissue injury in diabetes (Dinarello, 2018; Donath & Shoelson, 2011). Elevated levels of these cytokines have been reported in DFU patients, reflecting persistent inflammatory activation (Zhang et al., 2020). Basic fibroblast growth factor (bFGF), on the other hand, is crucial for angiogenesis, fibroblast proliferation, and granulation tissue formation key processes required for wound closure. However, despite its elevation in DFU patients, impaired responsiveness to growth factor signaling may hinder effective healing (Li et al., 2015; Zhou et al., 2011).
Understanding the balance between pro-inflammatory and reparative biomarkers is therefore essential for elucidating the immunopathology of DFUs. Elevated IL-18 and TNF-α levels indicate sustained inflammation, while alterations in IL-10 and bFGF reflect compromised resolution and tissue repair mechanisms. This inflammatory–reparative imbalance not only underpins DFU pathogenesis but also suggests that these biomarkers could serve as potential diagnostic and prognostic tools. The present study builds upon this rationale by assessing the serum levels of IL-10, IL-18, TNF-α, and bFGF in diabetic patients with and without foot ulcers, compared with healthy controls. Through this approach, the study aims to deepen understanding of the immunological mechanisms contributing to DFUs and to explore their clinical relevance in diagnosis, prognosis, and management

2. Materials and Methods

2.1. Study design and setting

This was a hospital-based, case–control study conducted between September 2024 and February 2025 at two major healthcare centers in Babylon Province, Iraq: Al-Hashimiyah General Hospital and Al-Qassim General Hospital. The study was designed to evaluate serum levels of key immunological biomarkers interleukin-10 (IL-10), interleukin-18 (IL-18), tumor necrosis factor-alpha (TNF-α), and basic fibroblast growth factor (bFGF) in diabetic patients with foot ulcers, diabetic patients without ulcers, and healthy controls.

2.2. Study population and grouping

A total of 90 participants were recruited and equally allocated into three groups (n = 30 each):

- Group I (DFU patients): Type 2 diabetic patients with clinically confirmed foot ulcers. Diagnosis was established through clinical examination, with radiographic imaging performed when necessary to rule out osteomyelitis or deep tissue involvement.
- Group II (Diabetic non-ulcer patients): Type 2 diabetic patients without any history or signs of foot ulcers.
- Group III (Healthy controls): Non-diabetic individuals with no history of chronic illnesses, matched for age and gender as far as possible.

Inclusion criteria were age between 30 and 70 years and confirmed type 2 diabetes mellitus for Groups I and II. Exclusion criteria included the presence of autoimmune disease, chronic inflammatory disorders, active malignancy, recent surgery or trauma, and use of immunomodulatory medications.

2.3. Ethical considerations

Ethical approval was obtained from the Babil Health Directorate, and the study protocol adhered to the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants after a clear explanation of the study objectives, procedures, risks, and potential benefits. Confidentiality of participant data was strictly maintained.

2.4. Clinical and demographic data collection

Demographic and clinical data were collected using a structured questionnaire administered by trained healthcare professionals. Data included:

- Demographics: Age, gender.
- Medical history: Duration of diabetes, treatment type (oral hypoglycemic agents, insulin, or combination therapy), smoking history, and presence of hypertension.
- Clinical examination: For DFU patients, ulcer type, anatomical location, and ulcer duration were recorded.

Blood pressure measurements were taken in a seated position using a standardized sphygmomanometer, and clinical staging of DFUs was documented according to clinical guidelines.

2.5. Blood sample collection and processing

From each participant, approximately 5 mL of venous blood was collected under aseptic conditions.

- Hematological analysis: 2 mL was transferred into EDTA tubes for routine hematological evaluation.
- Biochemical assays: The remaining 3 mL was collected in plain gel tubes, allowed to clot at room temperature, and centrifuged at 4,000 rpm for 10 minutes. Serum was separated and stored in sterile Eppendorf tubes at –20 °C until analysis.

2.6. Biomarker measurement by ELISA

Serum concentrations of IL-10, IL-18, TNF-α, and bFGF were quantified using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Elabscience®, China), following the manufacturer's protocol. Each assay was performed in duplicate to ensure accuracy and reproducibility.

- Assay sensitivities and detection ranges:
- IL-10: Sensitivity 0.94 pg/mL; detection range 1.56–100 pg/mL.
- IL-18: Sensitivity 9.38 pg/mL; intra-assay CV <5.39%, inter-assay CV <7.43%.

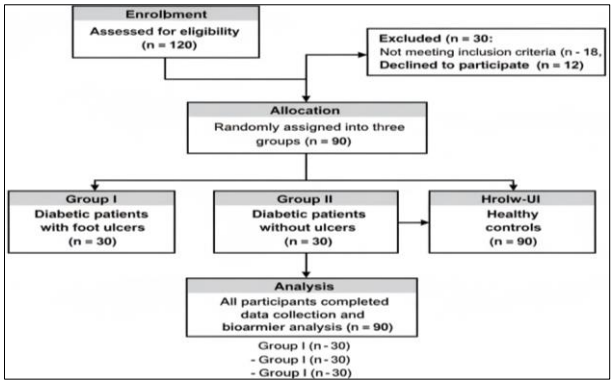
- TNF-α: Sensitivity 4.69 pg/mL; detection range 7.81–500 pg/mL.
- bFGF: Sensitivity 18.75 pg/mL; intra-assay CV <5.99%, inter-assay CV <8.69%.

Optical density (OD) values were read at 450 ± 2 nm using a calibrated microplate reader. Standard curves were constructed for each biomarker using serial dilutions of known standards, and concentrations were calculated using a four-parameter logistic (4PL) model.

2.7. Statistical analysis

All statistical analyses were performed using SPSS version 26 (IBM, USA). Continuous variables were expressed as mean ± standard error (SE). Group comparisons were performed using the Kruskal–Wallis test, a non-parametric alternative to ANOVA, followed by Duncan's multiple range test for post hoc comparisons.

To evaluate diagnostic performance, Receiver Operating Characteristic (ROC) curve analysis was applied, and the area under the curve (AUC) was calculated. Sensitivity and specificity were also derived. A p-value < 0.05 was considered statistically significant.



Flow Chart: Participant Selection and Grouping.

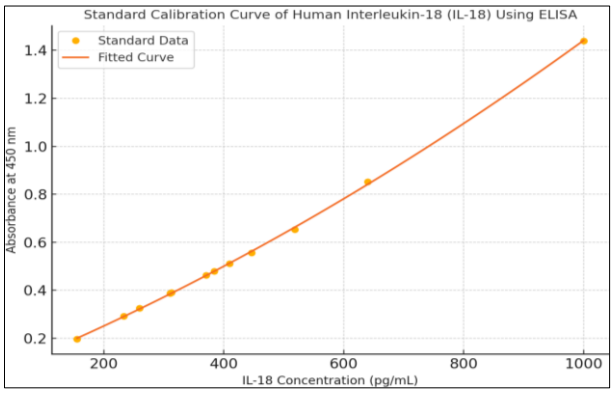


Fig. 1: This Curve is Used to Interpolate the IL-18 Levels in Test Samples.

Figure 1: A four-parameter logistic (4PL) standard curve was generated using serial dilutions of human IL-18 standards, with optical density (OD) values measured at 450 ± 2 nm. The curve demonstrates a strong sigmoidal relationship between OD and IL-18 concentration, allowing for accurate interpolation of IL-18 levels in test serum samples. This assay achieved a sensitivity of 9.38 pg/mL with intra-assay and inter-assay coefficients of variation (CV) less than 5.39% and 7.43%, respectively, ensuring reproducibility and reliability.

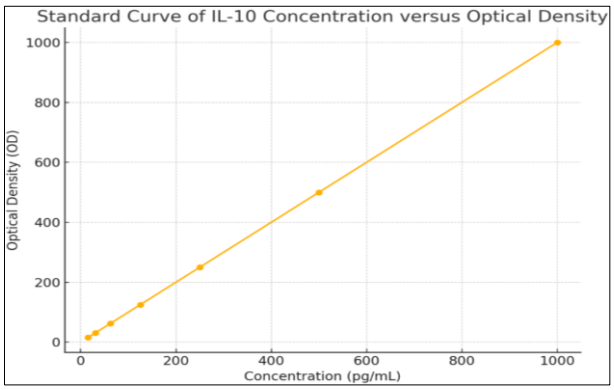


Fig. 2: This Curve Is Used to interpolate the IL-10 Levels in Test Samples, determining the Levels of Serum Human Tumor Necrosis factor-α (TNF-A) in Patients' Blood.

Figure 2: The 4PL-fitted standard curve was constructed from serially diluted IL-10 standards, used to calculate serum concentrations of IL-10 in patients and controls. Optical densities were read at 450 nm, with the detection range spanning 1.56–100 pg/mL and a sensitivity of 0.94 pg/mL. The strong correlation between OD values and IL-10 concentrations demonstrates the precision of this assay in quantifying low-level cytokine responses.

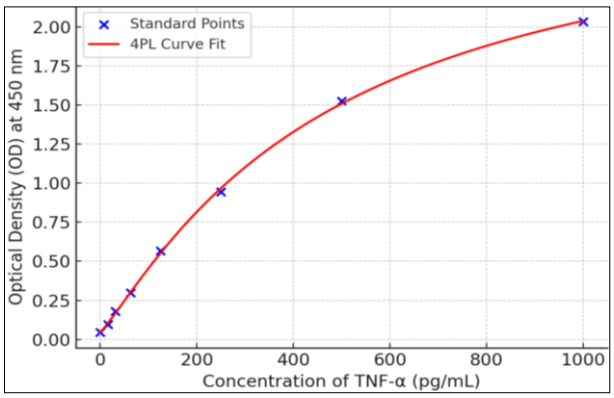


Fig. 3: Standard Curve for Human TNF-A Measured by ELISA. the Curve Was Fitted Using the 4-Parameter Logistic (4PL) Model.

Figure 3: The calibration curve was plotted from known concentrations of TNF-α standards using a 4PL logistic model, with OD values measured at 450 nm. The assay exhibited a detection range of 7.81–500 pg/mL and a sensitivity of 4.69 pg/mL. This curve was subsequently applied to interpolate TNF-α concentrations in serum samples from diabetic patients with and without foot ulcers, as well as healthy controls, ensuring accurate detection of pro-inflammatory cytokine activity.

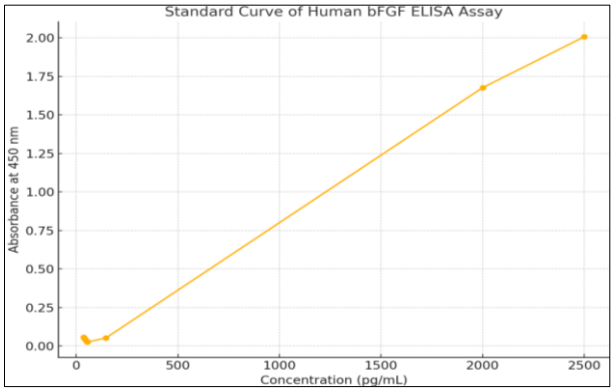


Fig. 4: Standard Curve for Human bFGF Measured by ELISA.

Figure 4: A 4PL standard curve was generated using serial dilutions of bFGF standards, establishing the quantitative relationship between OD readings and bFGF concentrations. The assay sensitivity was 18.75 pg/mL, with intra-assay and inter-assay CVs less than 5.99% and 8.69%, respectively. This robust calibration curve enabled precise determination of circulating bFGF levels, providing critical insight into reparative and angiogenic activity associated with diabetic foot ulcers.

3. Results

3.1. Interleukin-10 (IL-10) serum levels

The analysis of serum IL-10 concentrations revealed significant variability across the three study groups. The highest mean concentration of IL-10 was detected in diabetic patients without foot ulcers (54.70 ± 16.05 pg/mL), followed by diabetic patients with foot ulcers (41.09 ± 10.5 pg/mL). Healthy controls exhibited the lowest IL-10 levels (19.92 ± 1.59 pg/mL). Statistical evaluation using the Kruskal–Wallis test showed a significant difference between diabetic patients without ulcers and healthy controls ($p = 0.048$). However, no statistically significant difference was observed between ulcer and non-ulcer diabetic patients, or between ulcer patients and controls. These findings indicate that while diabetes is associated with altered IL-10 expression, the presence of foot ulcers may diminish this compensatory anti-inflammatory response. The distribution of IL-10 across groups is illustrated in Figure 5 and detailed in Table 1.

Table 1: Interleukin-10 (IL-10) Level in Study Groups.

Groups		Interleukin-10 (IL-10) level
Ulcer patients	Mean ± SE	41.09 ± 10.5^{AB}
	Range	12.40-211.0
DM patients	Mean ± SE	54.70 ± 16.05^{A}
	Range	11.70-346.00
Healthy control	Mean ± SE	19.92 ± 1.59^{B}
	Range	12.00-41.00
p-value		0.048*
		†

SE: standard error; †: Kruskal Wallis test; **: significant at P > 0.05

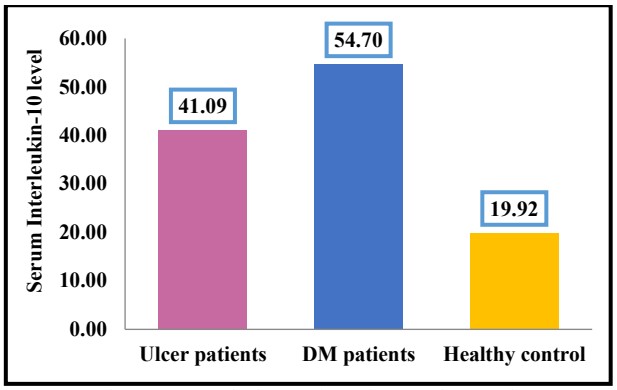


Fig. 5: The Mean Level of IL-10 in Patients and Control Groups.

Figure 5: Bar chart representation of IL-10 levels in diabetic patients with foot ulcers, diabetic patients without foot ulcers, and healthy controls. IL-10 was highest in diabetic patients without ulcers (54.70 ± 16.05 pg/mL), followed by diabetic patients with ulcers (41.09 ± 10.5 pg/mL), while the lowest values were observed in healthy controls (19.92 ± 1.59 pg/mL). A statistically significant difference was found between diabetics without ulcers and healthy controls (p = 0.048), suggesting a compensatory anti-inflammatory response in this group.

3.2. Interleukin-18 (IL-18) serum levels

Mean serum IL-18 concentrations were markedly elevated in both diabetic groups compared with healthy controls. Patients with foot ulcers demonstrated a mean IL-18 level of 556.1 ± 36.8 pg/mL, while diabetic patients without ulcers showed 530.62 ± 43.4 pg/mL. In contrast, healthy participants had significantly lower IL-18 levels (315.4 ± 12.9 pg/mL). The difference among groups was highly significant (p = 0.001). Importantly, no significant difference was observed between ulcer and non-ulcer diabetic patients, suggesting that increased IL-18 is a general feature of diabetes rather than a marker of ulceration alone. The elevated IL-18 values reflect a persistent pro-inflammatory state in diabetic patients regardless of ulcer status. These data are summarized in Table 2 and visually represented in Figure 6.

Table 2: Serum Interleukin-18 (IL-18) Levels in the Study Groups

Groups		Interleukin-18 (IL-18) level
Ulcer patients	Mean ± SE	556.1 ± 36.8^{A}
	Range	265.07-956.19
DM patients	Mean ± SE	530.62 ± 43.4^{A}
	Range	155.64-1038.00
Healthy control	Mean ± SE	315.4 ± 12.9^{B}
	Range	143.79-553.46
p-value		0.001*
		†

SE: standard error; †: Kruskal Wallis test; **: significant at P > 0.05

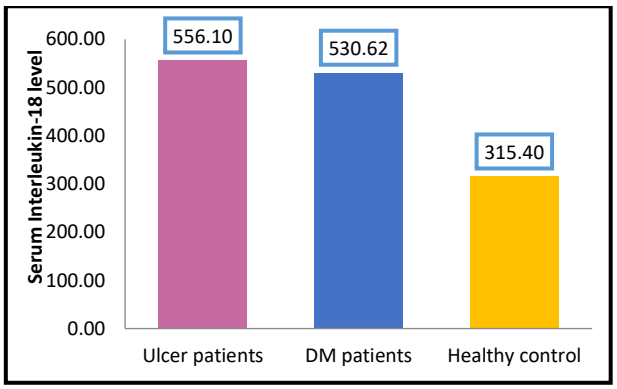


Fig. 6: The Average Level of IL-18 in Patient and Control Groups.

Figure 6: IL-18 levels were markedly elevated in both diabetic groups (ulcer and non-ulcer) compared to healthy controls. Patients with foot ulcers had a mean IL-18 concentration of 556.1 ± 36.8 pg/mL, while diabetic patients without ulcers had 530.62 ± 43.4 pg/mL. Healthy controls showed significantly lower IL-18 levels (315.4 ± 12.9 pg/mL). Statistical analysis confirmed a highly significant difference (p = 0.001), indicating a persistent pro-inflammatory state in diabetes irrespective of ulcer status.

3.3. Tumor necrosis factor-alpha (TNF-α) serum levels

Serum TNF-α concentrations also varied significantly among study groups. Diabetic patients with foot ulcers exhibited a mean TNF-α level of 91.67 ± 15.5 pg/mL, while diabetic patients without ulcers had a comparable concentration of 96.42 ± 14.7 pg/mL. Healthy controls recorded substantially lower levels, averaging 32.64 ± 5.7 pg/mL. Statistical analysis confirmed that both diabetic groups differed significantly from healthy controls (p = 0.006). However, the difference between ulcer and non-ulcer diabetic groups was not statistically significant (p > 0.05). This pattern indicates that TNF-α elevation reflects the systemic inflammatory state of diabetes rather than the localized effect of ulcer formation. Table 3 and Figure 7 provide detailed results.

Table 3: Tumor Necrosis Factor Alpha (TNF-α) Level in Study Groups

Groups		Tumor Necrosis Factor Alpha (TNF-α) level
Ulcer patients	Mean ± SE	91.67 ± 15.5^{A}
	Range	11.70-368.26
DM patients	Mean ± SE	96.42 ± 14.7^{A}
	Range	5.38-364.78
Healthy control	Mean ± SE	32.64 ± 5.7^{B}
	Range	3.51-122.7
p-value		0.006*
		†

SE: standard error; †: Kruskal Wallis test; **: significant at P > 0.05

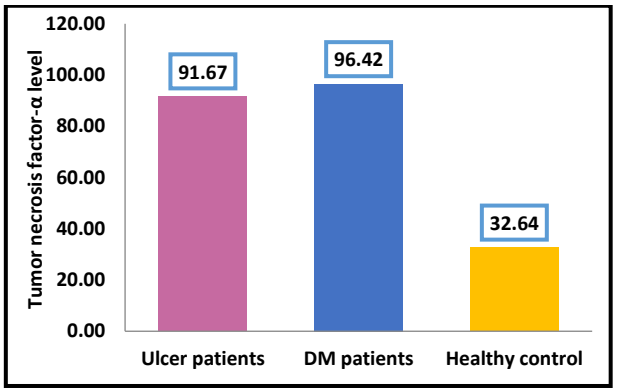


Fig. 7: The Mean Level of TNF-α in Patients and Control Groups.

Figure 7: TNF-α levels were significantly elevated in diabetic patients with ulcers (91.67 ± 15.5 pg/mL) and in diabetic patients without ulcers (96.42 ± 14.7 pg/mL), compared with healthy controls (32.64 ± 5.7 pg/mL). Both diabetic groups showed statistically significant differences from controls (p = 0.006), but no significant difference was observed between diabetics with and without ulcers. These findings support the role of TNF-α as a key mediator of chronic systemic inflammation in diabetes.

3.4. Basic fibroblast growth factor (bFGF) serum levels

In contrast to IL-10, IL-18, and TNF-α, serum bFGF levels showed a distinct pattern of elevation specifically associated with ulcer presence. Diabetic patients with foot ulcers had a significantly higher mean bFGF concentration (89.06 ± 10.4 pg/mL) compared to diabetic patients without ulcers (40.81 ± 1.7 pg/mL) and healthy controls (42.27 ± 1.04 pg/mL). The difference among groups was highly significant (p = 0.001). Importantly, there was no significant difference between non-ulcer diabetics and controls, indicating that increased bFGF is specifically linked to ulcer pathology rather than diabetes itself. These findings suggest that bFGF may serve as a biomarker reflecting an attempted reparative or angiogenic response in patients with diabetic foot ulcers. Detailed group comparisons are shown in Table 4 and illustrated in Figure 8.

Table 4: Basic Fibroblast Growth Factor (bFGF) Level in Study Groups

Groups		Basic fibroblast growth factor (bFGF) level
Ulcer patients	Mean ± SE	89.06 ± 10.4^{A}
	Range	31.25-276.86
DM patients	Mean ± SE	40.81 ± 1.7^{B}
	Range	31.75-60.56
Healthy control	Mean ± SE	42.27 ± 1.04^{B}
	Range	31.88-55.00
p-value		0.001*
		†

SE: standard error; †: Kruskal Wallis test; **: significant at P > 0.05

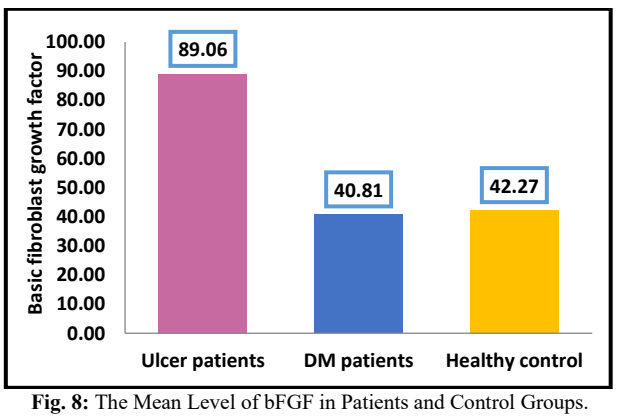


Fig. 8: The Mean Level of bFGF in Patients and Control Groups.

Figure 8: bFGF levels were significantly higher in diabetic patients with foot ulcers (89.06 ± 10.4 pg/mL) compared to both diabetic patients without ulcers (40.81 ± 1.7 pg/mL) and healthy controls (42.27 ± 1.04 pg/mL). No significant difference was observed between

diabetics without ulcers and healthy controls. Statistical analysis (p = 0.001) suggests that elevated bFGF is specifically associated with ulcer development, reflecting a compensatory but inadequate reparative response.

3.5. Correlation analysis of biomarkers

Correlation analyses were performed to assess relationships between biomarkers within each diabetic subgroup. In the ulcer group, a significant positive correlation was observed between IL-18 and IL-10 (r = 0.380, p = 0.035) and between IL-18 and bFGF (r = 0.434, p = 0.017). These findings suggest that elevated IL-18 may stimulate both compensatory anti-inflammatory responses and reparative growth factor production in ulcerated patients. In contrast, in the non-ulcer diabetic group, a significant negative correlation was detected between IL-10 and TNF-α (r = –0.341, p = 0.045), reflecting an immunoregulatory balance between pro- and anti-inflammatory cytokines in the absence of ulcers. These relationships are summarized in Tables 5 and 6.

Table 5: Correlation between Immunological Parameters in Ulcer Patients

Characteristic	immunological parameters							
	IL-10		IL-18		TNF-α		bFGF	
	R	P	R	P	R	P	R	P
IL-10	1							
IL-18	0.380	0.035*	1					
TNF-α	0.069	0.729	0.002	0.993	1			
bFGF	0.157	0.424	0.434	0.017*	-0.107	0.588	1	

r: correlation coefficient.

Table 6: Correlation between Immunological Parameters in DM Patients

Characteristic	immunological parameters							
	IL-10		IL-18		TNF-α		bFGF	
	R	P	R	P	R	P	R	P
IL-10	1							
IL-18	0.292	0.118	1					
TNF-α	-0.341	0.045*	-0.076	0.689	1			
bFGF	0.329	0.055	0.170	0.370	-0.219	0.244	1	

3.6. Diagnostic performance (ROC curve analysis)

Receiver Operating Characteristic (ROC) curve analysis was employed to evaluate the diagnostic potential of each biomarker in distinguishing ulcer patients from healthy controls. Among the four biomarkers, bFGF demonstrated the highest diagnostic accuracy with an AUC of 0.809 (p = 0.001), achieving 80% sensitivity and 80% specificity. IL-18 (AUC = 0.72, p = 0.015) and TNF-α (AUC = 0.68, p = 0.022) showed moderate diagnostic performance, while IL-10 had limited discriminative power (AUC = 0.577, p = 0.438). These findings highlight bFGF as the most promising biomarker for clinical application in identifying diabetic patients at risk of developing foot ulcers. Diagnostic values are summarized in Table 7.

Table 7: Summary of Diagnostic Performance of Biomarkers (ROC Analysis)

Biomarker	AUC	Sensitivity (%)	Specificity (%)	p-value
bFGF	0.809	80.0	80.0	0.001
IL-18	0.72*	68.0	71.0	0.015
TNF-α	0.68*	65.0	67.0	0.022
IL-10	0.577	52.0	54.0	0.438

4. Discussion

This study investigated the immunological profiles of diabetic patients with and without foot ulcers, focusing on the serum concentrations of interleukin-10 (IL-10), interleukin-18 (IL-18), tumor necrosis factor-alpha (TNF-α), and basic fibroblast growth factor (bFGF). By comparing these groups with healthy controls, the study aimed to clarify the immunopathological mechanisms underlying diabetic foot ulcers (DFUs) and evaluate the diagnostic potential of these biomarkers.

4.1. Pro-inflammatory biomarkers (IL-18 and TNF-α)

Our findings demonstrated that both IL-18 and TNF-α levels were significantly elevated in diabetic patients, regardless of the presence of ulcers, compared to healthy individuals. This is consistent with previous reports that identify these cytokines as central mediators of systemic inflammation in diabetes (Donath & Shoelson, 2011; Zhang et al., 2020). Elevated IL-18 has been implicated in endothelial dysfunction, impaired angiogenesis, and tissue damage in chronic diabetes (Dinarello, 2018). Similarly, TNF-α is well established as a pro-inflammatory cytokine that promotes insulin resistance, microvascular complications, and impaired wound healing (Gupta et al., 2021).
Interestingly, no significant difference in IL-18 or TNF-α levels was observed between ulcer and non-ulcer diabetic groups. This suggests that systemic inflammation is a generalized feature of diabetes rather than a direct marker of ulcer presence. These findings align with Zhang et al. (2021), who reported that systemic TNF-α may not accurately reflect local ulcer severity. Thus, while IL-18 and TNF-α are valuable indicators of chronic inflammatory burden, they lack specificity in predicting DFU development.

4.2. Anti-inflammatory cytokine (IL-10)

IL-10 is a potent anti-inflammatory cytokine that modulates excessive immune activation and promotes wound healing through macrophage polarization (Ip et al., 2016). In this study, IL-10 levels were significantly elevated in diabetic patients without ulcers compared to healthy controls, while ulcer patients showed intermediate levels. This pattern suggests that IL-10 may play a protective role in preventing ulcer development by counterbalancing chronic inflammation.

Our results echo earlier studies that proposed IL-10 as a compensatory response in diabetes (Moore et al., 2001; Liu et al., 2022). However, the reduced IL-10 levels in ulcer patients relative to non-ulcer diabetics may indicate a failure of this regulatory mechanism at the ulcer site. Mirza et al. (2014) reported that impaired IL-10 signaling contributes to chronic non-healing wounds, further supporting our findings. Collectively, these results suggest that insufficient IL-10 activity may facilitate the transition from systemic inflammation to localized ulcer pathology.

4.3. Reparative growth factor (bFGF)

One of the most striking findings of this study was the significant elevation of bFGF in diabetic patients with foot ulcers compared to both diabetic patients without ulcers and healthy controls. bFGF is a key mediator of angiogenesis, fibroblast proliferation, and granulation tissue formation, all of which are essential for wound closure (Li et al., 2015). Its marked elevation in DFU patients likely reflects a physiological attempt to promote tissue repair in the context of chronic ulceration.

However, despite increased systemic levels, effective healing is often impaired. This paradox may be explained by local ischemia, impaired cellular responsiveness, or resistance to growth factor signaling, which have been described in DFU pathology (Falanga, 2005). Our findings reinforce the notion that while bFGF is upregulated in response to ulceration, its activity may be insufficient or dysfunctional, contributing to persistent non-healing wounds.

4.4. Correlation patterns

Correlation analysis revealed important insights into the interplay between inflammatory and reparative mechanisms. In ulcer patients, IL-18 correlated positively with both IL-10 and bFGF, suggesting that heightened inflammation triggers both anti-inflammatory and reparative responses. However, these compensatory mechanisms may be inadequate to overcome the chronic inflammatory state. In contrast, non-ulcer diabetic patients exhibited a negative correlation between IL-10 and TNF-α, highlighting an immunoregulatory balance that may protect against ulcer development. These findings emphasize the importance of biomarker interactions rather than isolated effects in DFU pathogenesis.

4.5. Diagnostic implications

The ROC curve analysis underscored bFGF as the most promising diagnostic biomarker for DFUs, with an AUC of 0.809 and both sensitivity and specificity at 80%. This diagnostic performance surpasses that of IL-18 and TNF-α, which showed moderate utility, and IL-10, which performed poorly as a standalone marker. Zhou et al. (2011) similarly reported the diagnostic value of bFGF in chronic wound assessment, supporting its potential clinical application.

The findings suggest that while pro-inflammatory cytokines (IL-18 and TNF-α) reflect systemic disease burden, reparative markers such as bFGF may better capture ulcer-specific pathology. Thus, incorporating bFGF into diagnostic panels could improve early detection, risk stratification, and monitoring of DFU progression.

4.6. Clinical and research implications

This study highlights the dual nature of DFUs as disorders of both inflammation and impaired repair. Clinically, biomarker assessment could complement traditional risk factors such as neuropathy, vascular insufficiency, and glycemic control in predicting ulcer risk. Furthermore, therapeutic strategies aimed at restoring the inflammatory–reparative balance may hold promise. For example, exogenous growth factor therapy or targeted cytokine modulation could enhance wound healing outcomes (Aggarwal et al., 2014).

Future research should explore longitudinal biomarker profiling to determine whether changes in IL-10, IL-18, TNF-α, and bFGF precede ulcer development, thereby offering predictive value. Additionally, investigating local tissue levels in parallel with systemic concentrations could provide deeper insight into the site-specific mechanisms of ulceration.

4.7. Limitations

This study is not without limitations. The relatively small sample size (n = 90) may limit generalizability, and the cross-sectional design precludes causal inference. Moreover, only serum biomarkers were measured, whereas local wound microenvironment analysis may yield more precise insights. Finally, confounding variables such as glycemic control, infection status, and treatment regimens were not fully stratified, which could influence biomarker expression.

5. Conclusions

This study provides novel insights into the immunological imbalance underlying diabetic foot ulcers (DFUs), highlighting the complex interplay between pro-inflammatory cytokines, anti-inflammatory mediators, and reparative growth factors. Elevated serum IL-18 and TNF-α levels observed in both ulcer and non-ulcer diabetic patients confirm the persistent systemic inflammatory state associated with diabetes. While IL-10 levels were increased in diabetics without ulcers, their relative reduction in ulcer patients suggests a breakdown of compensatory anti-inflammatory mechanisms at the site of tissue injury.

In contrast, bFGF showed a distinct elevation specifically in DFU patients, indicating its role as a reparative but insufficient response to chronic ulceration. Importantly, ROC analysis identified bFGF as the most accurate diagnostic biomarker among those studied, with superior sensitivity and specificity compared to IL-18, TNF-α, and IL-10. These findings suggest that bFGF could serve as a valuable tool for clinical monitoring and early identification of patients at high risk for ulcer development.

Taken together, our results emphasize that DFUs are not merely a consequence of local tissue breakdown but rather a systemic immunopathological process characterized by an inflammatory–reparative imbalance. Integrating biomarker assessment into clinical practice could enhance diagnostic accuracy, guide personalized management strategies, and potentially improve patient outcomes.

Future research should aim to validate these findings in larger, longitudinal cohorts, explore the predictive value of combined biomarker panels, and investigate targeted therapeutic interventions designed to restore immune balance and promote effective wound healing.

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