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Pentraxin-3 and Interleukin-18: Potential Biomarkers for the Early Diagnosis and Severity Prediction of Diabetic Foot Ulcer

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Abstract

Background/Aim: As the global incidence of diabetes mellitus (DM) rises, diabetic foot ulcers (DFUs) have emerged as a critical complication associated with increased lower-limb amputations, reduced quality of life, and higher mortality rates. Early detection of DFUs is crucial, sparking interest in potential biomarkers. Interleukin-18 (IL-18) and Pentraxin-3 (PTX-3) are known contributors to inflammation, and recent studies suggest their involvement in diabetic foot events. This study aims to compare IL-18 and PTX-3 levels in type 2 DM (T2DM) patients, with or without foot ulcers (FUs), and healthy controls while exploring their association with DFU diagnosis and severity.

Methods: Eighty-four males participated in the study, including 28 patients with T2DM with FUs, 28 T2DM patients without FUs, and 28 healthy controls. Individuals with type 1 DM (T1DM) or any other types of diabetes, those with infectious or other chronic diseases, and inflammation (such as chronic inflammation and autoimmune diseases), were excluded from participation. Serum IL-18 and PTX-3 were examined using the ELISA method. Enzymatic and colorimetric techniques were used to investigate fasting blood glucose (FBG), hemoglobin A1c (HbA1c), creatinine, urea, alanine transaminase (ALT), and the lipid profile.

Results: The average serum levels of IL-18 and PTX-3 were higher in cases of DFU compared to both T2DM cases and controls ($P < 0.0001$), with the highest levels observed in the DFU group. There was a statistically significant difference in FBG, HbA1c, urea, and creatinine between the different studied groups. ROC curve results showed that the IL-18 cut-off value for predicting severity in patients with DFU compared to T2DM without DFU is ≥ 124.0 pg/ml ($P < 0.0001$). Meanwhile, the PTX-3 cut-off value for predicting DFU in patients with T2DM is > 8.67 ng/ml. The level of IL-18 differed significantly across the different groups based on perfusion, extent, sensation, infection, depth, and nephropathy conditions.

Conclusion: The results indicate that PTX-3 is a promising biomarker for predicting DFU, while IL-18 is a valuable biomarker for predicting severity.

Keywords: Diabetic foot ulcer, Interleukin-18, Pentraxin-3, Type 2 diabetes mellitus, biomarkers, Gaza Strip

1. Introduction

DM is considered the most prevalent endocrine disorder worldwide. It is expected that this disease will affect 642 million individuals by 2040 (Ogurtsova *et al.*, 2017). DM is distinguished by a long-term inflammatory response with elevated blood glucose levels. The development and consequences of DM are closely tied to low-grade inflammation and immunological stimulation (Navarro-Gonzalez and Mora-Fernandez, 2008). FUs occur in 15% to 25% of patients with DM at some point in their lives (Cavanagh *et al.*, 2005). A research paper published in 2017 reported that global DFU prevalence is 6.3%, which was higher in males (4.5%) than in females (3.5%), and higher in T2DM (6.4%) than in T1DM (5.5%) (Zhang *et al.*, 2017). Unfortunately, DFU will lead to amputations in a vast majority of patients with T2DM. Neutrophils' ability to phagocytose and kill bacteria is compromised by hyperglycemia, which also causes oxidative damage and a rise in cytokines linked to inflammation. In addition, hyperglycemia triggers the production of interleukin-6, transforming growth factor-β, and tumor necrosis factor-α. These undesirable effects are connected to a tendency for infections in DFU patients (Saltoglu *et al.*, 2015).

The global prevalence of DFU was 6.3%, according to Zhang et al., (2017), with male patients with T2DM experiencing the condition more frequently than female patients. Because DM is linked to long-term complications of the microvascular and macrovascular systems, the need to identify the problems must be urgent. Hospitalization, impairment, and fatalities for T2DM patients will all decrease if risk categorization of DFU can be attained earlier (Zhang *et al.*, 2017).

When it comes to early medical diagnosis, avoiding illness, and disease course estimation, finding related biomarkers is fundamental (Atkinson *et al.*, 2001). Targets and chemicals connected to the pathogenesis of DFU can be used for early diagnosis and prognosis of the disease. Identifying the biological elements and the course of recovery of DFU has led to the discovery of some novel potential biomarkers that can be applied in the clinical field (Ozer Balin *et al.*, 2019, Chen *et al.*, 2020).

Interleukin-18 (IL-18), initially recognized as an interferon-γ (IFN-γ)-inducing factor, belongs to the IL-1 cytokine superfamily and plays a crucial role in the inflammatory process, contributing to several autoimmune diseases (Yasuda *et al.*, 2019). Recent studies have linked the increase in serum levels of IL-18 to the progression of diabetic foot disease (Chen *et al.*, 2020). However, more research is needed to determine how IL-18 affects T2DM patients with and without FUs compared to healthy controls.

Moreover, previous studies have examined the effect of cytokines other than IL-18 and different acute-phase proteins on DFU (Ozer Balin *et al.*, 2019, Chen *et al.*, 2020). An example of an acute phase protein that was investigated includes the soluble pattern recognition receptor called pentraxin-3 (PTX-3). PTX-3 is produced directly at the site of inflammation by mononuclear phagocytes, fibroblasts, myeloid dendritic cells, granulosa cells, mesangial cells, endothelial cells, smooth muscle cells, and adipocytes. A significant quantity of PTX-3 is created in the vascular wall during an inflammatory process. This substance regulates endothelial function in thrombosis and ischemic vascular disease and binds to angiogenic fibroblast growth factor-2 to prevent angiogenesis (Abu Seman *et al.*, 2013).

Therefore, this study was conducted to assess the potential to use IL-18 and PTX-3 as biomarkers for early diagnosis and severity of DFU and to explore the association between IL-18, PTX-3, and DFU.

2. Materials & methods

2.1. Study Design and Ethical Approval

The present study is a case-control one. The study was approved by the Palestinian Health Research Council, Helsinki Committee (PHRC/1096/22). The participants involved in this study signed an informed consent form before sample collection. A face-to-face interview was conducted to fill in a questionnaire with questions about personal information such as age, educational level, family history of diabetes, smoking status, and clinical data. In addition, special questions were prepared for DFU patients related to skin conditions, perfusion, nephropathy, and the existence of infections which were filled in by the physician.

2.2. Sample size and sampling

This study involved 84 participants, classified as the following: 28 people suffering from T2DM with DFU, 28 people with T2DM without DFU, and 28 healthy people. Persons with the following criteria were excluded from participation in the study: those with T1DM and any other types of diabetes (gestational diabetes & other specific types), persons having infectious or chronic diseases, or who have Alzheimer's disease, or liver disease; and persons with any conditions characterized by inflammation, such as chronic inflammation and autoimmune diseases. Persons chosen as controls were healthy persons with normal blood pressure and non-smokers.

2.3. Sample collection

A venous blood sample was obtained from each participant after an overnight fast in clot activator tubes (Dragon Med, USA) for biochemical and ELISA assays and EDTA tubes for HbA1c test. The serum samples were separated by centrifugation (Gemmy, Taiwan) at 4000 rpm for 10 minutes at room temperature. Serum samples for the analysis of IL-18 and PTX-3 were stored at -80 °C for later use.

2.4. Biochemical analysis and ELISA assay

Total cholesterol, triglycerides, high-density lipoprotein (HDL), fasting blood glucose (FBG), urea, creatinine, alanine aminotransferase (ALT), and HbA1c tests were assayed using commercial analytical kits. Low-density lipoprotein (LDL) was calculated using the Friedewald equation: LDL (mg/dl) = cholesterol - (HDL + triglycerides/5) and then converted to mmol/L. HbA1c kit from (Hipro Biotechnology, China). The cholesterol, triglycerides, and HDL kits are from (Human Diagnostics Worldwide, Germany), and with Glucose kit is from (Dialab, Austria). Urea and creatinine are from (Elitech, France), and an ALT kit is from (Diasys, Germany). IL-18 and PTX-3 levels were measured using ELISA kits (R&D Systems Inc., USA), and the results were read using an ELISA reader (Snibe, China) at 450 nm. All test analyses were performed according to the manufacturer's instructions.

2.5. Statistical analysis

Categorical variables were summarized with frequencies and percentages, and numerical variables were summarized with the mean and standard deviation (SD), median and interquartile range (quartile 1 (Q1), quartile 3 (Q3)), and minimum (Min) and maximum (Max). Differences between groups in categorical variable distributions were tested using Pearson's Chi-square test or Fisher’s exact test, depending on the validity of the expected counts assumption for Pearson's Chi-square. On the other hand, differences in numerical variables between the two groups were tested using either the independent samples T-test or the Mann-Whitney U test (MW), depending on the validity of parametric assumptions. When more than two groups were compared, one-way analysis of variance ANOVA (with Tukey’s HSD post hoc test) or Kruskal-Wallis rank sum test (KW) (with Dunn test post hoc testing) was employed depending on the validity of the parametric test assumptions. The Kendall rank correlation coefficient (Kendall-tau) was used to measure the ordinal association between the grade of DFU and biomarker concentration. The perfusion, extent, depth, infection, and sensation (PEDIS) Score (Chuan *et al.*, 2015), which is a severity score for DFU, was calculated by summing the stages of perfusion, extent, depth, infection, and sensation. A PEDIS score of 7 and above indicates a higher risk of adverse outcomes for ulcers, and this cut-off was used to designate the DFUs as severe or non-severe. Using the coordinates of the receiver operating characteristic (ROC) curves, the maximum Youden index was used to identify the optimal thresholds for predicting the T2DM patients (either with or without FU), and the severity of FU (severe or non-severe) based on IL-18 or PTX-3 concentrations. Since the sample size was small (N = 28 or N = 14), we could not perform multivariable analyses. All analyses and figures were produced using R version 4.2.2 (R Core Team, 2013).

3. Results

3.1. 3.1 Demographic characteristics of the study groups

The results show that T2DM patients with FU were slightly older than the other two groups (63.5 ± 6.0 vs. 62.0 ± 6.7 respectively), and they were more predominantly living in South Gaza. Furthermore, 39.3% of T2DM with or without FUs were smokers or quit smoking compared to 60.7% for non-smokers. There was a significant difference between the study groups in terms of smoking status (P < 0.05), while there was no significant difference in BMI between the study groups (P = 0.362), as shown in Table 1.

Table 1: Demographic characteristics of the study groups.

	Normal (N=28)	T2DM (N=28)	T2DM with FU (N=28)	P-value
Age (years)				
Mean (SD)	60.9 (6.0)	62.0 (6.7)	63.5 (6.0)	
(Min, Max)	(50.0, 72.0)	(50.0, 72.0)	(52.0, 72.0)	0.298[#]
Median	60.5	62.0	64.0	
(Q1, Q3)	(56.5, 64.3)	(57.5, 68.3)	(59.0, 67.5)	
BMI (kg/m^2)				
Mean (SD)	24.2 (2.2)	24.5 (2.3)	24.9 (1.6)	
(Min, Max)	(21.0, 30.6)	(20.9, 29.0)	(21.9, 27.8)	0.520[#]
Median	24.2	25.0	24.9	
(Q1, Q3)	(22.5, 25.4)	(22.3, 26.2)	(24.0, 26.2)	
Smoking status				
Non-Smokers	28 (100%)	17 (60.7%)	17 (60.7%)	0.03*[$]
Quit Smoking	0 (0%)	6 (21.4%)	9 (32.1%)	
Smokers	0 (0%)	5 (17.9%)	2 (7.1%)	
Residence				
Gaza	10 (35.7%)	6 (21.4%)	5 (17.9%)	0.077[£]
Middle	3 (10.7%)	9 (32.1%)	3 (10.7%)	
South	15 (53.6%)	13 (46.4%)	20 (71.4%)	
Marital status				
Married	21 (75.0%)	20 (71.4%)	24 (85.7%)	0.413
Widowed	7 (25.0%)	8 (28.6%)	4 (14.3%)	
Education				
Illiterate	6 (21.4%)	5 (17.9%)	8 (28.6%)	
Preparatory	1 (3.6%)	2 (7.1%)	2 (7.1%)	0.922[$]
Secondary	10 (35.7%)	9 (32.1%)	10 (35.7%)	
University	11 (39.3%)	12 (42.9%)	8 (28.6%)	

BMI: Body mass index; Gaza, Middle, South: Governorates of Gaza strip. *: The test included only the T2DM patients and T2DM patients with FU groups since the normal group was chosen to be non-smokers; [#]: ANOVA test; [$]: Fisher test; [£]: Chi-square Chi-square.

3.2. Clinical characteristics and the biochemical parameters of the study groups

As illustrated in Table 2, the duration of diabetes in patients with FUs was slightly longer (8.2 ± 3.2 years) compared to T2DM patients without FUs (7.2 ± 3.4 years), but the difference was not statistically significant (P = 0.260).

Table 3 shows the levels of the different tested biochemical parameters. Both T2DM patients and T2DM patients with FUs had higher HbA1c and FBG than the normal group, and these differences were statistically significant (P < 0.0001). Cholesterol and LDL values were significantly lower in the T2DM patients with FUs group compared to the corresponding values for both T2DM patients without FUs and normal groups (P = 0.002 and P = 0.001, respectively). On the other hand, creatinine and urea were significantly elevated in T2DM patients with FUs compared to the other two groups (P < 0.0001).

Table 2: Clinical characteristics of the study groups.

	Normal (N=28)	T2DM (N=28)	T2DM with FU (N=28)	P value
Blood pressure				
Normal	28 (100%)	20 (71.4%)	16 (57.1%)	0.265#*
> 130 (mmHg)	0 (0%)	8 (28.6%)	12 (42.9%)	
Family history of DM				
Yes	18 (64.3%)	16 (57.1%)	17 (60.7%)	0.861#
No	10 (35.7%)	12 (42.9%)	11 (39.3%)	
Duration of diabetes (years)				
Mean (SD)	NA	7.2 (3.4)	8.2 (3.2)	0.260$
(Min, Max)	NA	(1.0, 13.0)	(4.0, 14.0)	
Median (Q1, Q3)	NA	7.0 (5.0, 9.2)	8.0 (5.8, 10.3)	

* The test included only the T2DM patients and T2DM patients with FU groups since the normal group was chosen to have normal blood pressure; # Chi square; $ T-Test.

Table 3: Comparison of the biochemical parameters among the study groups.

Parameter	Normal (N=28)	T2DM (N=28)	T2DM with FU (N=28)	P value	
				Overall	Post- hoc
HbA1c (%)					
Mean (SD)	5.7 (0.3)	9.0 (1.5)	9.8 (1.4)		<0.0001[a]
(Min, Max)	(5.2, 6.8)	(6.4, 12.2)	(7.8, 13.2)	<0.0001*	<0.0001[b]
Median (Q1, Q3)	5.7 (5.5, 5.9)	9.0 (7.9, 10.2)	9.6 (8.6, 10.5)		
FBG (mmol/L)					
Mean (SD)	5.8 (2.2)	10.3 (5.1)	11.2 (3.9)		0.0001[a]
(Min, Max)	(3.3, 13.9)	(3.6, 23.8)	(4.8, 21.4)	<0.0001*	<0.0001[b]
Median (Q1, Q3)	5.7 (4.2,6.2)	9.9 (6.7, 12.9)	10.9 (8.6, 13.7)		
Cholesterol (mmol/L)					
Mean (SD)	4.5 (0.9)	4.7 (1.0)	3.9 (0.7)		0.031[b]
(Min, Max)	(2.7, 6.1)	(2.9, 6.5)	(2.7, 5.3)	0.002#	0.002[c]
Median (Q1, Q3)	4.7 (3.8, 5.0)	5.0 (4.0, 5.4)	3.7 (3.4, 4.3)		
Triglycerides (mmol/L)					
Mean (SD)	1.8 (0.7)	1.7 (0.6)	1.8 (0.5)		
(Min, Max)	(0.7, 3.0)	(0.7, 3.1)	(0.6, 2.5)	0.669*	
Median (Q1, Q3)	1.9 (1.2, 2.2)	1.9 (1.1, 2.1)	1.8 (1.4, 2.2)		
HDL (mmol/L)					
Mean (SD)	1.1 (0.26)	1.1 (0.3)	1.0 (0.3)		
(Min, Max)	(0.7, 1.8)	(0.7, 1.6)	(0.7, 1.6)	0.884#	
Median (Q1, Q3)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	1.0 (0.8, 1.2)		
LDL (mmol/L)					
Mean (SD)	2.6 (0.8)	2.9 (0.9)	2.0 (0.6)		0.021[b]
(Min, Max)	(1.0, 4.0)	(1.2, 4.4)	(0.9, 3.1)	0.001#	<0.001[c]
Median (Q1, Q3)	2.7 (1.9, 3.2)	2.8 (2.1, 3.6)	2.0 (1.8, 2.5)		
Creatinine (µmol/L)					
Mean (SD)	79.6 (17.7)	97.3 (26.5)	128.2 (26.5)		<0.0001[b]
(Min, Max)	(53.1, 115.0)	(61.9, 176.8)	(70.7, 176.8)	<0.0001*	<0.0001[c]
Median (Q1, Q3)	88.4 (70.7, 88.4)	88.4 (70.7, 106.1)	132.6 (115.0, 141.5)		
Urea (mmol/L)					
Mean (SD)	11.4 (2.8)	14.0 (4.7)	21.7 (10.8)		<0.0001[b]
(Min, Max)	(6.1, 16.1)	(7.1, 10.2)	(8.2, 57.1)	<0.0001*	0.001[c]
Median (Q1, Q3)	10.9 (9.6, 13.7)	13.6 (11.1, 16.4)	19.6 (15.5, 23.7)		
ALT (U/L)					
Mean (SD)	20.7 (6.19)	24.1 (11.1)	22.0 (8.9)		
(Min, Max)	(11.0, 35.0)	(10.0, 52.0)	(11.0, 41.0)	0.863*	
Median (Q1, Q3)	19.5 (16.8, 21.8)	21.0 (16.0, 29.5)	20.0 (15.0, 26.0)		

ALT: Alanine transaminase; FBG: Fasting blood glucose; HbA1c: Hemoglobin A1c; HDL: high-density lipoprotein; LDL: Low-density lipoprotein. Significant P values (P < 0.05) are in bold. [a] Diabetic vs Controls; [b] T2DM with FU vs Controls & [c] T2DM vs T2DM with FU. * Kruskal-Wallis (KW) test, # ANOVA test.

3.3. IL-18 and PTX-3 levels

As shown in Figure 1, the levels of both biochemical markers were significantly elevated in the DFU group compared to the T2DM and the normal group (P < 0.001). On the other hand, no significant differences have been observed in PTX-3 and IL-18 levels between the normal group and the T2DM patients.

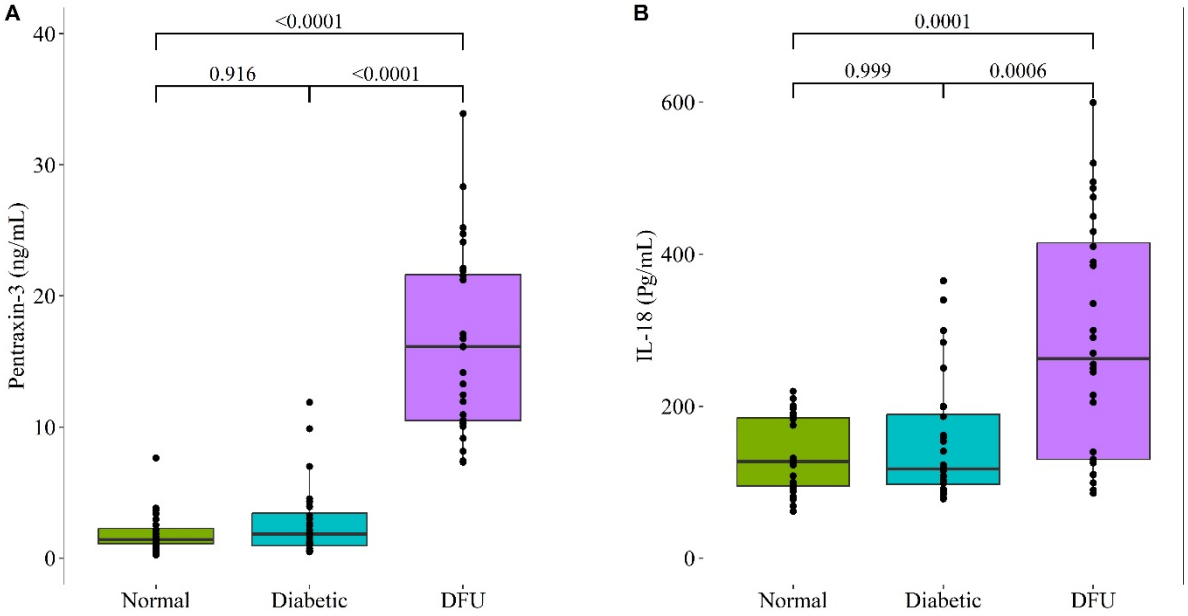

Figure 1: Boxplots of Pentraxin-3 and IL-18 levels in study groups. **A.** Pentraxin-3 levels. **B.** IL-18 levels. DFU: T2DM patients with FU. P values shown represent the Dunn test post hoc**.**

3.4. Association of IL-18 and PTX-3 levels with severity of FUs and stages of nephropathy

Table 4 indicates that there was a significant correlation between perfusion, extent, depth, infection, and IL-18, where IL-18 levels increased with increasing grades of these criteria (Kendall-Tau around 0.5 for all criteria and P < 0.001). In addition, PTX-3 and IL-18 levels were elevated in patients without sensation in their ulcers compared to those who have not lost sensation, but only the difference in IL-18 was significant (P < 0.0001). The sum of the grades of these five criteria represents the PEDIS score, which defines the risk for adverse outcomes (non-healing ulcer, amputation, or death). Patients with PEDIS scores of 7 and above had higher PTX-3 and IL-18 levels compared to those whose scores were lower than 7, and the difference in IL-18 was significant (P < 0.0001). Similarly, higher IL-18 levels were associated with an increasing stage of nephropathy (Kendall-Tau=0.576, P = 0.04).

Table 4: Association between concentrations of PTX-3 and IL-18 and severity of FU or stage of nephropathy in T2DM patients with FU (N=28).

Grade	n	PTX-3		IL-18	
		Median	Kendall-tau (P value)	Median	Kendall-tau (P value)
Perfusion					
0: No peripheral arterial disease	4	57.4	0.069 (0.57)	5.8	0.587 (<0.0001)
1: Peripheral arterial disease, no critical limb ischemia	14	32.9		12.8	
2: Critical limb ischemia	10	49.4		25.7	
Extent					
0: Skin intact	6	50.9		9.6	
1: <1 cm^2	4	55.0	0.069	7.2	0.479
2: 1–3 cm^2	12	35.9	(0.59)	16.4	(0.0002)
3: >3 cm^2	6	56.5		27.3	
Depth					
0: Skin intact	2	44.1*		8.7*	
1: Superficial	9	47.4	0.061	7.2	0.529
2: Fascia, muscle, tendon	11	36.8	(0.63)	16.7	(<0.0001)
3: Bone or joint	6	56.5		27.3	
Infection					
0: None	3	62.4		7.2	
1: Surface	9	35.0	0.061	7.8	0.505
2: Abscess, fasciitis, and/or septic arthritis	10	39.1	(0.63)	17.6	(<0.0001)
3: Systemic inflammatory response syndrome	6	56.2		26.0	
Sensation					
0: Sensation intact	16	37.9	0.15	7.5	<0.0001
1: Loss of sensation	12	49.4	MW	24.4	MW
PEDIS score					
< 7	14	43.2	0.430	7.5	<0.0001
≥ 7	14	48.2	T-Test	23.3	MW
Nephropathy					
0: None	9	39.1	0.167 (0.18)	7.2	0.576 (0.04)
1: stage 1	11	35.0		13.6	
2: stage 2	8	56.5		22.6	

A higher grade represents a more severe ulcer condition. PEDIS score is calculated by summing the grade of the preceding five criteria. *Median was calculated as the average of the two data points. Significant P values are in bold. The Kendall-tau P value column shows the Kendall-tau statistic and its P value except for Loss of sensation and PEDIS score.

3.5. Prediction of DFU by IL-18 and PTX-3

PTX-3 was a better predictor of group membership of T2DM patients regarding the presence or absence of FUs compared to IL-18 (AUC = 0.983 vs. 0.772; Figure 2A). The opposite was true when these biomarkers were used to classify patients with FUs into two groups based on ulcer severity, where a PEDIS score ≥ 7 was considered to be associated with a more severe case of DFU. In this regard, IL-18 was a very good predictor of ulcer severity, whereas PTX-3 was not useful in predicting ulcer severity (AUC = 0.954 vs. 0.597: Figure 2B).

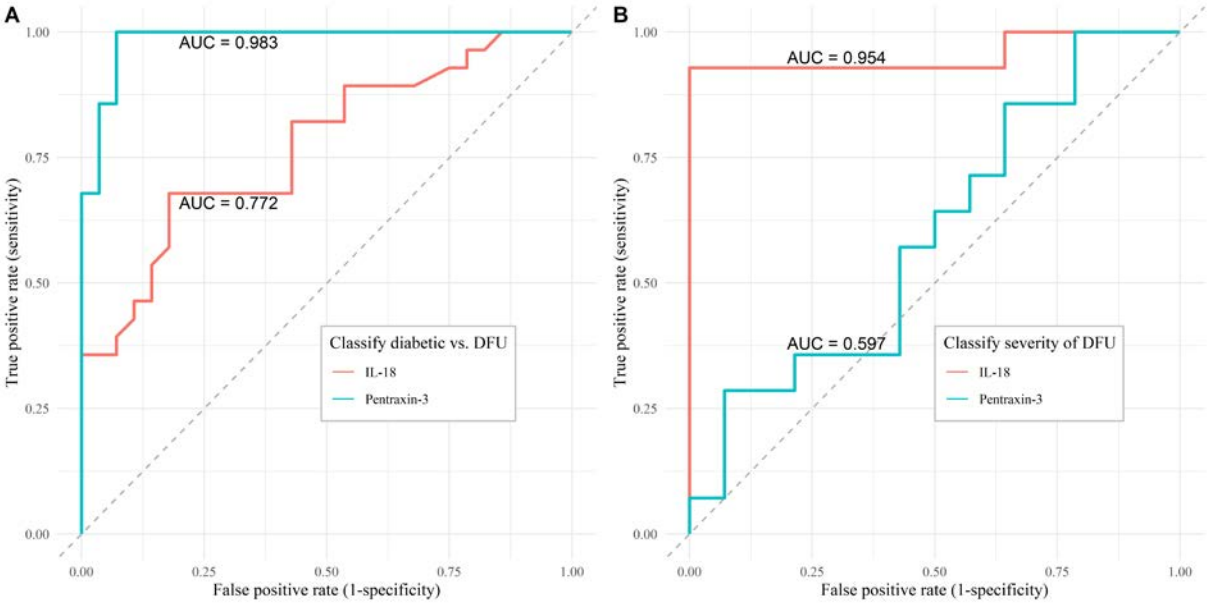

Figure 2: Receiver operating characteristic (ROC) curves of PTX-3 and IL-18 for prediction of the presence of T2DM with FU and severity of the DFU. **A.** ROC curves of the two biomarkers for prediction of T2DM patients with FUs. Each group has N=28. **B.** ROC curves of the two biomarkers for prediction of severe FUs. A PEDIS score of seven and above was taken to indicate a case of severe FU. Each group had N=14. AUC: Area under the ROC curve.

Additionally, Table 5 lists the diagnostic cut-offs and their performance for PTX-3 and IL-18 in predicting the presence of FUs in T2DM patients (Diabetic vs. DFU) and in predicting the severity of DFU. The cut-offs are based on the maximum Youden index, which gives the same weight to sensitivity and specificity. The cut-off value of 202.5 pg/ml IL-18 in classifying diabetics vs. DFU gives only 67.9% sensitivity.

Table 5. Performance of PTX-3 and IL-18 in predicting DFU group and severe FU based on maximum Youden index cut-offs.

	AUC (95% CI) P value	Cut-off	Sensitivity	Specificity	NPV	PPV	ACC
Classify Diabetic vs. DFU							
IL-18 (pg/ml)	0.772 (0.650, 0.894) <0.0001	202.5	67.9	82.1	71.9	79.2	75.0
PTX-3 (ng/ml)	0.983 (0.957, 1.000) <0.0001	7.17	100	92.9	100	93.3	96.4
Classify DFU severity							
IL-18 (pg/ml)	0.954 (0.864, 1.000) <0.0001	280	92.9	100.0	93.3	100.0	96.4
PTX-3 (ng/ml)	0.597 (0.383, 0.811) 0.383	-	-	-	-	-	-

ACC: Accuracy; AUC: Area under ROC curve; DFU: Diabetic foot ulcer; NPV: Negative predictive value; PPV: Positive predictive value. P value is the result of the statistical test between the AUC and an AUC of 0.5 (random classifier). No diagnostic criteria are shown for PTX-3 for severity classification since its AUC was not significantly different from 0.5.

3.6. Association between IL-18, PTX-3 and duration of diabetes

Since the duration of diabetes is an important factor in developing DFU, and because it affects the severity of the ulcer we also examined biomarker concentrations in the patients based on the duration of their duration of diabetes (Table 6). PTX-3 levels showed no correlation with the increasing duration of diabetes in T2DM patients with or without FUs. On the other hand, IL-18 concentration increased with increasing duration in both groups, and the correlation was strong (stronger association) in the case of T2DM patients with FUs (Kendall-Tau 0.63 vs. 0.41). None of the T2DM patients with FUs who had diabetes for longer than 10 years were in the non-severe FU group. Again, PTX-3 levels were not correlated with increasing duration of diabetes in patients with severe FUs, whereas IL-18 was significantly correlated with increasing duration in this group (although there was only one patient who had diabetes for less than 5 years).

Table 6. PTX-3 and IL-18 for T2DM patients with and without FU, and patients with severe and non-severe FU based on duration of diabetes.

Duration of diabetes (years)	Diabetic (N=28)		DFU (N=28)	
	PTX-3 (ng/ml) Median (Q1, Q3)	IL-18 (pg/ml) Median (Q1, Q3)	PTX-3 (ng/ml) Median (Q1, Q3)	IL-18 (pg/ml) Median (Q1, Q3)
<5	1.1 (0.66-2.7)	103 (82-134.5)	12.5 (11.4-14.8)	110 (97.5-117.5)
5-10	1.9 (1.0-2.7)	108 (96.5-155.3)	14.7 (10.4-22.1)	248 (132.5-323.8)
>10	2.9 (1.4-6.2)	267 (212.5-326)	16.8 (15.2-19.2)	487 (430-5.7.5)
Kendall-tau	0.20	0.41	0.11	0.63
(P value)	(0.188)	(0.007)	(0.496)	(<0.0001)
Duration of diabetes (years)	Non-severe DFU (N=14)		Severe DFU (N=14)	
	PTX-3 (ng/ml)	IL-18 (pg/ml)	PTX-3 (ng/ml)	IL-18 (pg/ml)
<5	14.5 (10.7-20.7)	120 (101.8-137.5)	12.5 (12.5-12.5)	125 (one patient)
5-10	14.7 (8.0-21.2)	210 (125-246.3)	17.5 (10.6-24.6)	387.5 (347.5-420)
>10	NA	NA	16.8 (15.2-19.2)	487 (430-507.5)
Kendall-tau	T-test	MW test	0.04	0.55
(P value)	(0.591)	(0.300)	(0.853)	(0.016)

4. Discussion

Diabetes is an illness of metabolism that affects about 9% of the global population in general. Uncontrolled DM can cause chronic problems, and a significant number of individuals already have complications when they are diagnosed (Weerasuriya *et al.*, 1998). Early diagnosis of complications is crucial since long-term microvascular and macrovascular consequences are linked to diabetes. A serious complication that affects T2DM patients is the DFU effect, which is considered a serious health issue. Lower limb amputations may result from nonhealing or slowly healing diabetic ulcers. DFU development involves numerous factors, peripheral artery disease, atherosclerotic plaque, alterations in blood circulation, and peripheral neuropathy are only a few of the intricate interactions that lead to DFU (Brownrigg *et al.*, 2013).

It is worth noting that multiple studies on potential indicators of diabetic foot are needed with the integration of diverse methodologies due to the rising incidence of diabetes and its complications worldwide. DFU biomarkers may aid in the improvement of early detection, avoidance of the disease, estimation of illness progression, and even treatment monitoring of DFU. So, we aimed to evaluate the role of some biomarkers in the early diagnosis of DFU in patients in the Gaza Strip. To the best of our knowledge, this is the first study to be done in the Gaza Strip to assess the association between IL-18, PTX-3, and DFU.

The most accurate measure of glycemic management is HbA1c. It is a crucial measure used to track blood glucose levels in people with diabetes (Diabetes Control Complications Trial Research Group, 1993). In this study, we found that both diabetic patients with and without FUs had higher HbA1c and FBG compared to the controls. These results are in agreement with what was found by (Kaleli *et al.*, 2019) in which they showed a higher level of HbA1c in T2DM patients and diabetic foot syndrome patients.

According to several reports, T2DM patients with DFU experience an increase in the frequency of cardiovascular disorders by 2-4 times (Tuttolomondo *et al.*, 2015). There is an association between inflammation and DM, as evidenced by the disparity in biochemical parameters such as LDL, triglycerides, and total cholesterol levels between normal and T2DM patients. Because of that, we decided to compare the lipid biomarkers between the study groups. We found that cholesterol and LDL values were significantly lower in diabetics with FUs group compared to the corresponding values for both diabetics without FUs and normal groups. These findings were not consistent with the study done by (Mushtaq *et al.*, 2020). Their study found that cholesterol, LDL, and triglyceride levels are significantly higher in DFU patients than in patients without DFU. These differences may be due to the limited number of participants in our work.

Furthermore, a significant increase in creatinine and urea levels was found in the DFU group when compared to the other groups. The same findings were discovered in a study conducted by (Al-karawi *et al.*, 2019). In another study, creatinine was also found to be higher in DFU patients compared to patients without FUs ($P < 0.0001$) (Aziz, 2020).

IL-18 is a pro-inflammatory cytokine that is released by dendritic cells, macrophages, and epithelial cells (Okamura *et al.*, 1995). IL-18 levels in T2DM patients were originally shown to be considerably higher than in those without diabetes (Esposito *et al.*, 2003). Moreover, increased IL-18 levels are considered a reliable indicator of T2DM and metabolic syndrome (Fischer *et al.*, 2005). IL-18 has also been demonstrated to impair insulin secretion and cause β-cell dysfunction in animal-based scientific investigations (Frigerio *et al.*, 2002). In addition, it causes endothelial cell dysfunction and plays a role in atherosclerosis (Gerdes *et al.*, 2002). In our study, we found that IL-18 was higher in the T2DM patients with FUs compared to the T2DM patients without ulcers and the healthy controls. To add to this, IL-18 was an excellent predictor of ulcer severity. Our results are consistent with (Sabuncu *et al.*, 2014) findings. Also, their study showed that IL-18 was positively correlated with high sensitivity-C reactive protein (CRP) and ESR which were the most well-known acute phase reactants. In contrast, (Weigelt *et al.*,

2009) reported that the levels of IL-18 in the serum of T2DM patients with acute FUs did not change when compared with those without DFU. When compared with the DFU group, the control group in the study by Weigelt et al. was significantly younger and had a higher HbA1c level; these variations could have affected their outcomes. Age-wise, our study's groups were closer.

We also found that IL-18 levels were elevated with increasing diabetes duration in both diabetics with and without FUs and in diabetics with severe FUs. This was in contrast with what was reported by (Aso *et al.*, 2003) where they found no significant association between IL-8 concentration and diabetes duration.

Both PTX-3 and CRP are acute-phase reactants that include five identical components collectively known as the pentraxin family. They are physically and functionally comparable molecules (Inforzato *et al.*, 2013). PTX-3 is an essential part of the innate immune system that removes dead or dying cells (Mantovani *et al.*, 2013). As it can be identified fairly early in the course of the disease, PTX-3 has been linked to the extent of several inflammatory conditions. It has been noted that bacterial and viral infections result in a considerable rise in PTX-3 levels (Muller *et al.*, 2001). Additionally, studies have demonstrated its value as a prognostic indicator in a variety of conditions, including community-acquired pneumonia (Kao *et al.*, 2013), inflammatory disorders, cardiovascular issues, and renal illnesses (Üstündağ *et al.*, 2011, Argani *et al.*, 2012). In addition, (Takashi *et al.*, 2018) reported that PTX-3 was positively linked with both the existence of diabetes and glycosylated hemoglobin.

In our study, we found that PTX-3 was very efficient in recognizing T2DM patients with FUs. Our results were compatible with what was reported by (Ozer Balin *et al.*, 2019), in which PTX-3 is an accurate indicator for the diagnosis of infectious DFU (IDFU). The subgroup analysis of IDFU in their study showed significant differences in terms of PTX-3 between mild, moderate, and severe disease subgroups.

5. Conclusion

The study of biomarkers is progressing quickly in a variety of domains. For example, various physical indicators are useful and have been taken into consideration for the diagnosis, risk classification, or monitoring of DFU. In order not to overestimate the value of the newly developed systems, it is ideal to combine the new markers with the clinical data and compare the results with the most accurate standard tests currently available for DFU. This study is the first to recommend a diagnostic application for blood IL-18 and PTX-3 levels in DFU patients in the Gaza Strip. The results show that PTX-3 is a good biomarker for the prediction of DFU, while IL-18 is a good biomarker for the prediction of the severity.

6. Limitations

However, our study has some limitations: first is the small sample size. Second, the study is cross-sectional, so it is difficult to explore the relationship between cause and effect in the correlation between systemic inflammation and FU. To further understand how inflammation affects the onset of foot ulcerations and their predictive value, longitudinal studies are required. Third, our results are valid for T2DM patients only because T1DM patients were excluded. The study of biomarkers in DFU is still in processing, and continuous attempts in this field will assist in increasing the knowledge of DFU diagnosis, prevention, and treatment.

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Conflict of interest

The authors declared that there is no conflict of interest.

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