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ORIGINAL RESEARCH

T-Natural Killers and Interferon Gamma/ Interleukin 4 in Augmentation of Infection in Foot Ulcer in Type 2 Diabetes

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Background: The link between immune system and type 2 diabetes mellitus (T2DM) pathogenesis attracted attention to demonstrate the role of immune cells and their secreted cytokines in T2DM development and its subsequent foot complications.

Objective: To investigate the relation between T Natural killer cell (TNK) %, Interleukin 4 (IL4) and Interferon gamma (IFN-γ) and diabetic foot infection (DFI) development in patients with diabetic foot ulcer (DFU).

Patients and Methods: Ninety patients with diabetes were included in this work, divided as T2DM group (n=30), DFU group (n=30), and DFI group (n=30). TNK% was detected using flow cytometry. Serum IL4 and IFN-γ were measured by ELISA. Diabetes biochemical parameters were also analyzed.

Results: Significant decrease was detected in TNK% and IFN-γ in DFI group compared to other 2 groups (P<0.001). Significant decrease was detected in serum levels of IL4 in DFI group compared to T2DM group (P=0.006). IFN-γ/IL4 was significantly decreased in DFI compared to DFU group (P=0.020). There was a significant correlation of TNK% with both IL4 and IFN-γ (r=0.385, P<0.001; r=0.534, P<0.001, respectively). Significant negative correlation of TNK% with HbA1c and LDL was revealed (r=−0.631, P<0.001; and r=−0.261, P=0.013, respectively), while a positive correlation was seen with HDL (r=0.287, P=0.006). A significant negative correlation of IL4 with HbA1c was found (r=−0.514, P<0.001;. As for IFN-γ, a significant negative correlation with HbA1c and LDL was detected (r=−0.369, P< 0.001; r=−0.229, P=0.030). TNK % and IFN-γ level showed negative correlations with disease duration/year (r=−0.546, P< 0.001; r=−0.338, P=0.001, respectively).

Conclusion: Decline in TNK frequency has essential role in T2DM pathogenesis and subsequent foot complications. Downregulation of TNK% and IFN-γ level have potential roles in predicting infection of diabetic ulcer and are correlated with disease duration.

Keywords: diabetic foot ulcer, diabetic foot infection, natural killer cell, interferon gamma, interleukin 4

Introduction

Epidemiology studies have shown that diabetes has the highest incidence of any chronic disease worldwide and is a huge threat to human health. Diabetes is clinically divided into types I and II.[1] The prevalence of type II diabetes mellitus (T2DM) increases with age across all regions and income groups.[2] Still, the prevalence is increasing in young age groups due to unhealthy life routines present since childhood.[3]

Insulin resistance (IR) associates with T2DM.[4] Diabetes-related foot complications are important causes for disability worldwide.[5] More than one-third of patients with diabetes worldwide will develop diabetic foot ulcer (DFU), which can progress to diabetic foot infection (DFI), and gangrene, consuming most of the healthcare costs dedicated for patients with diabetes.[6] Around 17% of DFI will require amputation.[7] Studies showed that patients with diabetes fear amputation more than death.[8]

The DFI patients will demand extensive debridement, and therapies which results in increasing the time of hospitalization, and the costs of treatment.[9] In addition, the recurrence rate is high, reaching 40% within 1 year.[10] Patients with DFI have a mortality incidence higher than patients with diabetes and without infection.[11]

Several factors predispose patients with diabetes to develop DFI, including immunopathology, as well as the potential to mount a normal inflammatory response. Also, due to loss of sweat and oil gland function in DM, the foot becomes dry and cracks more easily, leading to a portal for infection.[9]

The link between the immune system and diabetes pathogenesis has attracted the attention to highlight the pattern of some immune cells and their secreted cytokines associating T2DM progression.[12]

T-Natural killer (TNK) cells belong to the heterogeneous T-cell group. These cells recognize the specific lipid molecules presented by the CD1d molecules, as well as the polypeptides presented by non-major histocompatibility complexes. Therefore, TNK cells are also referred to as CD1d-dependent natural killer-like T-cells.[13] These cells express the T lymphocyte markers (CD3 and TCR), as well as the NK cellular markers (CD56 and CD16) on their surfaces.[14]

The TNK cell was noticed to act as a link between innate and adaptive immunity through their secreted cytokines after their major histocompatibility complex (MHC) class I recognizes lipid antigens.[15] Also, TNK cells directly act on target cells and regulate their biological functions.[13]

The TNK cell, if stimulated by lipid antigens, can produce Th1 and Th2 cytokines.[16] Those cytokines may initiate cell-mediated immunity or inhibit autoreactive immunity.[17] Among these TNK cytokines is interferon-gamma (IFN-γ), which is involved in pro-inflammatory response and is considered as a major player in phagocytosis and opsonization and which may point to refer to a protective role against development of local infection in DFI. Regarding interleukin 4 (IL4), it is reported to be involved in anti-inflammatory responses.[18] Hence the rationale of studying the ratio as an indicator of balance between pro-inflammatory/anti-inflammatory secreted cytokines.

Dyslipidemia plays a crucial role in T2DM which is characterized by elevated TG, reduced HDL, and predominant LDL, which also plays a crucial role in development of atherogenesis and foot ulcer development.[19] Since TNK cells mostly recognize lipid antigens, an altered lipid metabolic profile will also alter the repertoire of lipid antigens that can potentially affect TNK immunemodulatory function and cytokine secretion.[20]

This study aimed to investigate the link between circulating TNK % and serum IL4, IFN-γ levels, IFN-γ/IL4 ratio, and the development of DFI in patients with DFU. Also, we wanted to assess their relationship with diabetic biochemical parameters. Finally, we wanted to explore the association between TNK%, IL4, and IFN-γ levels and disease duration in DM patients.

Patients and Methods

Ninety patients with diabetes were enrolled in this cross-sectional study. Patients were divided into a T2DM group (n=30) including recently diagnosed patients, without any foot complications, recruited from Internal Medicine and Endocrinology Departments, a DFU group (n=30) recruited from the Vascular Department, and a DFI group (n=30) recruited from the Surgery Outpatient Clinic. All patients were recruited from Al-Zahraa University Hospital, during the period from September to December 2020.

The study was conducted in accordance with the Declaration of Helsinki. All patients were informed about the purpose of the study and written informed consent was obtained from all participants before proceeding with the study. Approval of the study proposal was granted by the Research Ethics Committee of the Faculty of Medicine for Girls (Cairo), Al-Azhar University (Approval No. 202008358).

Inclusion Criteria

T2DM was diagnosed based on the American Diabetes Association criteria.[21] The DFI group included patients with infected ulcers and palpable pulsations in the foot attending surgery outpatient clinic. Diagnosis of DFI was done according to the Infectious Diseases Society of America (IDSA) (the presence of infection is defined by

≥2 classic findings of inflammation or purulence including induration, erythema, raised temperature, increased pain, and purulent discharge).[22] Cultures were taken from the base of an appropriately debrided ulcer to yield true pathogens and more accurate results, and to exclude colonizers. All cases with DFI in our study showed clinically evident infection and this may be attributed to excluding patients with ischemia and the absence of its confounding effect on local and systemic inflammatory response.

DFU patients were recruited when there was no evidence of infection. The ulcer was classified according to Wagner classification and arterial duplex ultrasonography and Ankle-brachial index were done for all patients to exclude critical limb ischemia (CLI).

Exclusion Criteria

1) Any history of malignancy, autoimmune disorders, cardiac, liver, renal, pulmonary diseases, or use of anti-inflammatory drugs. 2) Type 1 DM (T1DM). 3) T2DM patients with morbid obesity (BMI>35 kg/m^2), as they are usually associated with severe co-morbidities. 4) T2DM patients diagnosed for more than 2 years, to exclude any vascular complications. 5) Patients with T2DM associated with critical limb ischemia (CLI) according to Norgren et al.[23]

Sample Collection, Processing, and Storage

Each patient provided 10 mL of his or her blood after 12 hours of fasting. Each blood sample was divided into four portions; 1) 4 mL of blood was added into an EDTA tube for assessing glycated hemoglobin (HbA1C) and Complete blood count. 2) 4 mL was added to a serum gel separator tube and centrifuged, then serum was separated, 200 µl of separated serum was stored at −80°C for IL4 and IFN-γ measuring by enzyme-linked immunosorbent assay (ELISA) till the time of assay, while the rest of the serum was used for the measurement of diabetic biochemical parameters. 3) 2 mL blood was added to the heparin tube for flow cytometry assay.

Laboratory Investigations

Complete blood count (Sysmex KX-21, Japan), Hemoglobin A1C (D10, BioRad, France) and diabetic biochemical parameters by chemistry auto-analyzer device (Cobas Integra 400 plus, Roche diagnostics, Germany). Biochemical parameters included serum creatinine and lipid profile (total cholesterol (CHO), triglyceride (TG), low-density lipoprotein (LDL), high-density-lipoprotein (HDL) . Measuring of serum levels of cytokines human IL4 and IFN-γ by ELISA assay using Sinogeneclon Co., Ltd China. ELISA kits (ref. no. SG-10264, Lot. no. 202010) (ref. no., SG-10011. Lot. No. 202011), respectively, according to manufacturer instructions.

Measurement of TNK Cells Frequency in Peripheral Blood by Flow Cytometry Assay

Flow cytometry assay was performed using FACS Calibur (BD Biosciences, San Jose, CA). Cell Quest Pro software (BD Biosciences) was used for data analysis. Isotype control was used for positive cutoff detection. Two tubes were used with 50 µl of fresh blood sample each. 5 µl of cocktail of mouse-stained anti-human controls IgG1 FITC/IgG2a PE (catalog no.34240, lot no.90642) was added to the first tube. 5 µl of FITC-conjugated anti-human CD3/PE-conjugated anti-human CD16+CD56 cocktail (catalog no.95131, lot no.6012680. BD Biosciences, USA) was added to the second tube. All tubes were incubated for 20 minutes. Then, red blood cells (RBCs) were lysed, before sample washing using FACS buffer and centrifugation at 500 g. The identification strategy of TNK in three examples of patients' groups is illustrated in Figure 1.

Microbiological cultures for deep tissue samples and purulent fluids were performed for all DFI group patients. Both aerobic and anaerobic cultures were done on routine culture media and identification of grown colonies was performed using Vitek 2 (Bio-Mérieux, France).

Plain x-ray was done for suspected cases of osteomyelitis (cases with moderate and severe DFI).

Statistical Methods

Statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY) was used for data coding. Mean and standard deviation were used for data summarization in normally distributed quantitative variables or median and interquartile range for non-normally distributed quantitative variables. Frequencies and relative percentages were used for categorical variables. Comparisons between groups were done using unpaired *t*-test or analysis of variance (ANOVA) test in quantitative variables with normal distribution while non-parametric Kruskal–Wallis test and Mann–Whitney test were used for non-normally distributed quantitative variables. For comparing categorical data, Chi square (χ^2) test was performed. Spearman correlation coefficient was done for correlations between quantitative variables. ROC curve was constructed to detect the best cutoff value of TNK%, IL4, IFN-gamma, IFN-gamma/IL4 for detection of DFI. *P*-values<0.05 were considered statistically significant.

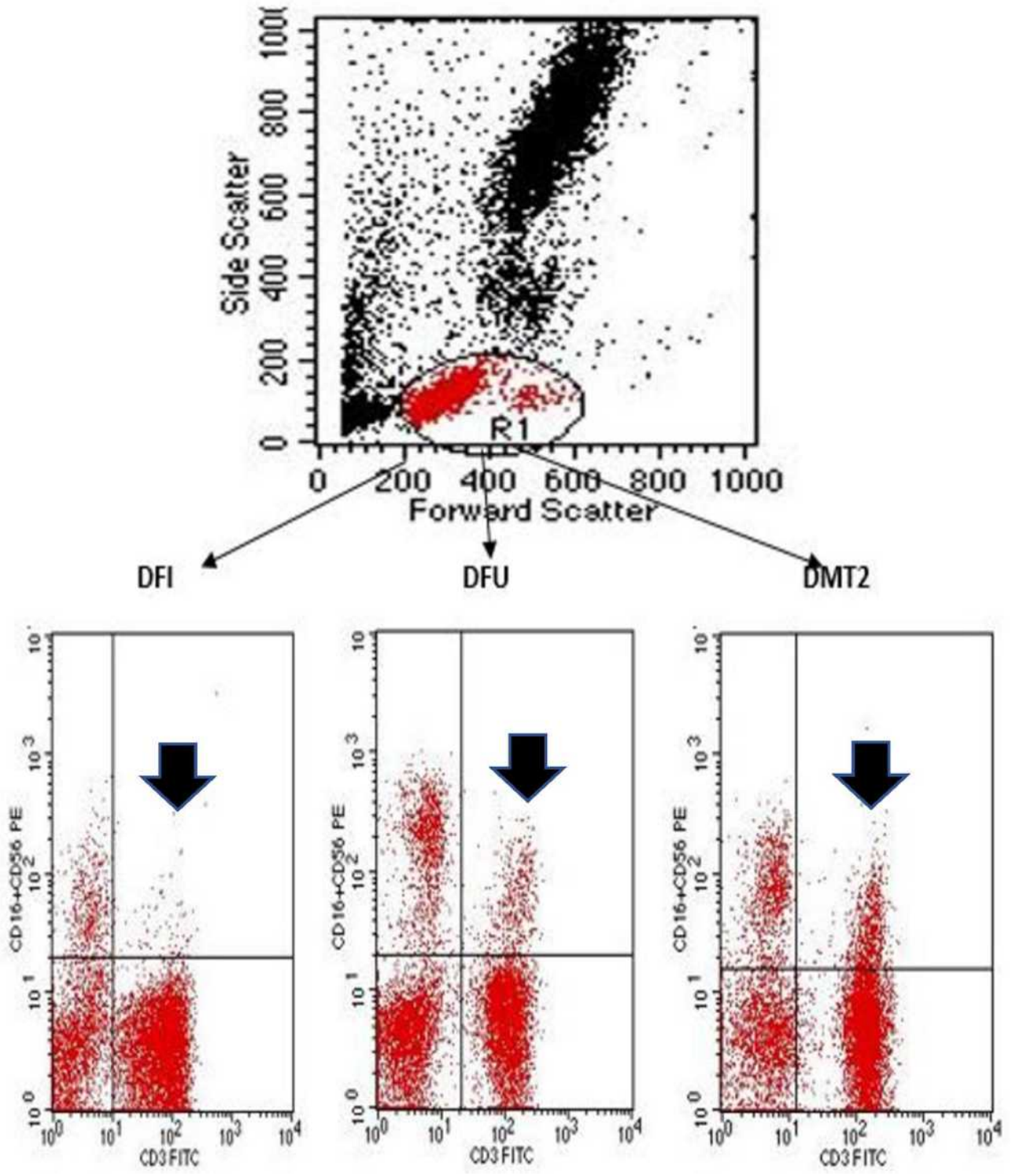

Figure 1 Gating strategy for TNK cells detection. Initial gating of lymphocyte region on forward scatter/side scatter (FS/SS) (R1). TNK were defined as cells co-expressing $CD16^{+}CD56$ and CD3 cells lying in upper right quadrant of the analysis plot, referred to by an arrow. An example of each group is illustrated.

Results

Ninety patients with diabetes type 2 with a mean±SD age of 51.7±11.51 years were included. Patients were divided into a DFI group (n=30), DFU group (n=30), and T2DM group without complications (n=30). In 100% of the patients, pedal pulses were palpable. In both DFU and DFI groups, ulcers were located below the ankle. No significant difference was seen between mean age values

between DFI and DFU groups (P=0.094). Demographic and clinical data of patients are shown in Table 1.

Comparative data between the three studied groups are displayed in Table 2.

Comparison of TNK% between DFI and DFU groups revealed a significant decrease in DFI group (P<0.001). Also, a comparison between DFI group and T2DM revealed a significant decrease in DFI group (P<0.001), while comparison between the DFU group and T2DM revealed a non-significant difference (P=0.082) (Figure 2A).

Comparison of IFN-γ/IL4 between the DFI group and DFU revealed a significant decrease in the DFI group (P=0.02), while comparison between the DFI group and T2DM group and comparison between the DFU group and T2DM revealed non-significant differences (P=0.357) and (P=0.748), respectively (Figure 2B).

Comparison of IFN-γ between the DFI group and the other two groups revealed a significant decrease in the DFI group (P<0.001). Comparison between the DFU group and T2DM revealed non-significant differences (P>0.999) (Figure 2C).

The DFI group showed a significant decrease in the IL4 level when compared to T2DM (P=0.006), while no statistical difference was shown in IL4 when compared to DFU (P=0.05). Also, IL4 level showed no significant difference when DFU was compared to T2DM (P>0.999) (Figure 2D).

Table 1 Demographic and Clinical Data of Study Participants

		Count	%
Gender	Females	37	41.1%
	Males	53	58.9%
Anti-diabetic drug	Insulin	60	66.7%
	Insulin + oral	1	1.1%
	Oral	29	32.2%
Comorbidities	HTN	22	24.4%
	HTN+IHD	5	5.6%
	IHD	8	8.9%
	NIL	55	61.1%
Ulcers grading in DFU	Grade 1	8	13.3%
	Grade 2	14	23.3%
Ulcers grading in DFI	Grade 3	20	33.3%
	Grade 4	18	30.0%
	Groups	Mean	SD
Age (years)	DFI	52.53	12.26
	DFU	58.23	10.39
	T2DM	44.33	6.86
DM disease duration/year	DFI	19.00	7.71
	DFU	15.23	5.72
	T2DM	1.84	1.74

Abbreviations: DM, diabetes mellitus; DFI, diabetic foot infection; DFU, diabetic foot ulcer; HTN, hypertension; IHD, ischemic heart disease.

Correlation Studies of Study Markers in All Study Participants (n=90)

Correlation of TNK% with both IL4 and IFN-γ revealed a significant correlation (r=0.385, P<0.001; and r=0.534; P<0.001, respectively).

Regarding the metabolic contribution of TNK%, correlation of TNK% with HbA1c and LDL revealed a significant negative correlation (r=−0.631, P<0.001; and r=−0.261, P=0.013, respectively) while a positive correlation was seen with HDL (r=0.287, P=0.006) (Figure 3). No significant correlation was seen with TG or CHO.

Regarding the metabolic contribution of IL4, correlation of IL4% with HbA1c revealed a significant negative correlation with both (r=−0.514, P<0.001, respectively), while no correlation was detected with HDL, LDL, TG, CHO. The IFN-γ correlation with HbA1c and LDL revealed significant a negative correlation with both (r=−0.369, P<0.001; r=−0.229, P=0.03, respectively), while no correlation was seen with HDL, TG, or CHO.

The TNK % showed a significant negative correlation with DM disease duration/year (r=−0.546, P<0.001). Also, IFN-γ level showed a significant negative correlation with DM disease duration/year (r=−0.338, P=0.001). While no significant correlation was seen with IL4 level and disease duration (r=−0.181, P=0.089).

The ROC curve output data of study markers as predictors of infection in diabetic ulcer are demonstrated in Table 3 and Figure 4.

Discussion

The relationship between the immune system and diabetes pathogenesis has attracted extensive attention.[24] DM is characterized by exhaustion of glycolytic ability due to prolonged hyperglycemia. Prolonged hyperglycemia leads to increased cytotoxic mediator secretion and inflammatory cytokines upon infection, a phenomenon known as diabetic chronic inflammation.[25]

Table 2 Comparative Laboratory Data Between Groups

	Group 1=DFI			Group 2=DFU			Group 3=T2DM			*P*-value
	Median	1st Quartile	3rd Quartile	Median	1st Quartile	3rd Quartile	Median	1st Quartile	3rd Quartile	
TNK%	2.65	1.70	4.00	6.00	5.00	8.00	8.25	6.50	10.00	<0.001*
IFN-gamma (pg/mL)	9.10	7.40	14.80	20.30	15.40	30.00	19.50	17.90	27.60	<0.001*
IL4 (pg/mL)	18.00	12.40	25.50	25.00	15.00	44.00	31.50	18.00	50.00	0.005*
IFN/gamma/IL4	0.53	0.40	0.82	1.20	0.39	1.90	0.74	0.39	1.40	0.025*
HbA1C %	12.05	9.80	16.20	10.65	7.00	12.30	7.05	6.40	8.00	<0.001*
TLC (10^3/μL)	9.00	7.00	13.70	9.55	7.00	11.60	7.65	7.00	9.00	0.093
PLT (10^3/μL)	306.50	251.00	423.00	290.50	220.00	363.00	251.50	219.00	310.00	0.039*
S. Creatinine (mg/dL)	0.88	0.70	0.96	0.73	0.59	1.01	0.72	0.64	0.86	0.199
S. LDL (mg/dL)	110.00	96.00	145.00	88.50	45.00	127.00	85.00	65.00	124.00	0.102
S. HDL (mg/dL)	31.00	26.00	35.00	30.35	25.00	40.00	39.50	34.00	49.00	0.001*
S. TG (mg/dL)	141.50	89.00	217.00	123.00	93.00	190.00	143.50	99.00	207.00	0.655
S. CHO (mg/dL)	149.50	111.00	191.00	136.00	112.00	183.00	186.50	167.00	223.00	0.003*

Note: *Statistically significant (*P*-value<0.05).
Abbreviations: TLC, total leucocyte count; HbA1C, glycated Hgb; S. TG, serum triglyceride; S. HDL, serum high-density lipoprotein; S. LDL, serum low-density lipoprotein; S. CHO, serum cholesterol; IFN-γ, interferon-gamma; IL4, interleukin-4, IL4; TNK, T-natural killer cell.

In the current study, comparison of TNK% in DFI and DFU groups revealed a significant decrease in TNK% in the DFI group. Also, the comparison between the DFI group and T2DM revealed a significant decrease in the DFI group while comparison between the DFU group and T2DM revealed non-significant differences. These findings highlight an immunoregulatory function for TNK cells which agreed with Gómez-Díaz et al,[26] who found that TNK abnormalities associate with type 1 diabetes progression. Van-Kaer and Wu[27] stated that invariant T natural killer (iTNK) cells comprise most of the TNK cells. They are important players in immune regulation as they promote self-tolerance, in addition to their cytotoxic properties. Tard et al[16] reported that iTNK cell defects are associated with T2DM development as iTNK are rapidly able to produce IL4 and IFN-γ after the stimulation of their TCR in addition to their cytotoxic ability and added that iTNK can induce anergy of pathogenic T-cells.

Comparison of IFN-γ between the DFI group and DFU and T2DM groups revealed a significant decrease in the DFI group (P<0.001). This finding agreed with the findings of Sunandhakumari et al [18] that IFN-γ is involved in triggering phagocyte-dependent inflammation through macrophage activation, which is responsible for complement fixation and opsonization. Also, Mahmoud et al[28] proved that declining of IFN-γ predicts infection in foot ulcer and added that IR shifts T-helper differentiation toward Th2 in the expense of Th1 which is concerned with cytotoxic response. Also, increased IL6 in DM suppresses IFN-γ gene expression during T-cell activation, which does not allow T1 differentiation.

Tsiavou et al[29,30] and Kartika et al documented the reduction in IFN-γ production in T2DM. Also, Schmohl et al[31] reported that IFN-γ was not detected in wound fluid extracted from DFU with or without infection, whereas 100% recovery was found for IFN-γ during wound recovery.

This low IFN-γ level detected in association with infection in DFI group can explain the increased susceptibility to local infection, as IFN-γ is concerned with boosting microbicidal functions through NADPH oxidase and nitric oxide (NO) synthase production. Both are major players in bacterial and fungal killing. Also, IFN-γ reinforces MHCII expression, which is crucial for Ag presentation.[32] Also, Foss-Freitas et al[33] reported lower IFN-γ levels in the T2DM group compared to the normal control and proposed that IFN-γ

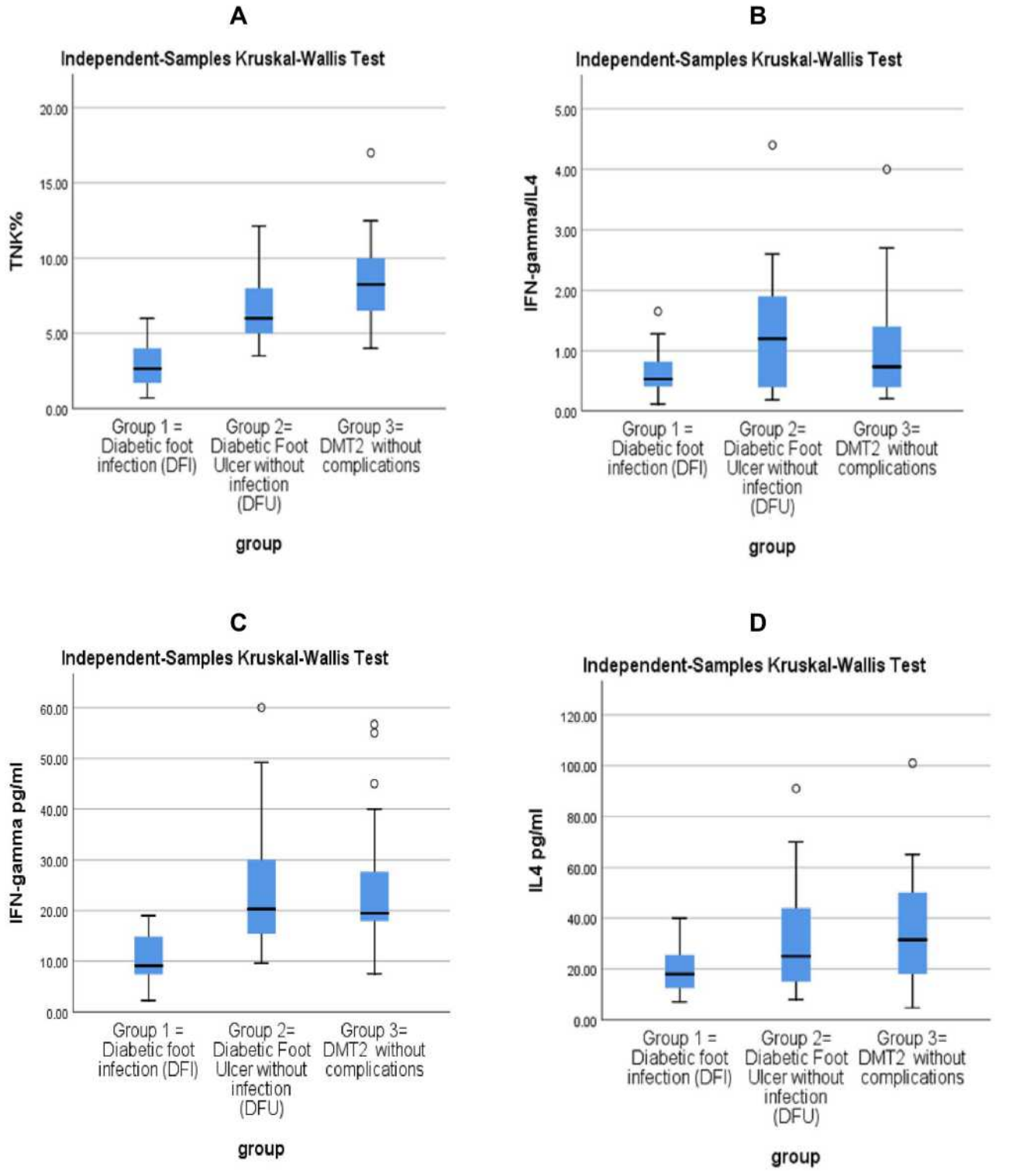

Figure 2 (**A**) Comparison of TNK% in studied groups. (**B**) Comparison of IFN-gamma/IL4 in studied groups. (**C**) Comparison of IFN-gamma in studied groups. (**D**) Comparison of IL4 in studied groups.

improves the capacity of granulocyte activation and phagocytic capacity, reducing the susceptibility to infections.

Comparison of IFN-γ between the DFU group and T2DM revealed non-significant differences. This was not in line with Theocharidis et al[34], who observed inhibition of IFN-γ in DFU when compared to T2DM due to dysregulation of biological processes that included cell movement of monocytes, migration of dendritic cells, and

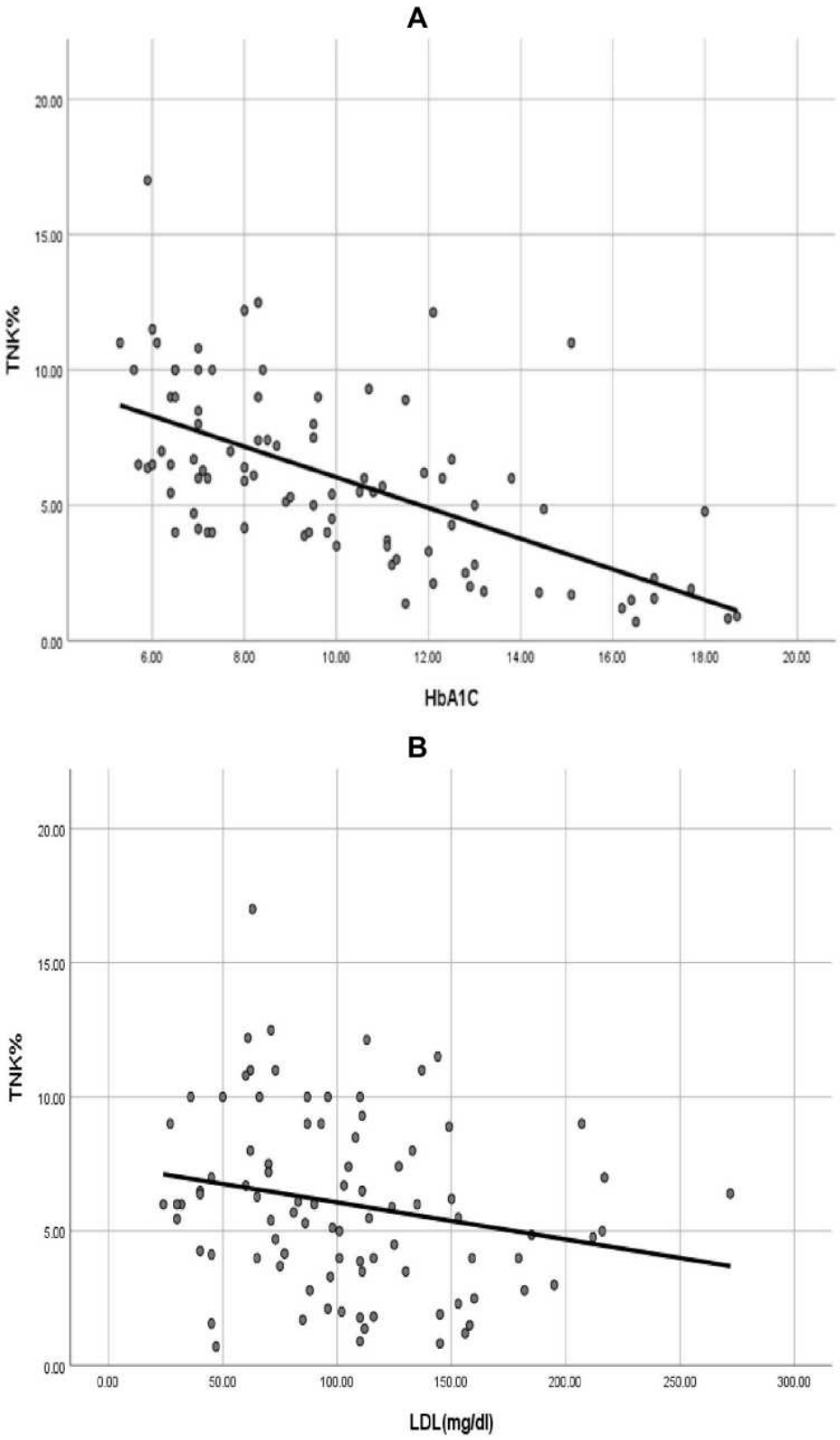

Figure 3 (**A**) Correlation of TNK% with HbA1c revealed a significant negative correlation (−0.631, *P*<0.001). (**B**) Correlation of TNK% with LDL revealed a significant negative correlation (r=−0.261; *P*=0.013).

Table 3 Output Data of ROC Curve for Discriminative Ability of Study Markers as Predictors of Infection in Diabetic Ulcer

	AUC	*P*-value	95% Confidence Interval		Cutoff	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
TNK%	0.946	<0.001*	0.894	0.997	4.065	83.3	93.3
IL4 (pg/mL)	0.684	0.007*	0.549	0.819	19.60	63.3	66.7
IFN-γ (pg/mL)	0.913	<0.001*	0.846	0.980	16.65	96.7	70
IFN-γ/IL4	0.707	0.004*	0.567	0.847	1.090	90	56.7

Note: *Statistically significant (*P*-value<0.05).
Abbreviations: IFN-γ, interferon-gamma; IL4, interleukin-4; TNK, T-natural killer cell.

chemotaxis of antigen-presenting cells pointing to an impaired migratory profile of immune cells in DM skin and proposed that up-regulation of IFN-γ is a sign of healing onset in DFU.

Also, Xu et al[35] suggested that IFN-γ may correlate with DFUs onset but reported a significant upregulation in IFN-γ extracted from the skin tissue surrounding the ulcer in DFU when compared to the control group, using Western blot analysis. This could be explained by differences in techniques used and sample analyzed for IFN-γ level as we tested its serum level using the ELISA technique.

The DFI group showed a significant decrease in IL4 level when compared to T2DM, while no difference was seen in IL4 when the DFI group was compared to the DFU group. IL4 is concerned with M2 macrophage stimulation.

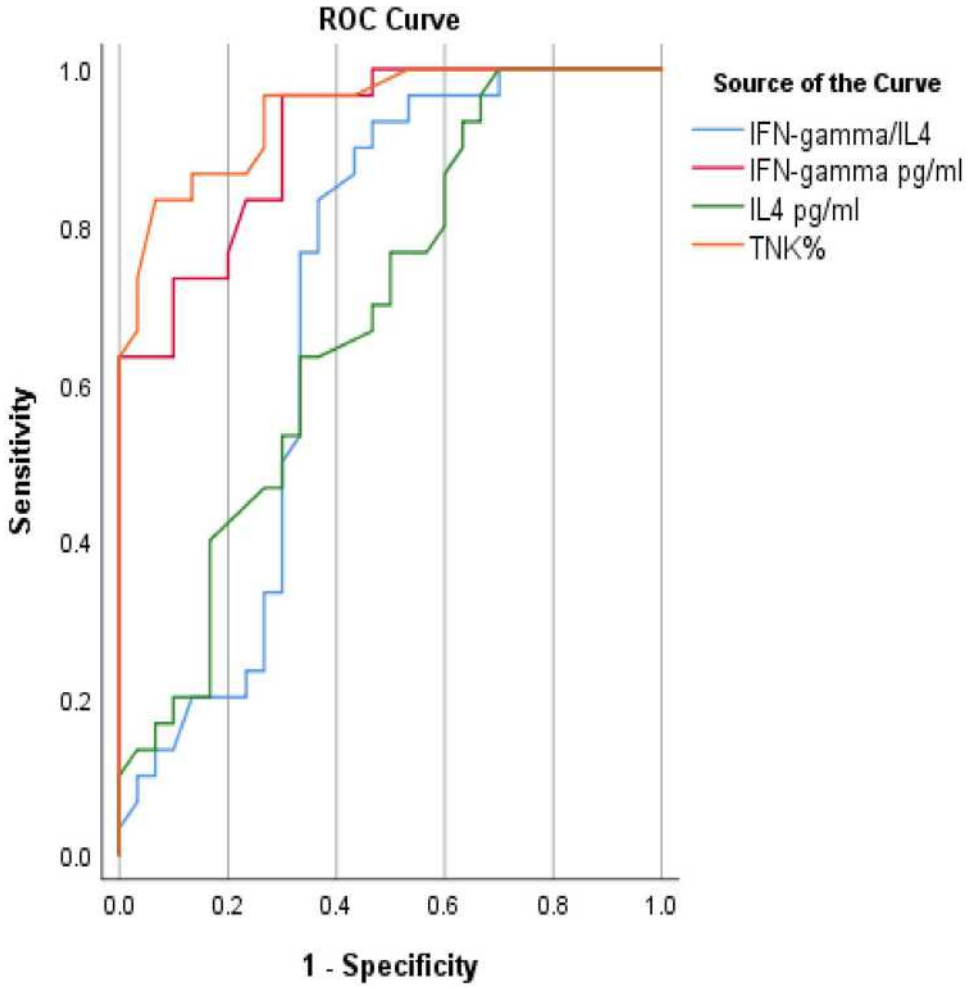

Figure 4 ROC curve for TNK%, IL4, IFN-gamma, IFN-gamma/IL4 as discriminators of infection between DFU and DFI.

M2 macrophages enhance insulin sensitivity, while M1 enhance IR.[36] Also, the decrease of TNK cells in DFI also enhances the M1 phenotype switch.[37]

Comparison of IFN-γ/IL4 between the DFI group and DFU revealed a significant decrease in the DFI group while comparison between the DFI group and T2DM and comparison between the DFU group and T2DM revealed non-significant differences (Figure 2B). These findings highlight the role of IFN-γ/IL4 ratio in the development of infection in foot ulcers. As all cases of DFI had ulcer grades of 3 or 4 while DFU had ulcer grades of 1 or 2, this ratio may also play a role in ulcer grade progression.

The TNK % showed a negative correlation with DM disease duration/year. Correlation of TNK% with HbA1c and LDL revealed a significant negative correlation, while a positive correlation was seen with HDL (Figure 3). These observed findings strengthen the theory of the regulatory role of TNK cells in T2DM.

Correlation of serum level of IL4 with HbA1c revealed a significant negative correlation. These data agreed with that of Yang et al,[38] which stated that the IL4 improves insulin sensitivity and glucose tolerance. This regulatory role was attributed to the ability of IL4 to suppress the production of the cytokines enhancing the IR as IL-6 and TNF-α.[39]

Surprisingly, IFN-γ revealed a significant negative correlation with HbA1c and LDL. This was in contrast to Kartika et al[30], who demonstrated that IFN-γ is known to stimulate proinflammatory macrophage of M1 phenotype which potentiates the development of T2DM.

Correlation of TNK% with both IL4 and IFN-γ revealed a significant positive correlation. This agreed with Tard et al[16], who reported that under the stimulation of antigens, TNK can interact with immune systems through the production of IL4 and IFN-γ cytokines.

The TNK % and IFN-γ level both showed a significant negative correlation with DM disease duration/year ($r=-0.546$, $P<0.001$; $r=-0.338$, $P=0.001$, respectively). This could highlight the link between TNK % and its secreted IFN-γ along DM progression with time.

Output data of ROC curves demonstrated in Table 3 and Figure 4 reveled that downregulation of TNK% and IFN-γ level have a role in occurrence of infection in DFU with IFN-γ being the more sensitive, which could be attributed to the protective role of IFN-γ in reducing the susceptibility to infections, as mentioned above.[33] While TNK% are more specific, these given results introduce both TNK% and IFN-γ as potential immune therapy agents.

Study Limitations

Due to funding limitations the current study did not assess the level of intracellular production of IL4 and IFN-γ cytokines in TNK using flow cytometry to ascertain that their origin is the TNK. This point should be covered in future studies. Still, the presence of a positive correlation between the TNK% and both cytokines determine the relationship between them and linking TNK mediated T2DM pathogenesis and progression of DFI, through the production of IL4 and IFN-γ cytokines. Also, due to a lack of financial resources, frequency of TNK after appropriate management of infection was not studied, which should also be covered in future studies.

Conclusion

1) Decline in TNK frequency plays a role in T2DM pathogenesis and augmentation of subsequent foot complications.

2) Downregulation of TNK% and IFN-γ level have a potential role in occurrence of infection of diabetic ulcer with IFN-γ being the more sensitive, with TNK% being more specific.

3) Altered IL4 level has less role in augmentation of infection in foot ulcer than IFN-γ.

4) The TNK% and IFN-γ are downregulated in T2DM in a disease duration dependent manner.

5) The role of IFN-γ level downregulation is more sensitive and specific in augmentation of foot ulcer infection than altered IFN-γ/IL4 ratio.

6) The TNK and IFN-γ are potential agents for immune therapy to prevent foot complications in T2DM.

Disclosure

The authors declare that they did not receive any fund for this work and no conflicts of interest.

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