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Cytokine profiling for Egyptian diabetic patients with nephropathy

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Abstract

Background

Evidence suggests that poor glycemic control is significantly associated with the development of microvascular complications of diabetes such as nephropathy. Studies have indicated that interleukin 17 (IL-17), interferon gamma-inducible protein 10 (IP-10), and IL-10 are important risk factors for nephropathy. This study aimed to evaluate the cytokine profile for diabetic patients with nephropathy.

Patients and methods

The study included 72 diabetic patients who were divided into two groups. Group 1 included diabetic patients without complications with an albumin creatinine ratio of less than or equal to 30, while group 2 included 36 diabetic patients with an albumin creatinine ratio more than 30 and 28 subjects as a control group. Two diabetic groups were divided into two subgroups according to C-peptide levels into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Serum samples were collected and tested for C-reactive protein, IL-17, IL-10, and IP-10 by ELISA.

Results

The IL-17 and IP-10 levels were significantly increased in type 2 diabetic nephropathy patients compared with controls, but IL-10 levels were significantly increased in diabetic patients with T2DM compared with controls and T1DM. There was a significant increase for nephropathy T1DM patients than non-nephropathy patients.

Conclusion

Monitoring of cytokines help evaluate the immune status inflammation of diabetic nephropathy patients.

Keywords: Diabetes mellitus, interleukin 10, interleukin 17, interferon gamma-inducible protein 10, nephropathy

Introduction

Diabetes nephropathy (DN) is historically regarded as a nonimmune disorder; rising evidence shows that inflammatory mechanisms play a crucial function in disease pathogenesis and progression. Indeed, the degeneration of renal characteristics in diabetic patients with proteinuria is related to tubulointerstitial inflammation. Therefore, investigations into the mechanisms underlying intrarenal inflammation may also provide new therapeutic goals for anti-inflammatory techniques toward DN [1].

Clinical DN is predicted by microalbuminuria, which is a strong predictor of the development of a reversible cahange but can lead to kidney failure if neglected. Therefore, early diagnosis can help prevent the kidney disease from getting worse [2]. Identification of the T-helper 17 (Th17) group not only alters the classical Th1/Th2 paradigm in T-cell immune responses but also gives fresh insights into the pathophysiology of various autoimmune disorders [3].

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Type 1 diabetes mellitus (T1DM), one of the most common autoimmune disorders generally assumed to be mediated by Th1 cells, is now being linked to Th17 cells [3]. Th17 cells mainly generate cytokine interleukin (IL)-17A (also known as IL-17), and IL-17-C-reactive protein (CRP) signaling may have a role in chronic inflammatory diseases [3,4]. Th17 cells play an important role in the development of T1DM [5].

Peripheral blood cluster differentiation 4+ (CD4+) T-cells from children with newly diagnosed T1DM generates higher levels of IL-17 and IL-22, as well as enhanced RORC2 and FoxP3 gene expression [5], while there was no evidence of elevated interferon gamma (IFN-γ) level expression in T1DM patients [3]. A higher number of IL-17-producing CD4 + T cells were also determined in children with newly diagnosed T1DM [6]. More critically, when circulating CD4+T cells were activated by β-cell autoantigens and GAD65 peptides, they generated IL-17 in T1DM patients [3]. Increased IL-17 levels in T1DM may be attributable to the existence of a proinflammatory cytokine milieu that promotes Th17 differentiation [7].

Indeed, T1DM patients' monocytes spontaneously release significantly higher amounts of IL-6 and IL-1, which increase IL-17 production by memory CD4+T cells. In response to polyclonal stimulation, CD4+T cells with a memory phenotype from T1DM patients' pancreatic-draining lymph nodes secrete more IL-17 but not IFN-γ or IL-4. Furthermore, in response to diabetes-related antigens, proinsulin, and GAD65, these pancreatic-draining lymph nodes memory CD4+T cells produce more IL-17 [8]. The importance of IL-17A not only as a primary cytokine in autoimmune diseases but also raises concerns about the function of cytokines in the pathophysiology of T2DM and its consequences [9].

IL-17 played a critical part in the pathophysiology of insulin resistance generated by angiotensin 2 type 1 receptor-ligand interaction, which resulted in an increase in the formation of renal nitric oxide (NO) in DN and activation of metalloproteinase. IL-17 activated JAK1, JAK2, PI3K, and NF-ƙB pathways through their respective receptors. It has been shown that STAT3 regulates IL-17 signaling, and JAK2/STAT3/SOC-1 signaling is crucial to hepatic insulin resistance because STAT3 regulates β-cell apoptosis so that IL-17 induces fibrosis and apoptosis in liver cells [9].

In addition to IL-17A and IL-17F, Th17 cells produce IL-22, which activates many of the same innate inflammatory genes. There is evidence that IL-17 and IL-22 work cooperatively or synergistically to induce inflammatory gene expression, especially in epithelial cells. Furthermore, IL-17 is produced by different cell types other than CD4+Th17 cells. In addition to CD8+ and CD4+T cells, NKT cells are also important sources of IL-17 [10]. Th17 cells can be directly influenced by IL-10 [11]. It has now been demonstrated that IL-10 is an anti-inflammatory and immunosuppressive cytokine that can inhibit the synthesis of many cytokines, including IL-6, IL-1α, IL-1β, and tumor necrosis factor-α in activated macrophages and IFN-γ by T cells [12]. In humans, the gene encoding IL-10 can be found on chromosome 1q31-1q32, which has five exons and four introns. IL-10 is associated with the degree of renal damage in DN, so that IL-10-1082A/G polymorphism may increase the risk of DN [12].

Mesangial and endothelial cells express IL-10, which acts as an autocrine growth factor. IL-10 promotes the proliferation of these cells by inducing the production of growth factors, cytokines, and chemokines. The result is structural changes within the glomerulus and tubulointerstitial changes, such as cell hypertrophy, thickening of the basement membrane, mesangial matrix accumulation, and overt proteinuria. These pathological changes, in turn, lead to renal failure and end-stage renal disease (ESRD). Because IL-10 controls inflammation, it seems paradoxical that the low expression of IL-10 could be detrimental to renal function, considering that the loss of anti-inflammatory protection would result in renal dysfunction [13,14].

In DN patients, interferon gamma-inducible protein 10 (IP-10) mRNA was overexpressed in tubulointerstitial compartments of the renal tissues. In addition, human mesangial cells expressed CXCR3 and IP-10, which stimulated cell proliferation. IP-10 might be involved in the pathogenesis of DN [15].

Patients and methods

A total of 72 DM patients were recruited from the National Institute for Diabetes and Endocrinology 'NIDE,' Cairo, Egypt and 28 control subjects of comparable socioeconomic, age, and sex were selected from the relatives of the diabetic patients. All subjects gave informed written consent for participation and the study was approved by the ethics committee of the General Organization for Teaching Hospitals and Institutes (GOTHI) (no. IDE00218).

Subjects were classified into three main groups:

(1) Control group: a population of 28 healthy individuals with CRP of less than 6, normal fasting plasma glucose (FPG), and normal kidney function test.

(2) Normoalbuminuric group: a population of 36 diabetic patients without complications; this group was divided into two subgroups according to C-peptide:
   (a) Group 1a: 18 T1DM patients without DN.
   (b) Group 1b: 18 T2DM patients without DN.

(3) Nephroalbuminuric group: a population of 36 diabetic patients with nephropathy complications. This group was divided into two subgroups according to C-peptide:
   (a) Group [2a: 18 T1DM patients with DN.
   (b) Group 2b: 18 T2DM patients with DN.

All patients and controls were subjected to full history which included age, sex, diabetic duration, family history of DM, and current daily oral antidiabetic regimen or any current medications. BMI was calculated as (weight kg)/(height m^2) [16].

Exclusion criteria for subjects' selection:

(1) Obese patients (BMI ≥30).
(2) Patients have other complications such as retinopathy, neuropathy, or diabetic foot.
(3) Patients have HCV, HBV, HIV, CMV, and EBV.
(4) Patients have other chronic diseases such as ESRD, CVD, cancer, neither smokers nor alcoholics, nor hypertensive nor hyperlipidemic.
(5) Not receiving any medications affecting the endocrine–metabolic system or immunological system (e.g. anti-thyroids, glucocorticosteroids, etc.).
(6) Patients have systolic blood pressure/diastolic blood pressure less than 135/85.

Sample collection

Whole blood and serum samples were collected in the morning from fasting patients, 8 h first drawn and 12 h second drawn. Two serum tubes were separated by centrifugation at 5000 rpm for 5 min, and the first tube was frozen at −20°C, and blood samples were collected with K3-EDTA tubes for glycosylated hemoglobin (HbA1c) determination. Na-fluoride containing tubes were used for FPG determination and, finally, urine samples were collected for the determination of albumin creatinine ratio and albuminuria.

Biochemical analysis

Serum was used to assess urea, creatinine, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, triacylglycerol, and CRP. These measurements were performed using standard methods on the BT 3500 (Biotechnica Instruments SPA, Italy, Venice Rome TOSOH Corporation, Japan, Tokyo). The EDTA containing whole blood samples were used to measure HbA1c by the chromatographic method on the TOSOH G8 G8 Hemoglobin testing system (TOSOH Corporation, Japan). First-morning urine samples were obtained from the patients and centrifuged. The supernatants were used to measure albumin and creatinine. Urinary albumin was quantified using an immunoturbidimetric assay on the BT 3500 automated analyzer. The results were expressed as the albumin creatinine ratio as a tool to match the levels of albumin by the concentration of urine [17]. The estimated glomerular filtration rate (eGFR) was calculated using the creatinine equation obtained from the chronic kidney disease epidemiology collaboration [18].

Immunological parameters

Frozen −20°C sera were used to determine insulin, C-peptide, IL-17, IL-10, and IP-10 by using commercial ELISA kits (R&D Systems Inc., Minneapolis, Minnesota, USA). All tests were performed according to the instructions recommended by the manufacturer.

Statistical analysis

Statistical results were analyzed using the mean and SE. Analysis of variance with post Tukey test and the categorical data were expressed as percentages, and differences between the groups were compared using the χ^2 test. All data were presented in the form of mean ± SE. Statistical analysis was performed using the statistical software package Statistical Package for the Social Sciences (SPSS), version 23, 2016 (SPSS Inc., Chicago, Illinois, USA) for Windows and Graphpad (prism), version 14.

Results

Demographic data

The serum samples for this study belonged to the following groups, as summarized in Table 1.

The samples have been drawn from patients with normal blood pressure and BMI less than or equal to 30. There is no significant difference between groups for demographic data; *P* value is 0.5. Patients and controls have been selected with normal blood pressure, but the results showed that there was an increase significantly for DN patients than DM without complications.

Biochemical test

There was a statistical difference between the diabetic patient groups and the control groups regarding HbA1c and FPG. Table 2 shows a statistically significant difference between the different patients' groups for HbA1c and FPG compared with the control group. There was a significant decrease for C-peptide in T1DM groups compared with T2DM groups. There was a statistical difference between the diabetic patient groups and the healthy group as regards lipid profile and from the above data, there was a statistically significant difference in the mean of cholesterol, triglycerides, and HDL for the T2DM patients' groups with and without nephropathy compared with the healthy control. There was a statistically significant difference in low-density lipoprotein in T2DM without complications in the patient groups compared with the healthy control, but there was a nonsignificant difference for T2DN patients. Finally, there was a nonsignificant difference between the T1DM patient groups and the control group for lipid profile except for HDL for T1DN patients and there was a significant difference compared with the control group.

Kidney function test

The groups were rearranged according to DM types to T1DM and T2DM.

Table 3 shows the statistical difference between the diabetic patient groups and the healthy group as regards the kidney function test and from the above data, there was a statistically significant difference in creatinine and eGFR between T1DM and T2DM with nephropathy patient groups compared with the control group and also when comparing T1DM and T2DM with nephropathy patient groups with T1DM and T2DM without nephropathy patient groups a highly significant difference was observed.

Immunological markers

Table 4 shows the comparison of serum IL-17 between the diabetic patient groups and the control group. From the above data, the mean levels of IL-17 did not change significantly in all studied patient groups except group 2a and group 2b

Table 1: Demographic characteristics of the studied subjects

Groups	Control subjects (*n*=28)	Diabetes patients without complications normoalbuminuric (*n*=36)	Diabetes patients with nephropathy nephroalbuminuric (*n*=36)	*P*
Age (years) (mean±SE)	32.9±4.4	31.1±3	30.7±2.9	0.9
Duration of diabetes (years) (mean±SE)	-	6.2±0.9	7.5±0.73	0.3
Sex [*n* (%)]				
Male	12 (42.8)	12 (33.3)	15 (41.7)	0.9
Female	16 (57.2)	24 (66.7)	21 (58.3)	
BMI (kg/m^2) (mean±SE)	24.8±1.1	24.2±0.9	25.8±0.72	0.3
SBP (mmHg) (mean±SE)	120±0.4	116±1.9	128.7±1.85	0.001
DBP (mmHg) (mean±SE)	80±0.2	76.3±1.2	83.5±1.1	0.001

DBP, diastolic blood pressure; SBP, systolic blood pressure

Table 2: Biochemical laboratory investigations in normoalbuminuric diabetes mellitus and nephroalbuminuric diabetes mellitus subgroups compared with controls

Groups	Control subject (*n*=28)	Normoalbuminuric DM (*n*=36)		Nephroalbuminuric DM (*n*=36)		*P*
		T1DM without nephropathy group 1a (*n*=18)	T2DM without nephropathy group 1b (*n*=18)	T1DN with nephropathy group 2a (*n*=18)	T2DN with nephropathy group 2b (*n*=18)	
FPG (mg/dl)	92±2.4	259±39.2[a]	159±15.8	250±31.8[a]	236±17.1[a]	0.001
HbA1c (%)	5.7±0.16	10.8±0.7[a]	8.5±0.5[a]	11.6±0.57[a]	9.9±0.6[a]	0.001
HOMA-IR	1.2±0.1	9.3±1.9[a]	4.9±0.63	9.3±2[a]	10.3±1.9[a]	0.002
C-peptide (ng/dl)	2.6±0.12	0.25±0.03[a]	1.9±0.4[ab]	0.3±0.03[a]	1.9±0.4 [ac]	0.001
SGOT (U/l)	25.8±1.01	24.9±1.07	25.1±0.6	25.5±0.8	24.2±0.7	0.8
SGPT (U/l)	24.3±0.9	26±1.7	24.6±0.9	24±0.7	25.6±1.5	0.8
TC (mg/dl)	179±9.7	191±7.1	220.1±9[a]	193±11	222±6.3[a]	0.006
TG (mg/dl)	88±11.7	84±7.3	135.9±12.8[ab]	100.3±6.5	140±10.9[ac]	0.008
LDL-C (mg/dl)	116±8.8	119.1±7.6	150.2±2.7[ab]	119.9±2.7	143.6±1.5	0.01
HDL-C (mg/dl)	53±3.1	52.28±2.9	40.33±7.3[ab]	45.33±5.3[a]	40.3±7[a]	0.009
CRP (mg/l)	3.8±0.5	10±2.3[a]	10±2.2[a]	10±2.2[a]	11±2.7[a]	0.04

CRP, C-reactive protein; DM, diabetes mellitus; DN, diabetes nephropathy; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride.,[a]Significant between controls and diabetic patient groups.,[b]Significant between group 1a and group 1b. [c]Significant between group 2a and group 2b.

Table 3: Kidney functions test in patients with type 1 diabetes mellitus and type 2 diabetes mellitus compared with the control group (mean±SE)

Groups	Control subjects (*n*=28)	Type 1 diabetic T1DM (*n*=36)		Type 2 diabetic T2DM (*n*=36)		*P*
		Without complication group 1a (*n*=18)	With nephropathy group 2a (*n*=18)	Without complication group 1b (*n*=18)	With nephropathy group 2b (*n*=18)	
Creatinine (mg/dl)	0.9±0.06	0.87±0.02	1.3±0.04[a]	0.93±0.04	1.4±0.06[a]	0.001
P value between DW and DN		0.001		0.001		
Urea (mg/dl)	28±1.9	27.2±1.9	30.5±1.8	26.6±2.2	33.2±2.4	0.1
P value between DW and DN		0.8		0.2		
eGFR ml/min/1.73 m^2	104±9.8	110±4.3	67±2.1[a]	90±5.6	57.2±1.5[a]	0.003
P value between DW and DN		0.001		0.001		
Albumin urea mg/24 h	15.4±3.1	11.3±1.9	331±49.2[a]	12.6±2	406±48[a]	0.001
P value between DW and DN		0.008		0.001		
ACR (mg/g Cr)	13.1±2.4	11.6±1.9	275±61.8[a]	10.4±2.1	284±29[a]	0.001
P value between DW and DN		0.001		0.001		

ACR, albumin creatinine ratio; DN, diabetes nephropathy; eGFR, estimated glomerular filtration rate; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.,[a]Significant between control and diabetic patient groups.,[b]Significant between group 1a and group 1b.,[c]Significant between group 2a and group 2b.

compared with the control group and serum IL-10 between the diabetic patient groups and the healthy group. From this data we noticed that the T1DM patients without nephropathy marked the lowest IL-10 level. There was a significant

Table 4: Immunological marker in type 1 diabetic patients and type 2 diabetic patients compared with the control group (mean±SE)

Groups	Control subjects (*n*=28)	Type 1 diabetic (*n*=36)		Type 2 diabetic (*n*=36)		*P*
		Without complication group 1a (*n*=18)	With nephropathy group 2a (*n*=18)	Without complication group 1b (*n*=18)	With nephropathy group 2b (*n*=18)	
IL-17 pg/ml	111±3.9	144±8	160±9.3[a]	151±7.7	168±16.1[a]	0.006
P value between DW and DN		0.8		0.8		
IL-10 pg/ml	131±11.4	78.3±4.4	196±7.2	215±14.6[ab]	284±33.1[ac]	0.001
P value between DW and DN		0.000		0.049		
IP-10 pg/ml	87.4±3.5	195±21.4[a]	262±21.2[a]	148±9	179±19.6[ac]	0.001
P value between DW and DN		0.06		0.7		

DN, diabetes nephropathy; IL-10, interleukin 10; IL-17, interleukin 17; IP-10, interferon gamma-inducible protein 10.,[a]Significant between control and patient groups.,[b]Significant between group 1a and group 1b.,[c]Significant between group 2a and group 2b.

difference between group 1a and group 2a and also between group 1b and group 2b. In addition to this data, there was a significant difference between the control group compared with group 1b and group 2b. Besides that, there was a significant difference between group 1a and group 1b and also between group 2a and group 2b. In the comparison of serum IP-10 between the diabetic patient groups and the healthy group, there were significantly increased levels of IP-10 in all studied patient groups except group 1b compared with the control group. From the above data, there was a significant increase between group 2a and group 2b, which means in the T1DM nephropathy patients IP-10 significantly increased than in T2DM nephropathy patients.

IL-17, IL-10, and IP-10 have diagnostic values for diabetic patients with nephropathy.

Discussion

DN is one of the most DM complications; it is increased protein excretion in urine. DN has been developed due to functional and structural changes. These changes occur because metabolic and hemodynamic abnormalities in diabetes interact with each other and pathways linked to reactive oxygen species. Early stage is characterized by a small increase in urinary albumin excretion that progresses to macroalbuminuria at the renal function test [19]. GFR starts to decline due to diabetic glomerulopathy and is known to occur in both type 1 and type 2 patients [20]. According to Lemann *et al.* [21], who eGFR based on creatinine, GFR is a good estimate among patients with other kidney diseases and for DN patients, so that GFR does not specify DN early. GFR declined leading to the progression of the ESRD. Regarding serum creatinine, a highly significant difference among the studied groups was observed, where it was higher in albuminuric patients than in normoalbuminuric patients.

Kidney inflammation is promoting DN development and progression[1] so that CRP was measured here. Arık *et al.*[22] and Elhefnawy *et al*.[23] showed that there was a highly significant difference regarding CRP among their study's groups, where it was higher in the macroalbuminuric and microalbuminuric group than in the normoalbuminuric group. CRP, which is a nonspecific marker of inflammation [24], has been reported to be associated with the risk of DM complications. However, our results showed a nonsignificant difference in CRP level between the microalbuminuria, macroalbuminuria group and normoalbuminuria group for T1DM and T2DM, so we are profiling other inflammatory markers. On the other hand, there was a significant difference in CRP between T1DM or T2DM patient groups and healthy control groups.

Inflammatory reactions are highly correlated with the increase in oxidative stress[25] by increases in oxidative stress and increases in the production of inflammatory cytokines and likewise, an increase in inflammatory cytokines can stimulate the production of free radicals [26].

One of these inflammatory cytokines is IL-17 which is produced by both CD4+ and CD8+T cells [27]. According to DN for T1DM and T2DM, our study proved that there is a significant difference in IL-17between DN patients for T1DM or T2DM and healthy controls, which agreed with Shiuchi *et al.* [28], who interpreted that IL-17 mediates the immune response by triggering the production of other proinflammatory cytokines. In the inflammatory condition of T1DM and T2DM patients, such as DN, the Treg/Th17 balance is shifted toward Th17 cells, promoting inflammatory response [29,30]. IL-17 may contribute to the accelerated progression of diabetic microvascular complications. It was shown that under inflammatory conditions, the suppressive function of T-regs may be lost, and these cells start to produce IL-17 [31,32].

Matsumoto and Kanmatsuse[33] found increased urinary excretion of IL-17 in nephrotic patients, and Klimontov *et al.*[34] proved that IL-17 significantly increased for albumin urea chronic kidney disease chronic kidney disease patients than normal controls. Our results agree with Parhi *et al.* [35], who thought that IL-17 is a contributory factor to the inflammatory process in T2DM and its complications.

Sumarac-Dumanovic *et al.* [36], Ohshima *et al.* [37], Santalahti *et al.* [38], and Demir *et al.*[39] demonstrated that IL-17 played a crucial role in the pathogenesis of metabolic syndrome and

insulin resistance induced by angiotensin II type 1 receptor. It has been evidenced that angiotensin II type 1 receptor/ligand interaction leads to an increase in the production of renal NO in DN. NO production, as an active free radical, results in renal tissue damage [40]. Hence, it seems that IL-17A induced type 2 complications through the induction of free radical production and induced reactive oxygen species [41].

There were controversial results about the serum levels of IL-17 and the severity of renal damage. Interestingly, circulating IL-17A levels were diminished in T2DM patients with or without DN when compared with normal glucose-tolerant subjects [42]. On the other hand, Arababadi *et al*. [43], Surendar *et al.* [44], and Mohamed *et al.* [45], and Tjahjono *et al.*[46] found that plasma and urine IL-17 significantly decreased for DN patients for T1DM and T2DM than control. Although the previous studies and our study found a relation between IL-17 and DN, Phoksawat *et al.*[47] proved that there is a nonsignificant difference for IL-17 of CD3, CD56 in DN patients compared with controls.

Marwaha *et al.* [6], Arababadi *et al.* [43], Arif *et al.* [48], Zareian and Dizgah [49], Li *et al.* [50], and Baharlou *et al*.[51] found that there was a significant increase for IL-17 between DM groups for T1DM or T2DM and healthy controls, this result has disagreed with our study. Roohi *et al*.[52] observed no significant difference between cases and controls, and there is a lack of evidence for the role of Th17 cells in T1D and T2D systemic inflammation. These suggested that serum IL-17 levels were not affected in diabetic patients and we agreed with these results in our study. We found that, although the level of IL-17 increased in T1D or T2D patients without DN than the healthy control group, there is a nonsignificant difference between T1D or T2D without DN and healthy controls. Sumarac-Dumanovic *et al.*[53] and Jagannathan-Bogdan *et al*.[54] found that blood concentration of the proinflammatory Th17 cytokine IL-17 in newly diagnosed patients with T2D decreased after 3 months of the therapy consisting of lifestyle modification and metformin. While confirming the recent findings on the increase in the activity of the Th17 response in T2D, both lifestyle changes and/or metformin displayed a positive effect on weight control in diabetic patients by a reduction of BMI.

One of the other important inflammatory markers related to DM is IL-10; in this study, IL-10 increased with T1DM nephropathy patients, which agreed with Mysliwska *et al.* [55], who correlated elevated IL-10 concentrations with DN in T1DM. We reported in this study the association of elevated IL-10 concentrations with DN in T2DM than T2DM without nephropathy, which agreed with the many other studies [56–61].

In addition, Inal *et al.*[62] proved that IL-10 might play an important role in the pathogenesis of T2DM; Li *et al*.[63] found that DN patients express a lower level of serum IL-10. IL-10 induces the proliferation of mesangial cells, which occupies a central position in the renal glomerulus. This leads to structural intraglomerular and tubulointerstitial changes, such as cell hypertrophy, thickening of the glomerular basement membrane, mesangial matrix accumulation, and overt proteinuria. These pathological changes, in turn, lead to renal failure and ESRD [56].

IL-10 plays a key anti-inflammatory role in regulating the immune system and cellular activities, and IL-10 effectively prevents the expression and production of proinflammatory cytokines [64]. The higher level of circulating IL-10 may be clarified the long bearing of the disease, the fairly well-conserved renal function of DN patients. The immoderate production of IL-10 in DN patients may indirectly engage the development of DN [60]. The increased concentration of IL-10 in serum samples from patients with DN seems to depend on the severity of the nephropathy by examining the level of circulating IL-10 and relating it to the grade of albuminuria in DN patients due to T1DM [55].

Ma *et al.* [65], Peng *et al.* [66], Erdogan *et al.* [67], and Ezzidi *et al*.[68] suggest that the IL-10-1082G/A polymorphism is correlated with the development of DN. Studies with a larger sample size are required to confirm the role of IL-10 polymorphisms in the risk of developing DN.

In line with Geerlings *et al.* [69], Foss-Freitas *et al.* [70], and Jasem [71], in this study we did not find significant differences in the production of IL-10 between T1DM and normal individuals.

Here, we disagreed with Saleh [72], who observed that IL-10 cytokines are important in the outcome of autoimmune diabetes, which was indicated by a significant elevation of serum levels in T1DM patients than controls but Rapoport *et al*.[73] and Wulandari *et al.*[74] observed that IL-10 decreased in T1DM than in normal controls. Exel *et al.*[75] found that there is an association between IL-10 low production and high serum glucose in T2DM patients.

However, Foss-Freitas *et al*.[70] and Gupta *et al.*[76] observed a nonsignificant difference between T2DM and controls or nonglycemic tolerance subjects in IL-10 levels for type 2 diabetic patients than controls, which disagree with our results. Al-Shukaili *et al.* [77], Francisco *et al.* [78], and Randeria *et al*.[79] agree with our results that there is a significant increase in IL-10 production in type 2 diabetic patients compared with healthy controls.

Finally, in this study cytokine was detected the serum IP-10; their concentrations were increased in T1DM with or without nephropathy as compared with appropriate controls. Both Shimada *et al.*[80] and Nicoletti *et al.*[81] reported elevated serum IP-10 levels in recent-onset adult T1DM and Devaraj and Jialal[82] confirmed increased circulating levels as well monocytic levels of IP-10 in T1DM with and without microvascular complications compared with controls. Shigihara *et al.*[83] reported elevated serum levels of IP-10 in T1DM than controls, which agree with our results, and it was observed here that there was a significant increase for diabetes

with and without nephropathy T1DM when compared with controls. In addition, Antonelli *et al.* [84], Nicoletti *et al.* [81], and Ahmadi *et al.*[85] reported raised IP-10 levels in subjects at high risk for T1DM, suggesting that serum IP-10 may be a useful predictor of the development of type 1 diabetes. Another study with individuals at high risk for T1DM (i.e. first-degree relatives with multiple autoantibodies) demonstrated no change in IP-10 [86]. The study by Rotondi *et al.*[87] found that the range of serum IP-10 concentrations was much broader in newly diagnosed T1DM patients than in healthy controls and the highest concentration found in patients was more than five times higher than in controls. Daneva[88] found a genetic polymorphism in the gene encoding the chemokine IP-10.

Herder *et al.*[89] demonstrated that IP-10 significantly decreased between nonobese diabetic patients and nonobese healthy controls [88]. Herder *et al.*[89] and Dalmas *et al.*[90] found that IP-10 significantly increased for obese and T2DM patients which was interpreted by Kochumon *et al.* [91], who observed a significant increase in the gene expression of CXCLs in subcutaneous adipose tissue from obese subjects relative to that of lean individuals. Xu *et al.*[92] suggested that IP-10 may have an etiopathogenic role in T2DM and DN as one of the downstream effectors of proinflammatory cytokines, which agrees with this study's results.

In conclusion, inflammatory cytokines could play a crucial role in the pathogenesis of DN. Monitoring of cytokines helps evaluate the immune status inflammation of DN patients and identify those patients at high risk of developing diabetes to select the best therapeutic option.

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Conflicts of interest
There are no conflicts of interest.

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