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Role of nuclear factor kappa B, interleukin-19, interleukin-34, and interleukin-37 expression in diabetic nephropathy

Doaa Esam[1], Adel Abdel-Moneim[2*], Basant Mahmoud[1] and Mohamed Abdel-Gabbar[1]

Abstract

Background: The long-term effects of diabetes mellitus (DM) can impair several organs, including the kidney, resulting in serious health problems. Diabetic nephropathy (DN), a primary contributor in end-stage renal failure worldwide, affects 20–30% of patients with type 2 DM (T2DM). This study was designed to assess the contribution of nuclear factor kappa B (NF-κB) and interleukin (IL)-6, IL-19, IL-34, and IL-37 in the development of DN.

Methods: The study included 160 participants, of which 130 were allocated into the patients with diabetes group, patients with chronic kidney disease (CKD), and patients with diabetic chronic kidney disease (DCKD), and 30 were healthy controls.

Results: The obtained data revealed a significant ($p < 0.05$) increase in IL-19, IL-34, and NF-κB mRNA expression and serum IL-6 levels in patient groups (CKD and DCKD) compared with the healthy control group, whereas IL-19, IL-34, and NF-κB mRNA expression showed a marked elevation in the DCKD group when compared with patients with CKD. Conversely, IL-37 mRNA expression and serum superoxide dismutase (SOD) activity were significantly ($p < 0.05$) decreased in both groups relative to the healthy controls, whereas the decrease was markedly higher in the DCKD group when compared with the CKD group.

Conclusion: The obtained results could indicate the potential implication of NF-κB, IL-19, IL-34, and IL-6 levels, along with the decrease in IL-37 expression and serum SOD activity, in the pathophysiology of kidney disease in diabetes. Moreover, designing drugs targeting these cytokines and/or their signal pathways may prevent or alleviate the progression of kidney disease.

Keywords: Diabetic nephropathy, Insulin resistance, Antioxidant enzymes, Cytokines, NF-κB

1 Background

Diabetes mellitus (DM) can impact several organ systems, resulting in critical problems [1]. Diabetic nephropathy (DN) is among the main complications of diabetes mellitus and has been a major factor in renal failure [2]. DN or diabetic renal diseases are disorders characterized by persistent albuminuria and a progressive decline in renal function, arterial hypertension, and a reduction in glomerular filtration [3]. Accordingly, DN is one of the more common types of diabetic microangiopathy. DN pathogenesis has been linked to inflammatory mediators, such as adhesion molecules, nuclear factors, cytokines, growth factors, and immune cells [4].

A family of DNA-binding proteins called nuclear factor kappa B (NF-κB) is crucial for the transcription of more than 400 genes, including cytokines, chemokines, and their modulators, immune and non-immune receptors, as well as different enzymes; all these molecules control

*Correspondence: adel_men2020@yahoo.com; adel.hassan@science.bsu.edu.eg

[2] Molecular Physiology Division, Faculty of Science, Beni-Suef University, Salah Salim St., Beni-Suef 62511, Egypt
Full list of author information is available at the end of the article

several biological processes [5]. NF-κB has vital role in controlling the immune response; thus, angiotensin II-induced production of pro-inflammatory molecules in renal damage is important [6]. The antioxidant defense system may have a key role in diabetic vascular injury. Superoxide dismutase (SOD) is the principal antioxidant enzyme for superoxide elimination, which can convert superoxide to hydrogen peroxide (H_2O_2) and molecular oxygen [7]. In addition, SOD gene polymorphism is involved in human DN risk and it might be used in the prediction of kidney-related complications in diabetic patients as an independent genetic factor [8].

Elevated interleukin-6 (IL-6) expression impairs renal function by worsening kidney cell infiltration, and DN has higher IL-6 levels [9]. IL-19 is a newly discovered cytokine of the IL-10 family. In diabetic patients with microalbuminuria, IL-19 levels may rise as a result of pre-existing atherosclerosis [10]. In addition, IL-34 is a newly discovered cytokine whose increased levels are independently linked with kidney disease [11]. Moreover, IL-37, a member of the IL-1 family, is significantly reduced, and the overexpression of IL-37 reduces the development of diabetes [12] and may prevent renal damage after renal ischemia and transplantation [13].

However, the involvement of cytokine-induced inflammatory mechanisms in DN requires further investigation. Hence, the study aims to explore the implications of an inducible transcription factor, NF-κB, and pro-inflammatory cytokines, IL-6, IL-19, and IL-34, as well as the anti-inflammatory cytokine, IL-37, in DN.

2 Patients and methods

2.1 Patients

The study population comprised diabetic patients (diagnosed according to the 1999 World Health Organization criteria) or those without renal impairment or hemodialysis. Overall, 130 patients from the Kidney Hospital, Beni-Suef, Egypt, were enrolled in the current study. In addition, healthy control participants enrolled in the study were free from type 1 diabetes mellitus or T2DM and had no first-degree relatives to diabetes and kidney dysfunction, while the enrolled diabetic chronic kidney disease (DCKD) patients have had diabetes for several years prior to developing nephropathy, whereas chronic kidney disease (CKD) patients are non-diabetic subjects who have had nephropathy for several years. Diabetes was diagnosed according to the World Health Organization's 1999 criteria. For all enrolled subjects (healthy controls and patients), the key exclusion criteria included the presence of thyroid diseases, autoimmune disorders, infectious diseases, asthma, eczema, allergies, respiratory disorders, chronic liver, and cardiac diseases, and malignancies. Before participation in the experiment, written consent was obtained from all patients. The research protocol was approved by the Kidney Hospital, Beni-Suef, Egypt, ethical committee in compliance with the Declaration of Helsinki and the recommendations for good clinical practice (BSU/2018/7).

2.2 Experimental design

The study population included 160 participants of both sexes, allocated into the following four groups according to biochemical biomarkers:

Group 1: Healthy control group ($n=30$).

Group 2: Diabetes group (glycosylated hemoglobin (HbA1c) > 6.4%, serum creatinine < 1.5 mg/dl, and/or glomerular filtration rate (GFR) ≥ 90 mL/min/1.73 m^2] ($n=30$).

Group 3: CKD group (HbA1c < 6%, serum creatinine > 2 mg/dl, and/or GFR < 90 mL/min/1.73 m^2) ($n=50$).

Group 4: DCKD group (HbA1c > 6.4%, serum creatinine > 2 mg/dl, and/or GFR < 90 mL/min/1.73 m^2) ($n=50$).

Blood samples were obtained from subjects who had fasted overnight and incubated at room temperature for 30 min and then centrifuged. The sera were immediately separated and stored at − 20 °C for biochemical analysis. The second component of the blood sample was placed in a sodium fluoride tube to determine the plasma glucose level, and the third part of the blood sample was added to ethylenediaminetetraacetic acid to measure HbA1c% and mRNA expression.

2.3 Laboratory assays

Fasting blood glucose, serum creatinine, urea, and uric acid levels were assayed using kits obtained from Spinreact (Spain). The percentage of glycosylated hemoglobin in the blood was tested using reagent kits from Stanbio (USA). The homeostasis model assessment for insulin resistance (HOMA-IR), homeostasis model assessment for insulin sensitivity (HOMA-IS), and homeostasis model assessment for beta-cell function (HOMA-β) were measured according to Wallace et al. [14]. The MDRD equation was used to determine the GFR [15]. Serum insulin, SOD, and IL-6 levels were measured using a standard sandwich enzyme-linked immune-sorbent assay kit purchased from MyBioSource (USA) according to the manufacturer's instructions.

2.4 RNA isolation and qRT-PCR

Total RNA was isolated from the blood samples using a Qiagen extraction kit purchased from Qiagen (Germany). cDNA synthesis was performed using

High-Capacity cDNA Reverse Transcription Kits (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RT-PCR was conducted on a 20 µL system comprising 10 µL 1 × SsoFast EvaGreen Supermix (Bio-Rad, Hercules, CA, USA), 2-µL cDNA, 6 µL of RNase/DNase-free water, and 500 nM of the following primer pair sequences: NF-κB: F: 5′TACTCT GGCGCAGAAATTAGGTC3′ and R: 5′CTGTCTCGG AGCTCGTCTATTTG-3′; IL-19: F: 5′-CGAGCTCTC CCAGGGATT-3′ and R: 5′-CAGAGTCATCCATGA CAACTATGAT-3′; IL-34: F: 5′-GCCACCCATCCT GGAAGTA-3′ and R: 5′-GACAACACGGATTCCACC TT-3′; IL-37: F: 5′CGGGATCCATGGTTCACACAA GTCCA3′ and R: 5′CCCAAGCTTCTAATCGCTGAC CTACT3′; and β-actin: F: 5′-CTGTCTGGCGGCACC ACCAT-3′ and R: 5′-GCAACTAAGTCATAGTCC GC-3.′ The conditions of the thermal cycler were as follows: 3 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C. A melting curve was used to conduct a 60 °C–95 °C ramp for each reaction. The threshold time at which the fluorescent signal surpassed an arbitrarily determined threshold near the midpoint of the log-linear amplification phase was calculated for each process, as was the relative quantity of mRNA. The amplification data were analyzed using the manufacturer's program, and the results were normalized to β-actin.

2.5 Statistical analysis

The results were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 22.0, Chicago, IL, USA). A one-way analysis of variance was performed to compare the data across the different groups, followed by Duncan's test to assess the significant differences between the groups. A simple linear correlation study was conducted using Pearson's method to estimate the dependency degree between the variables. A p value < 0.05 was considered statistically significant.

3 Results

The demographics of the study groups are shown in Table 1. Group 1 included 30 healthy individuals (13 males and 17 females), aged 43.10 ± 12.11 years, who served as healthy controls. Group 2 included 30 subjects with diabetes (15 males and 15 females), aged 44.67 ± 13.72 years. Group 3 had 50 patients with CKD (27 males and 23 females), aged 49.54 ± 14.38 years. Group 4 included 50 patients with DCKD (30 males and 20 females), aged 56.96 ± 12.92 years. Body mass index (BMI) values exhibited a marked elevation in both groups with diabetes compared with the healthy control group (Table 1).

Regarding blood pressure (systolic and diastolic blood pressure), the results showed a significant ($p < 0.05$) elevation in all patients with kidney disease when compared to healthy controls (Table 1). The fasting glucose level and HbA1c% were markedly ($p < 0.05$) increased in diabetic and DCKD groups compared to the healthy controls. In addition, HOMA-IR revealed a significant increase in the groups with diabetes and DCKD, while HOMA-IS and HOMA-β were significantly decreased in the groups with diabetes and DCKD compared with the healthy control group (Table 1). Moreover, the serum levels of urea,

Table 1 Demographic and biochemical characteristics of the healthy control group and patient groups

Parameter		H. control	Diabetic	CKD	DCKD
Number of subjects		30	30	50	50
Gender (No)	Male	13	15	27	30
	Female	17	15	23	20
Age (Years)		43.10 ± 12.11^{a}	44.67 ± 13.72^{a}	47.54 ± 14.38^{a}	46.96 ± 12.92^{a}
BMI (Kg/m^2)		24.46 ± 2.19^{a}	31.38 ± 5.65^{b}	24.98 ± 3.91^{a}	30.67 ± 5.08^{b}
SBP (mmHg)		117.99 ± 7.79^{a}	135.43 ± 12.42^{b}	139.36 ± 16.5^{b}	146.66 ± 19.69^{c}
DBP (mmHg)		80.00 ± 5.29^{a}	88.23 ± 7.44^{b}	93.7 ± 11.28^{b}	91.64 ± 11.16^{b}
FBS (mg/dL)		90.67 ± 8.76^{a}	211.53 ± 44.86^{c}	89.76 ± 9.07^{a}	174.0 ± 47.63^{b}
HbA1c (%)		5.44 ± 0.40^{a}	7.97 ± 1.12^{b}	5.66 ± 0.50^{a}	7.62 ± 1.18^{b}
HOMA-IR		1.93 ± 0.34^{a}	4.36 ± 0.88^{b}	2.33 ± 0.69^{a}	4.47 ± 1.24^{b}
HOMA-IS		13.9 ± 2.80^{c}	5.88 ± 1.38^{a}	11.68 ± 4.11^{b}	6.06 ± 2.17^{a}
HOMA-β		115.16 ± 55.45^{c}	44.83 ± 15.81^{b}	105.36 ± 99.5^{c}	32.01 ± 30.2^{a}

Data are expressed as means ± SD. Values that share the same superscript symbol(s) are not significantly different. H: healthy, CKD: chronic kidney disease, DCKD: diabetic chronic kidney disease. SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, HbA1c: glycosylated hemoglobin, FBS: fasting blood sugar, HOMA-IR: homeostasis model assessment for insulin resistance, HOMA-IS: homeostasis model assessment for insulin sensitivity, HOMA-β: homeostasis model assessment for beta-cell function

creatinine, and uric acid were significantly increased in both groups with kidney disease (CKD and DCKD). However, creatinine levels in DCKD group showed a marked increase compared to the CKD group, whereas GFR levels were noticeably decreased in both groups (CKD and DCKD) compared with the healthy control group, with a noticeable decrease in DCKD as compared with the DCKD group (Fig. 1).

The obtained data also revealed a marked increase in NF-κB, IL-19, and IL-34 mRNA expression and IL-6 production levels in the groups with diabetes, CKD, and DCKD compared with the healthy control group, whereas NF-κB, IL-19, and IL-34 mRNA expression showed a significant elevation in the DCKD group when compared with CKD group. Conversely, IL-37 mRNA expression and serum SOD activity levels revealed a noticeable ($p<0.05$) decrease in both the CKD and DCKD groups relative to the healthy controls. Notably, the results of the DCKD group revealed a noticeable decrease compared to the CKD group (Figs. 2, 3).

Regarding correlation analysis, BMI (obesity marker) exerted a significant correlation with IL-37 ($r=-0.256$; $p=0.005$), IL-34 ($r=0.209$; $p=0.008$), IL-19 ($r=0.163$; $p=0.039$), SOD ($r=-0.255$; $p=0.001$), HOMA-IS ($r=-0.378$; $p=0.000$), and HOMA-IR ($r=0.468$; $p=0.000$) (Table 2). HbA1c% also revealed a significant correlation with IL-37 ($r=-0.419$; $p=0.000$), IL-34 ($r=0.393$; $p=0.000$), IL-19 ($r=0.290$; $p=0.000$), IL-6 ($r=0.246$; $p=0.002$), NF-κB ($r=-0.224$; $p=0.004$), SOD ($r=-0.406$; $p=0.000$), HOMA-IS ($r=-0.628$; $p=0.000$), and HOMA-IR ($r=0.676$; $p=0.000$). Moreover, creatinine exhibited a significant correlation with IL-37 ($r=-0.655$; $p=0.000$), IL-34 ($r=0.652$; $p=0.000$), IL-19 ($r=0.508$; $p=0.000$), IL-6 ($r=0.523$; $p=0.000$), NF-κB ($r=-0.430$; $p=0.000$), SOD ($r=-0.578$; $p=0.000$), HOMA-IS ($r=-0.260$; $p=0.001$), and

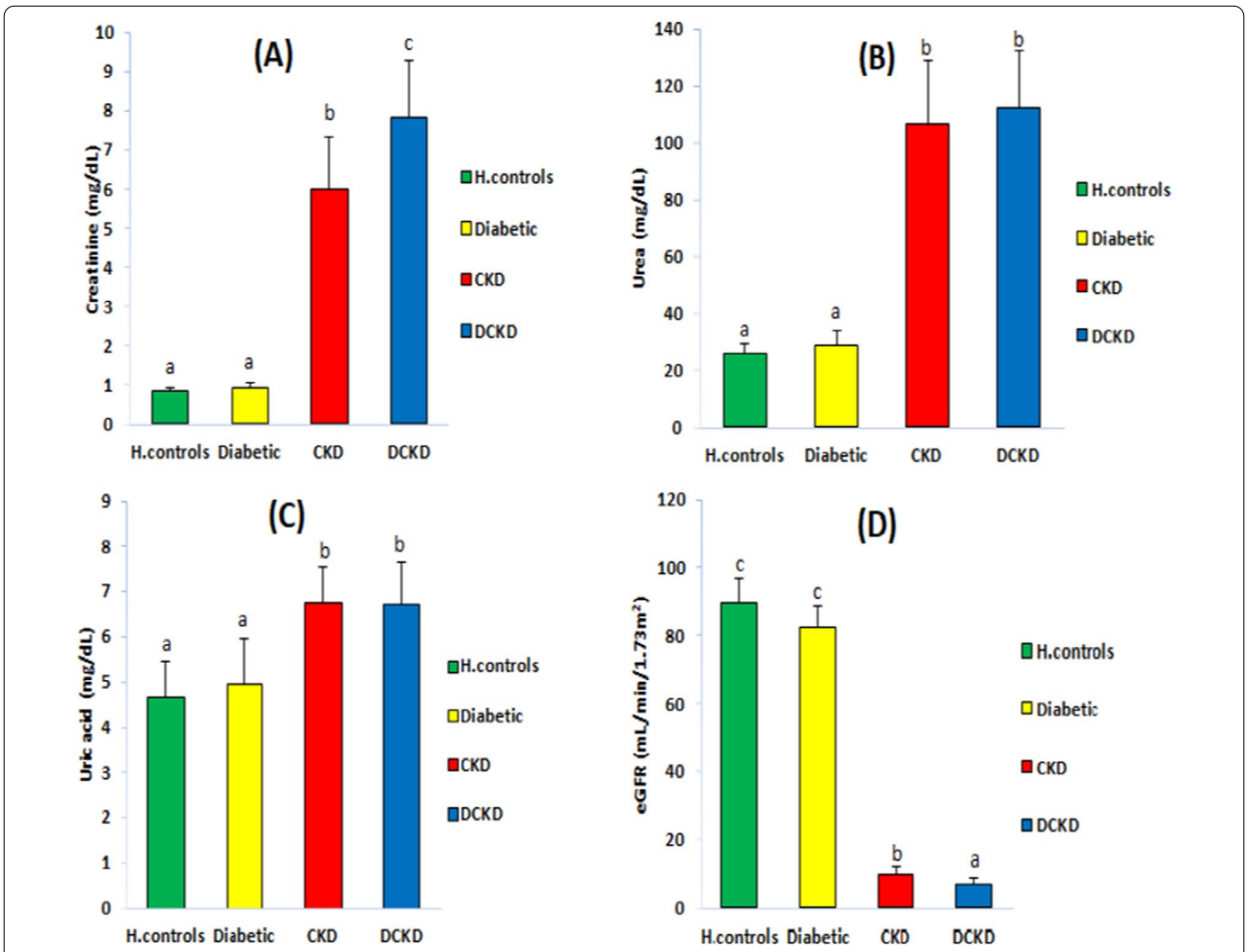

Fig. 1 The levels of **A** creatinine, **B** urea, **C** uric acid, and **D** eGFR in healthy control, diabetic, chronic kidney dysfunction (CKD), and diabetic chronic kidney dysfunction (DCKD) groups. Data are represented as mean ± SD. Different letters indicate significant differences ($p<0.05$). H.: Healthy, and GFR: estimated glomerular filtration rate

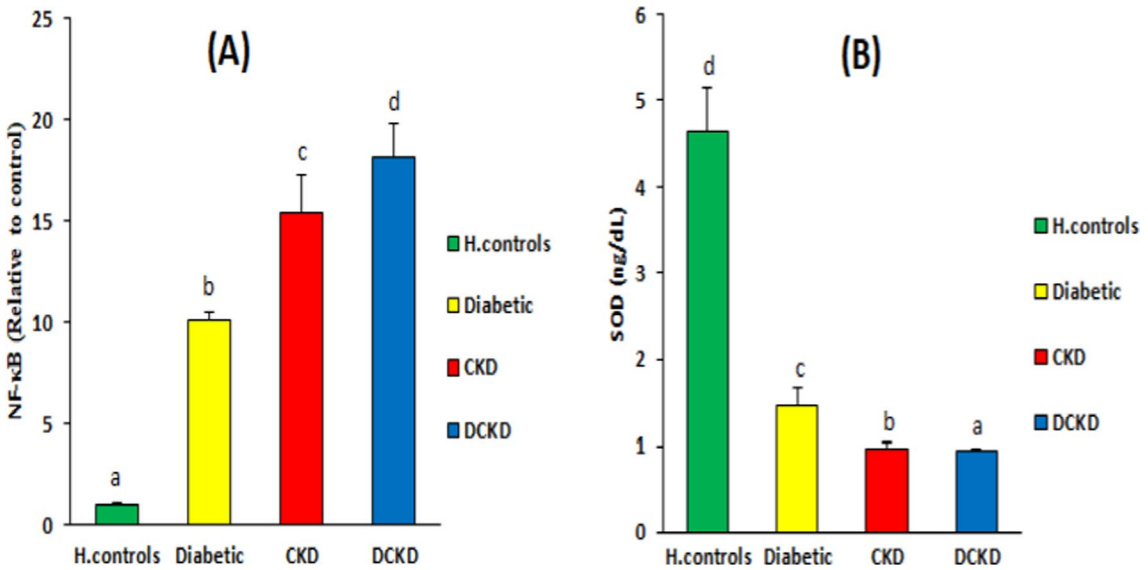

Fig. 2 The levels of **A** NF-κB mRNA expression, **B** SOD activity in healthy control, diabetic, chronic kidney dysfunction (CKD), and diabetic chronic kidney dysfunction (DCKD) groups. Data are represented as mean ± SD. Different letters indicate significant differences ($p < 0.05$). H.: Healthy, SOD: superoxide dismutase activity, NF-κB: nuclear factor kappa B

HOMA-IR ($r = 0.247$; $p = 0.002$). Moreover, GFR showed a significant correlation with IL-37 ($r = 0.703$; $p = 0.000$), IL-34 ($r = -0.665$; $p = 0.000$), IL-19 ($r = -0.700$; $p = 0.000$), IL-6 ($r = -0.669$; $p = 0.000$), NF-κB ($r = -0.666$; $p = 0.000$), SOD ($r = 0.695$; $p = 0.000$), and HOMA-IS ($r = 0.164$; $p = 0.039$) (Table 2).

4 Discussion

The prevalence of CKD has increased in recent decades, along with an increase in diabetes and hypertension, the two leading causes of CKD [16]. Herein, we investigated the contribution of NF-κB and IL-6, IL-19, IL-34, and IL-37 in the pathogenicity of DN. Our data revealed that HbA1c% increased in all groups with diabetes compared with healthy controls. In addition, there was a marked increase in the serum levels of urea, uric acid, and creatinine, as well as a decrease in GFR levels, in both groups with kidney dysfunction. The increase in creatinine levels and the decrease in GFR levels were markedly observed in the DCKD group. Inflammation, which is closely associated with renal illness, is described as a complex network of interactions between renal parenchymal cells and local immune cells such as macrophages and dendritic cells, as well as recruitment of circulating monocytes, lymphocytes, and neutrophils [17].

Hyperglycemia is thought to reflect a state of elevated oxidative stress due to excessive production of ROS and a deficient antioxidant response [18]. Our data showed that serum SOD activity levels were markedly decreased in patient groups compared with healthy controls. Enhanced ROS production in the glomerular microcirculation lowers nitric oxide bioavailability, leading to mesangial contraction dysfunction and arteriolar tone, as well as persistent oxidative stress, which causes endothelial disorder, leukocyte adhesion, and apoptosis of glomerular cells [19]. In agreement with our findings, Liu et al. [20] demonstrated upregulation of renal SOD activity in diabetic mice. The antioxidant enzyme SOD is an important part of the cell's defense against increased oxidative stress. It is therefore speculated that the decreased levels of SOD in patients with diabetes could lead to cytotoxic levels of superoxide overproduction, which may result in diabetic renal tissue damage [21]. However, oxidative damage, inflammation, and cell death caused by hyperglycemia could be alleviated by SOD. Consequently, the reduction in serum SOD levels may contribute to the onset of DN.

The stimulation of the transcription factor NF-κB by oxidative stress caused by hyperglycemia may increase the levels of pro-inflammatory cytokines, which could promote DN [5, 6]. The current data revealed a noticeable increase in NF-κB expression levels in the groups with diabetes, CKD, and DCKD compared to the healthy control group. Moreover, NF-κB mRNA expression showed a positive correlation with HbA1c and creatinine and a negative correlation with GFR levels. Hyperglycemia leads to the production of ROS in mesangial cells,

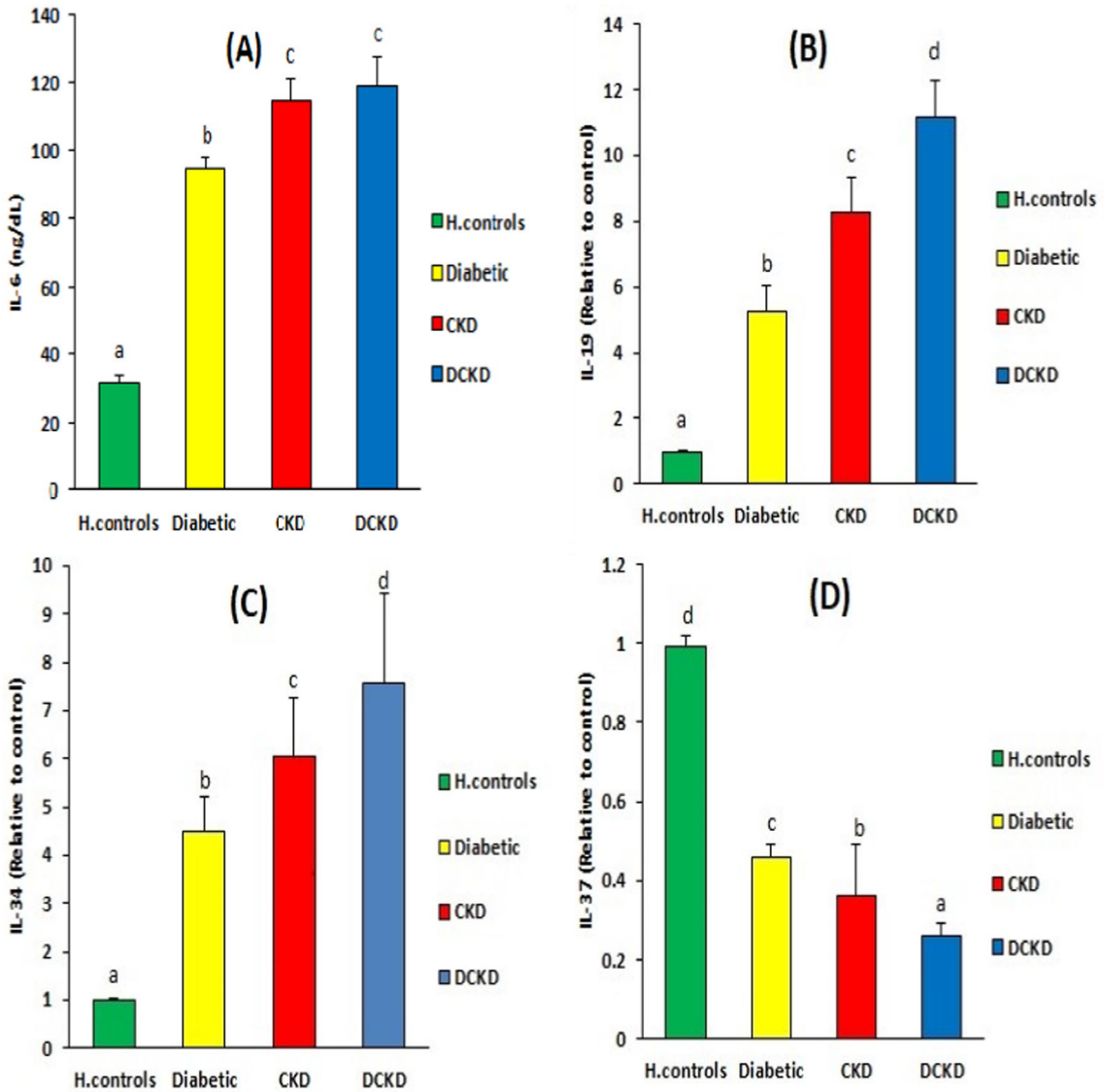

Fig. 3 The levels of **A** IL-6, **B** IL-19 expression, **C** IL-34 expression, **D** IL-37 expression in healthy control, chronic kidney dysfunction (CKD), and diabetic chronic kidney dysfunction (DCKD) groups. Data are represented as mean ± SD. Different letters indicate significant differences ($p < 0.05$). H.: Healthy, IL-6: interleukin-6, IL-19: interleukin-19, IL-34: interleukin-34, IL-37: interleukin-37

which upregulates NF-κB. Interestingly, NF-κB inhibitors protect against kidney injury and may be useful in inflammatory diseases [22]. Notably, inhibiting the RAS and/or the NF-κB system may become an effective therapeutic asset to detain renal injury [23]. Angiotensin II elevation, which is directly connected to hypertension, is a stimulus for NF-κB activation and the production of inflammatory responses. Additionally, NF-κB has been linked to the pathophysiology of kidney injury caused by hypertension [24]. Importantly, the stimulation of NF-κB signaling regulates a variety of pro-inflammatory genes, including those that encode chemokines and cytokines like IL-6 [25].

IL-6 is a pro-inflammatory cytokine that strongly promotes the progression of insulin resistance and the pathophysiology of T2DM through the regulation of differentiation, proliferation, and cell death [26]. In our study, serum IL-6 levels significantly increased in the groups with diabetes, DCKD, and CKD. In addition, IL-6 production showed a positive correlation with HbA1c and creatinine but a negative correlation with GFR levels. According to our findings, pro-inflammatory cytokines, such as IL-6, IL-1b, and TNF-α, have been linked with

Table 2 Correlation analysis between interleukins, SOD, NF-κB, insulin resistance, BMI, and kidney and glucose profiles

		HOMA-IR	HOMA-IS	SOD	NF-κB	IL-6	IL-19	IL-34	IL-37
BMI	R	0.468***	−0.378***	−0.255**	0.116	0.128	0.163*	0.209**	−0.256**
	p value	0.000	0.000	0.001	0.146	0.107	0.039	0.008	0.005
FBS	R	0.636***	−0.591***	−0.308***	0.136	0.166*	0.221**	0.321***	−0.321***
	p value	0.000	0.000	0.000	0.087	0.036	0.005	0.000	0.000
HbA1c	R	0.676***	−0.628***	−0.406***	0.224**	0.246**	0.290***	0.393***	−0.419***
	p value	0.000	0.000	0.000	0.004	0.002	0.000	0.000	0.000
Creatinine	R	0.247**	−0.260**	−0.578***	0.430***	0.523***	0.508***	0.652***	−0.655***
	p value	0.002	0.001	0.000	0.000	0.000	0.000	0.000	0.000
urea	R	0.117	−0.133	−0.589***	0.453***	0.584***	0.493***	0.581***	−0.635***
	p value	0.140	0.095	0.000	0.000	0.000	0.000	0.000	0.000
Uric acid	R	0.088	−0.0100	−0.546***	0.431***	0.531***	0.466***	0.442***	−0.544***
	p value	0.269	0.207	0.000	0.000	0.000	0.000	0.000	0.000
eGFR	R	−0.148	0.164*	0.695***	−0.666***	−0.669***	−0.700***	−0.665***	0.703***
	p value	0.061	0.039	0.000	0.000	0.000	0.000	0.000	0.000

***correlation is significant at the 0.001 level; **correlation is significant at the 0.01 level; *correlation is significant at the 0.05 level, *r*: Pearson correlation, BMI: body mass index, FBS: fasting blood sugar, HbA1c: glycosylated hemoglobin, HOMA-IR: homeostasis model assessment for insulin resistance, HOMA-IS: homeostasis model assessment for insulin sensitivity, GFR: glomerular filtration rate, SOD: superoxide dismutase activity, NF-κB: nuclear factor kappa B

the severity of CKD. These are produced by adipose tissue (along with lymphocytes), which becomes dysfunctional during CKD [27]. Notably, elevated IL-6 levels are not only a consequence of renal disease, but more importantly, it also acts as a trigger for the progression of renal disease and its related complications [28]; these results indicate that IL-6 contributes to the higher prevalence of CKD. Interestingly, it can be concluded that IL-6 impacts renal resident and infiltrating cells, affecting vascular permeability and promoting mesangial cell proliferation, glomerular basement membrane thickening, extracellular matrix synthesis, and neutrophil infiltration into the tubulointerstitium, resulting in DN progression [4]. As a result, increased IL-6 levels in human sera are both an early and strong indicator of DN initiation.

It is worth mentioning that IL-6 trans-signaling induced by Ang II might activate STAT3 in podocytes, which would impact podocyte differentiation, cell cycle, and other physiopathologic processes [28]. Importantly, increased IL-6 production by podocytes activated the JAK2/STAT3 pathway via enhanced phospho-JAK2 binding to gp130 and STAT3 phosphorylation. Likewise, hyperglycemia enhanced IL-6 production and signal transduction in podocytes, which was inhibited by an IL-6-neutralizing antibody or IL-6 siRNA. Therefore, the inhibition of IL-6 and its downstream mediators, including IL-6R and gp130, may reduce the onset of DN [29].

IL-19, a pro-inflammatory cytokine, promotes the T-helper 2 response [10]. Indeed, Cuneo et al. revealed that IL-19 is activated by pro-inflammatory cytokines and is expressed in damaged vascular smooth muscle cells [30]. IL-19 may be implicated in the development of vascular disease in diabetes. Serum IL-19 concentrations were positively correlated with Ang II and showed a significant association with insulin resistance and HbA1c%, suggesting that IL-19 is strongly linked with impaired glucose metabolism and vascular disorders in patients with T2DM [31]. Regarding IL-19 expression, increased IL-19 expression in the groups with diabetes and both CKD and DCKD groups was observed. Furthermore, IL-19 mRNA expression showed a positive correlation with BMI, HbA1c, and creatinine but a negative correlation with GFR levels. Similarly, patients with DN had considerably greater serum levels of IL-19 compared with the controls [32] and revealed a positive correlation with HbA1c%, urea, creatinine, and CRP. Li et al. [10] proposed that chronic hyperglycemia may enhance the expression of IL-19 by activating endothelial cells, resulting in local inflammation and accelerating endothelial damage and atherosclerosis.

In mouse monocytes, IL-19 is elevated in an acute systemic inflammatory state and promotes the generation of IL-6, TNF-α, and apoptosis. In addition, IL-19 upregulates TGF-β1 and MCP-1 expression in renal cortical collecting duct cells (M-1) and also activates caspase-3 and caspase-9 to promote cell apoptosis through the p38 MAPK pathway in M-1 cells [33], which can lead to interstitial fibrosis caused by excess extracellular matrix deposition. In M-1 cells, IL-19 can activate ERK 1/2, JNK, and p38 MAPK and inhibit p38 MAPK signaling, which reduces IL-19-induced apoptosis; these results suggest that IL-19 causes apoptosis in renal epithelial cells via

these three pathways [34]. Moreover, Jennings et al. [35] revealed a substantial rise in IL-19 levels in the urine of patients with CKD, which is closely associated with calculated GFR levels, indicating that IL-19 was a possible novel translational marker of renal damage. Therefore, IL-19 or its receptor may represent novel therapeutic targets for addressing kidney impairment following DN.

The newly discovered pro-inflammatory cytokine, IL-34, has several functions in the modulation of inflammation and the immune response. Indeed, vascular diabetes complications may be predicted by serum IL-34 levels [36]. Moreover, multiple chronic inflammatory conditions can be caused by IL-34, and it has a significant role in the development of insulin resistance in T2DM patients [37]. In addition, IL-34 has been linked to impaired kidney function and severe anemia in patients with CKD [11]. Our study revealed upregulation of IL-34 expression in the patients with DN groups. In addition, IL-34 mRNA expression revealed a positive correlation with BMI, HbA1c, and creatinine and a negative correlation with GFR levels. Importantly, IL-34 stimulates the protein tyrosine phosphatase ζ receptor; during acute kidney damage, both receptors and cytokines are upregulated. Moreover, IL-34 increased both intrarenal macrophage and bone marrow cell proliferation, increasing the levels of circulating neutrophils and monocytes and their recruitment to the kidney [38]. It has been shown that TNF-α increased CSF-1 and IL-34 expression in intestinal epithelial cells via the NF-κB signaling pathway [39]. In mice, redacting IL-34 improves nephritis via macrophage- and autoantibody-mediated pathways both inside and outside the kidney. IL-34 is a possible therapeutic target for tubular epithelial cell damage, and IL-34 inhibition may have reno-protective effects [40]. Notably, IL-34 may be an effective biomarker and therapeutic target for treating inflammatory diseases, including DN.

IL-37 generates a complex with IL-18R and IL-1R8 that transduces anti-inflammatory signals by suppressing NF-κB and MAPK and activating the Mer-PTEN-DOK pathway. IL-37 exerts broad and complex anti-inflammatory and immunomodulatory effects, inhibits excess inflammation, and prevents tissue damage mediated by inflammation [41]. Importantly, IL-37 is a possible therapeutic target for managing obesity-induced insulin resistance and T2DM [42]. The biological effects of IL-37 can be attributed to its ability to inhibit NF-κB; the IL-6/STAT3 pathway; and TNF-α, IL-1β, and IL-6 synthesis [43]. In addition, it has been shown that a high IL-37 concentration is correlated with increased systemic lupus erythematous activity and impaired renal manifestations [44].

IL-37 mRNA expression was found to be decreased in the patients with diabetes and both kidney dysfunction groups relative to the healthy control group. In addition, IL-37 mRNA expression revealed a negative correlation with BMI, HbA1c, and creatinine and a positive correlation with GFR levels. Notably, IL-37 can greatly reduce podocyte inflammation, oxidative stress, and apoptosis caused by hyperglycemia and can also block the STAT3-CypA signaling pathway [45]. Moreover, IL-37 decreased the kidney expression of TNF-α, IL-6, and IL-1β and alleviated mononuclear cell infiltration and kidney damage in a mouse ischemia injury model. Thus, IL-37 may represent an effective approach for suppressing renal damage responses and promoting renal function after renal ischemic damage [13]. In addition, understanding the role of IL-19, IL-34, and IL-37 in the pathogenicity of DN has been deemed crucial in creating novel anti-inflammatory agents to prevent or delay the onset of DN.

Hyperglycemia and insulin resistance are responsible for the micro- and macrovascular complications of diabetes. The metabolic insult of hyperglycemia and podocyte insulin resistance is a major source of podocyte damage, leading to albuminuria and proteinuric glomerulopathies in early DN [46]. Importantly, IL-19, IL-34, and NF-κB expression levels and the levels of IL-6 in diabetic and DCKD patients were associated with insulin sensitivity and insulin resistance. The sustained rise in inflammatory cytokines, such as IL-19, IL-34, and NF-κB, as well as serum IL-6 levels, is involved in the progression of insulin resistance in diabetic and DCKD patients. In patients with diabetes, prolonged activation of pro-inflammatory and pro-fibrotic cell types causes excess extracellular matrix deposition [47]. These elements are involved in the stimulation and proliferation of smooth muscle actin-positive myofibroblasts, which are essential for the excessive synthesis of extracellular matrix, ultimately leading to DN [48]. Thus, IL-6, IL-19, IL-34, IL-37, and NF-κB may represent therapeutic targets for preventing or alleviating diabetic kidney disorders as well as their complications.

The current study has several limitations, including the small study population in the patient groups and the fact that the findings were separated depending on renal stages and disease duration. In addition, the lack of determination of IL-19, IL-34, and IL-37 at protein levels, immunohistochemistry staining slides for interleukin levels in the patients, and the addition of more inflammatory and anti-inflammatory cytokines could influence inflammation processes, pathogenesis, and DN development. Importantly, clinical investigations in larger cohorts are required to deeply understand the

pathways that influence IL-19, IL-34, and IL-37 in the etiology and pathogenicity of DN.

5 Conclusion

Hyperglycemia, insulin resistance, and chronic inflammation are responsible for the development of diabetic complications, such as DN. The obtained results could indicate the potential implication of NF-κB, IL-19, IL-34, and IL-6 levels, along with the decrease in IL-37 expression and serum SOD activity, in the pathophysiology of kidney disease in diabetes. Thus, IL-6, IL-19, IL-34, IL-37, and NF-κB or their receptors may be a research target for the pathogenesis and therapies of DN.

Abbreviations

CKD: Chronic kidney disease; DN: Diabetic nephropathy; DCKD: Diabetic chronic kidney disease; GFR: Glomerular filtration rate; HbA1c: Glycosylated hemoglobin; HOMA-IR: Homeostasis model assessment for insulin resistance; HOMA-IS: Homeostasis model assessment for insulin sensitivity; HOMA-β: Homeostasis model assessment for beta-cell function; IL: Interleukin; NF-κB: Nuclear factor kappa B; PCR: Polymerase chain reaction; RT: Real time; SOD: Superoxide dismutase; T2DM: Type 2 diabetes mellitus.

Author contributions

All authors contributed to the study's conception and design. Data collection, material preparation, and analysis were performed by BM, DE, and MAG. The draft of the article was written by BM, AAM, and MAG. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Written informed consent was given by all participants, and the research protocol was approved by the Kidney Hospital, Beni-Suef, Egypt, ethical committee in compliance with the Declaration of Helsinki and the recommendations for good clinical practice (BSU/2018/7).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Biochemistry Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt. [2]Molecular Physiology Division, Faculty of Science, Beni-Suef University, Salah Salim St., Beni-Suef 62511, Egypt.

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