

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(4): 733-739
www.biochemjournal.com
 Received: 02-02-2026
 Accepted: 07-03-2026

Ammar K Madlool
 Department of Aesthetic and
 Laser Techniques, College of
 Health & Medical
 Techniques/Kufa, Al-Furat
 Al-Awsat Technical
 University, Iraq

Serum interleukin-35 as a prognostic biomarker in type 2 diabetes and diabetic nephropathy

Ammar K Madlool

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i4j.8293>

Abstract

Diabetic kidney disease (DKD) is a prevalent and serious complication of diabetes mellitus that contributes significantly to end-stage renal disease worldwide. This study aimed to evaluate the diagnostic potential of serum interleukin-35 (IL-35), an immunosuppressive cytokine, in patients with DKD. A total of 90 participants were enrolled and categorized into three groups: 30 with DKD, 30 with type 2 diabetes mellitus without nephropathy (DM), and 30 healthy controls (HC). Serum IL-35 levels were measured using enzyme-linked immunosorbent assay (ELISA), and biochemical parameters such as serum creatinine, urea, and fasting glucose were assessed. IL-35 levels were significantly elevated in DKD patients compared to both DM and HC groups ($p < 0.05$), and positively correlated with serum creatinine and urea, indicating a potential link with renal dysfunction. Receiver operating characteristic (ROC) analysis revealed moderate diagnostic accuracy for IL-35 in distinguishing DKD patients from controls. These findings suggest that IL-35 may serve as a promising non-invasive biomarker for the early detection and monitoring of diabetic kidney disease progression.

Purpose: This study aimed to investigate the diagnostic potential of serum interleukin-35 (IL-35) levels in patients with diabetic kidney disease (DKD) and to determine its association with renal function markers in comparison to diabetic patients without nephropathy and healthy controls.

Rationale: Early detection of diabetic kidney disease is critical for effective management and prevention of progression to end-stage renal failure. Inflammatory cytokines such as IL-35 may serve as novel biomarkers for DKD due to their role in immune modulation and renal inflammation. By evaluating serum IL-35 levels in well-defined clinical groups, this study seeks to provide evidence supporting its use as a reliable, non-invasive biomarker for DKD diagnosis and monitoring. The results may contribute to improving precision in diagnosing renal complications in diabetic patients.

Limitations: This study was limited by its cross-sectional design, which restricts conclusions about causality between IL-35 levels and DKD progression. Additionally, the study was conducted at a single center with a relatively modest sample size, which may limit the generalizability of the findings. Further longitudinal and multicenter studies are needed to validate IL-35 as a prognostic marker and explore its mechanistic role in DKD.

Keywords: Diabetic kidney disease, Interleukin-35, biomarkers, chronic kidney disease, inflammation, ELISA

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia. It is classified into many types such as type 1 diabetes (T1D), type 2 diabetes (T2D), other factors which causes special type of diabetes, and gestational diabetes mellitus (GDM) [1, 2]. Diabetes can be caused by impaired β -cell pancreas due to the autoimmune-mediated destruction (insulin deficiency), impaired insulin receptor (resistance to the peripheral actions of insulin), or both [3]. Several complications may be caused for patients with T2D. The most common complications of T2D are nephropathy [4]. Diabetic nephropathy is a chronic inflammation of the kidney which is considered a serious complication of T2D causing proteinuria (albuminuria) and decline in renal functioning [5, 6]. The average course of the disease is 10-20 years, though this might vary from patient to another based on a variety of factors, including glycemic management [7]. Previous studies explain that diabetic nephropathy may up to 30% in T1D patients and 40% in T2D patients, which means that 20-50% of individuals enrolled in renal failure programs have diabetes. It is the primary cause of end-stage renal disease (ESRD) prompting dialysis and kidney transplantation [8-10]. Actually, the pathobiology of the diabetic nephropathy is even more complicated, involving

Corresponding Author:
Ammar K Madlool
 Department of Aesthetic and
 Laser Techniques, College of
 Health & Medical
 Techniques/Kufa, Al-Furat
 Al-Awsat Technical
 University, Iraq

modifications to kidney structure, function, and metabolism that are still not fully understood [11]. One of the many linked causes of disorder initiation is hyperglycemia, which triggers alternatively activated glucose pathways and their cytotoxic by products [12]. Oxidative stress [13], immunological response and oxidized lipids all have affecting diabetic nephropathy [14]. All of these combined factors cause impaired to the kidney cells due to producing pro-inflammatory cytokines by DAMPs [15]. Although the body benefits from inflammation in the short term, prolonged inflammation can be highly harmful and lead to a variety of problems kidney [16].

Inflammation is caused by an array of cytokines, and one of them is the IL-35. IL-35 is a novel heterodimeric cytokine in the IL-12 family [17]. An earlier study reported that IL-35 is a heterodimer of two subunits, EBI3 and P35 [18, 19]. Cell-specific receptor subunit heterodimerization is required for IL-35 binding, i.e., GP130-GP130 (for T-cell), IL-12Rb2-IL12Rb2 and IL-12Rb2-GP130 (20, 21). IL-35 binds its while receptors then activate signaling pathway contain STAT1, STAT3 and STAT4 as well as JAK1 and JAK2 molecules [22, 23]. IL-35, which is produced by regulatory T cell (Treg) and other lymphocytes, plays an immunosuppressive role through suppressing the generation of T-helper type-17 (Th17) cells and facilitating proliferation of Treg cells [24, 25]. IL-35 has been associated with a variety of diseases from autoimmune diseases, cancer, to inflammatory response and sepsis [26, 27]. In the past few years, researchers have created IL-35 transgenic mice with islet b-cell-specific expression of IL-35 and discovered that IL-35 has an inhibitory effect on type I diabetes [28]. The main mechanism for suppress of IL-35 is to inhibit the infiltration and proliferation of CD4 + T and CD8 + T [29]. Nevertheless, the expression of IL-35 in T2D and DN as well as its role in prognosis is largely unknown. Thus, in the present study, we investigated differences in patients with T2D and DN as well as whether serum IL-35 levels were associated with renal function hypothesizing that IL-35 be used as a potential biomarker for disease diagnosis and progression.

Materials and Methods

Study Design and Participants

This case-control study included a total of 90 participants recruited from Al-Sader Educational Hospital, from 1st of November of 2023 to the end of March of 2024 and categorized into three equal groups (n = 30 each): (1) patients diagnosed with diabetic kidney disease (DKD) based on clinical and laboratory findings; (2) patients with type 2 diabetes mellitus (DM) but without nephropathy; and (3) healthy controls (HC) without any known metabolic or renal disorders. Inclusion criteria for the DKD group included persistent albuminuria, reduced estimated glomerular filtration rate (eGFR), and a history of diabetes mellitus for at least five years. Exclusion criteria included the presence of other chronic illnesses, infections, autoimmune diseases, or malignancies.

Sample Collection and Biochemical Analysis

Fasting venous blood samples (5 mL) were collected from all participants using sterile vacutainer tubes. Samples were allowed to clot, centrifuged at 3,000 rpm for 10 minutes, and serum was separated and stored at -80 °C until analysis. Serum creatinine, blood urea nitrogen (BUN), and fasting

glucose levels were measured using standard colorimetric methods.

Interleukin-35 Measurement

Serum levels of IL-35 were quantified using a commercially available ELISA kit (BT Lab Company, China) in accordance with the manufacturer's instructions. Optical densities were measured at 450 nm using a microplate reader (Model, Manufacturer). All samples were assayed in duplicate to ensure analytical consistency.

Statistical Analysis

Data analysis was performed using SPSS version 26.0 (IBM, USA). Continuous variables were expressed as mean $\pm$ standard deviation. Comparisons between groups were made using one-way ANOVA followed by Tukey's post hoc test. Pearson's correlation was used to assess associations between IL-35 levels and renal function markers. ROC curve analysis was employed to evaluate the diagnostic performance of IL-35. A p-value of <0.05 was considered statistically significant.

Experiments

Experimental Groups

- **Group 1 (DKD):** Patients with confirmed diabetic kidney disease (n = 30)
- **Group 2 (DM):** Diabetic patients without nephropathy (n = 30)
- **Group 3 (HC):** Healthy, age-and sex-matched individuals (n = 30)

Primary Outcome

The primary experimental outcome was the comparison of serum IL-35 levels among the three groups.

Secondary Outcomes

- Correlation between IL-35 and serum creatinine, BUN, and glucose levels
- Diagnostic accuracy of IL-35 as assessed by ROC curve analysis

Experimental Procedure

All laboratory procedures were conducted under standardized conditions. The ELISA assay was validated for sensitivity and specificity, and calibration curves were generated with each run. Assay variability was monitored by including internal controls in every batch.

Results

The general characteristics of the enrolled participants is presented in table 1, including control subjects, patients with diabetes mellitus (DM), and those with diabetic nephropathy (DN). Several statistically significant differences were observed among the groups. Patients with DN were significantly older than both control (p = 0.001) and DM groups (p = 0.026). Body mass index (BMI) showed a marked decline across groups, being highest in controls and lowest in DN patients (p < 0.001 for all comparisons). Fasting serum glucose (FSG) levels were significantly elevated in both DM and DN groups compared to controls (p < 0.001), while no difference was observed between the DM and DN groups (p = 0.736). Fasting insulin (FI) levels were significantly lower in both DM and DN groups compared to controls (p < 0.001), with a borderline

difference between DM and DN ($p = 0.050$). Similarly, HOMA-IR and HOMA- β were significantly altered between control and disease groups ($p < 0.05$), though the difference between DM and DN was not statistically significant for HOMA- β ($p = 0.699$).

Triglycerides (TG) and very low-density lipoprotein cholesterol (VLDL-C) levels were significantly higher in controls than in both DM and DN patients ($p < 0.05$), with borderline differences between DM and DN ($p = 0.059$). In contrast, total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels were significantly elevated in the DN group compared to both DM and control groups

($p < 0.05$). High-density lipoprotein cholesterol (HDL-C) was markedly reduced in both DM and DN groups relative to controls ($p < 0.001$). Notably, serum IL-35 levels were significantly decreased across the groups, with the lowest levels observed in DN patients. IL-35 levels were significantly lower in both DM and DN groups compared to controls ($p < 0.001$), and further significantly decreased in DN compared to DM patients ($p = 0.001$). While several parameters such as waist circumference (WS), hip circumference (HIP), and %HbA1c showed expected trends, their comparisons between certain groups did not reach statistical significance.

Table 1: General characteristics of enrolled participants stratified by study group.

Parameter	Control (n = 30) Mean $\pm$ SD	Diabetes Mellitus (n = 30) Mean $\pm$ SD	Diabetic Nephropathy (n = 30) Mean $\pm$ SD	P-value (a/b/c)
Anthropometric and Demographic				
Age (years)	49.03 $\pm$ 6.25	51.23 $\pm$ 7.30	55.13 $\pm$ 6.44	a = 0.206 b = 0.001 c = 0.026
BMI (kg/m ²)	32.52 $\pm$ 1.11	28.85 $\pm$ 2.50	21.77 $\pm$ 2.17	a = 0.001 b = 0.001 c = 0.001
Waist Circumference (cm)	101.80 $\pm$ 12.10	106.56 $\pm$ 13.53	96.96 $\pm$ 13.63	a = 0.163 b = 0.157 c = 0.006
Hip Circumference (cm)	103.10 $\pm$ 12.75	108.60 $\pm$ 14.22	97.33 $\pm$ 13.49	a = 0.118 b = 0.102 c = 0.002
Glycemic Markers				
FSG (mg/dL)	91.00 $\pm$ 5.20	207.60 $\pm$ 50.54	211.53 $\pm$ 59.13	a = 0.001 b = 0.001 c = 0.736
FI (μ IU/mL)	4.20 $\pm$ 1.41	2.82 $\pm$ 1.08	2.24 $\pm$ 0.90	a = 0.001 b = 0.001 c = 0.050
HOMA-IR	0.88 $\pm$ 0.34	1.46 $\pm$ 0.70	1.17 $\pm$ 0.61	a = 0.001 b = 0.050 c = 0.050
HOMA- β	3.12 $\pm$ 1.22	0.43 $\pm$ 0.21	0.35 $\pm$ 0.22	a = 0.001 b = 0.001 c = 0.699
HbA1c (%)	4.01 $\pm$ 0.59	8.79 $\pm$ 1.92	8.81 $\pm$ 2.21	a = 0.001 b = 0.001 c = 0.970
Lipid Profile				
TG (mg/dL)	255.63 $\pm$ 31.04	216.03 $\pm$ 69.55	186.60 $\pm$ 69.82	a = 0.001 b = 0.047 c = 0.059
TC (mg/dL)	138.87 $\pm$ 33.15	180.03 $\pm$ 60.08	205.13 $\pm$ 59.68	a = 0.003 b = 0.001 c = 0.068
HDL-C (mg/dL)	47.63 $\pm$ 6.68	33.17 $\pm$ 9.09	31.07 $\pm$ 8.84	a = 0.001 b = 0.001 c = 0.329
LDL-C (mg/dL)	60.10 $\pm$ 33.07	103.66 $\pm$ 68.51	136.74 $\pm$ 66.54	a = 0.005 b = 0.001 c = 0.031
VLDL-C (mg/dL)	31.12 $\pm$ 6.20	43.20 $\pm$ 13.91	37.32 $\pm$ 13.96	a = 0.001 b = 0.047 c = 0.059
Inflammatory Cytokine				
IL-35 (pg/mL)	67.72 $\pm$ 3.65	30.62 $\pm$ 6.61	20.46 $\pm$ 2.65	a = 0.001 b = 0.001 c = 0.001

Data are presented as mean $\pm$ standard deviation. BMI, body mass index; FSG, fasting serum glucose; FI, fasting insulin; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; IL-35, interleukin-35. a = Control vs. DM; b = Control vs. DN; c = DM vs. DN. Bold p-values indicate statistical significance ($p < 0.05$).

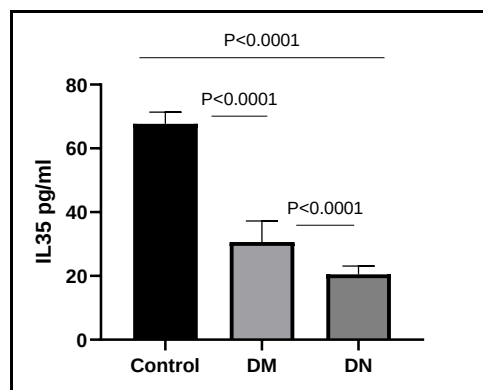


Fig 1: Comparison of IL-35 for DN, DM and control

The correlation analysis between serum IL-35 levels and various clinical and biochemical parameters in patients with diabetic nephropathy (DN) is summarized in table 2. Among the studied variables, age demonstrated a statistically significant positive correlation with IL-35 levels ($r = 0.376$, $p = 0.040$), indicating that IL-35 levels tend to increase slightly with advancing age in this patient group. All other evaluated parameters including anthropometric indices (BMI, waist circumference, hip circumference, and waist-to-hip ratio), glycemic markers (fasting serum glucose, fasting insulin, HOMA-IR, HOMA- β , and HbA1c), and lipid profile components (TG, TC, HDL-C, LDL-C, and VLDL-C) are showed non-statistically significant correlations with IL-35 levels ($p > 0.05$).

Table 2: Pearson correlation coefficients between serum IL-35 (pg/mL) and clinical parameters in patients with diabetic nephropathy.

Category	Parameter	r	p-value	Interpretation
Demographic				
Demographic	Age (years)	+ 0.376	0.040*	Significant positive
Anthropometric				
Anthropometric	BMI (kg/m ²)	+ 0.043	0.820	Non-significant
Anthropometric	Waist Circumference	+ 0.129	0.497	Non-significant
Anthropometric	Hip Circumference	+ 0.192	0.309	Non-significant
Anthropometric	Waist-to-Hip Ratio	-0.267	0.153	Non-significant
Glycemic Markers				
Glycemic	FSG (mg/dL)	+ 0.274	0.143	Non-significant
Glycemic	Fasting Insulin (μ IU/mL)	+ 0.024	0.899	Non-significant
Glycemic	HOMA-IR	+ 0.162	0.392	Non-significant
Glycemic	HOMA- β	-0.127	0.502	Non-significant
Glycemic	HbA1c (%)	+ 0.123	0.516	Non-significant
Lipid Profile				
Lipid Profile	TG (mg/dL)	+ 0.052	0.786	Non-significant
Lipid Profile	TC (mg/dL)	-0.058	0.762	Non-significant
Lipid Profile	HDL-C (mg/dL)	+ 0.151	0.425	Non-significant
Lipid Profile	LDL-C (mg/dL)	-0.083	0.664	Non-significant
Lipid Profile	VLDL-C (mg/dL)	+ 0.052	0.786	Non-significant

Pearson correlation coefficients (r) between serum IL-35 and clinical parameters in diabetic nephropathy patients ($n = 30$). FSG, fasting serum glucose; BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol. * Statistically significant at $p < 0.05$.

Table 3: Schematic summary of correlations between serum IL-35 levels and glycemic markers in diabetic nephropathy patients. Each panel (A-E) shows the Pearson correlation coefficient (r) and associated p-value. Arrows denote direction of association; all correlations were non-significant ($p > 0.05$).

Correlation of Serum IL-35 with Glycemic Markers in Diabetic Nephropathy (n = 30)				
Pearson Correlation Analysis · Arrow direction indicates correlation sign · All associations non-significant ($p > 0.05$) except age				
(A) ↗ Fasting Insulin $r = + 0.024$ $p = 0.899$ Positive	(B) ↗ Fasting Glucose $r = + 0.274$ $p = 0.143$ Positive	(C) ↗ HbA1c $r = + 0.123$ $p = 0.516$ Positive	(D) ↗ HOMA-IR $r = + 0.162$ $p = 0.392$ Positive	(E) ↘ HOMA- β $r = -0.127$ $p = 0.502$ Negative
Note: None of the glycemic parameters showed a statistically significant correlation with serum IL-35 levels in the DN group (all $p > 0.05$), suggesting that IL-35 may reflect immune-mediated renal damage rather than glycemic dysregulation.				

Diagnostic Performance of Serum IL-35 in Discriminating Disease Groups

The receiver operating characteristic (ROC) curve analysis is performed to assess the diagnostic potential of serum IL-35 as a biomarker for diabetes mellitus (DM) and diabetic nephropathy (DN). Three comparisons were made: healthy controls versus DM patients, controls versus DN patients, and DM versus DN patients. When comparing controls and DM patients, IL-35 demonstrated good discriminative power with an area under the curve (AUC) of 0.87. The optimal cutoff value for IL-35 was 46.12 pg/mL, which yielded a sensitivity of 93% and a specificity of 87%, indicating a strong ability to distinguish diabetic individuals

from healthy controls. In the comparison between controls and DN patients, IL-35 exhibited excellent diagnostic performance, achieving an AUC of 0.93. At a cutoff value of 26.81 pg/mL, IL-35 was able to differentiate DN patients from healthy subjects with both sensitivity and specificity of 93%, suggesting its potential as a reliable biomarker for detecting advanced disease. For differentiating DM patients from those with DN, IL-35 levels also demonstrated high discriminative accuracy, with an AUC of 0.88. The optimal cutoff value of 24.77 pg/mL provided a sensitivity of 90% and a specificity of 87%, underscoring its value in detecting the progression from uncomplicated diabetes to nephropathy.

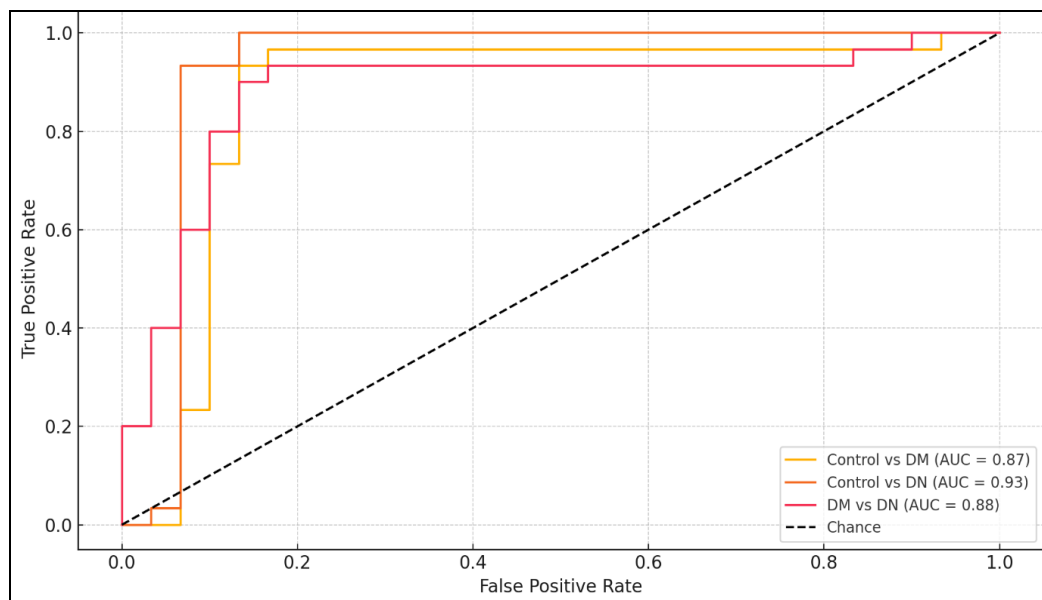


Fig 2: Receiver Operating Characteristic (ROC) Curve of Serum IL-35 Levels in Discriminating Diabetes Mellitus and Diabetic Nephropathy

Discussion

Diabetic nephropathy (DN) is one of the most common causes of kidney failure, and it is characterized by progressive kidney damage due to long-term high blood sugar levels. In this study, we investigated the serum levels of interleukin-35 (IL-35) among healthy controls, diabetic patients, and individuals with diabetic nephropathy (DN). Our findings revealed a significant and progressive decline in IL-35 levels from healthy individuals to those with diabetes and further in patients with DN. These results reinforce the growing understanding of IL-35 as an immunosuppressive cytokine involved in the regulation of chronic inflammation, which underpins the pathogenesis and progression of diabetic complications, including DKD.

IL-35, a member of the IL-12 cytokine family, is secreted predominantly by regulatory T cells and has demonstrated anti-inflammatory properties. The marked reduction of IL-35 observed in our DN group supports previous evidence that IL-35 levels are diminished in renal inflammatory conditions and may reflect disease severity. In a recent study, IL-35 levels were found to be significantly decreased in diabetic patients with renal involvement compared to those without nephropathy, implicating its role in modulating renal inflammation [30]. Though most prior research has focused on autoimmune renal diseases like lupus nephritis, IL-35's diagnostic potential extends to broader renal pathologies. Nassif *et al.* reported that IL-35 levels in both serum and urine were significantly lower in lupus nephritis patients, suggesting its utility as a non-invasive biomarker of renal inflammation [31].

The results align with experimental models in which IL-35 administration ameliorated renal inflammation. For instance, Yao *et al.* observed that IL-35 levels decreased in septic mice with acute kidney injury, and IL-35 supplementation mitigated renal damage through suppression of inflammatory cytokines and oxidative stress [32]. These findings suggest that diminished IL-35 may contribute to unchecked inflammation in chronic diabetic states, accelerating renal injury. Similarly, in a recent rodent model, IL-35 pretreatment significantly alleviated lipopolysaccharide-induced kidney injury by inhibiting nuclear factor-kappa B (NF- κ B) activation, a key regulator

of inflammatory gene expression [33]. While our study is cross-sectional, the inverse correlation between IL-35 levels and disease severity reinforces IL-35's proposed nephroprotective role.

Interestingly, our data showed no significant correlation between IL-35 and glycemic markers (fasting serum glucose, HbA1c, HOMA indices), suggesting that IL-35 may reflect immune-mediated damage more than metabolic dysregulation. This echoes findings in type 1 diabetes models where IL-35 was shown to modulate immune responses independent of glycemic control, offering protection against β -cell destruction [34]. Moreover, Neubauer-Geryk *et al.* identified IL-35 alterations in young patients with type 1 diabetes, correlating the cytokine's levels with cutaneous microvascular dysfunction a complication also tied to systemic inflammation [35]. Such results support the role of IL-35 as a sensitive marker for inflammatory microangiopathic changes, a hallmark of diabetic complications.

In the correlation analysis, age was the only variable that showed a statistically significant positive relationship with IL-35 levels in DN patients. This finding contrasts with expectations, as aging typically promotes a pro-inflammatory milieu ("inflammaging"). One plausible explanation could be a compensatory immune-regulatory mechanism in older individuals, particularly in chronic disease states, although more data are required to confirm this hypothesis. The lack of association between IL-35 and lipid parameters, BMI, or waist-hip ratio indicates that IL-35 may be a more specific marker of immunologic and renal perturbation than general metabolic status.

This study reinforces the potential of serum interleukin-35 (IL-35) as a non-invasive biomarker for differentiating among stages of diabetes mellitus (DM) and its progression to diabetic nephropathy (DN). The ROC curve analysis demonstrated robust discriminative performance of IL-35, with an AUC of 0.87 in distinguishing DM from healthy controls, 0.93 in DN versus controls, and 0.88 in DM versus DN comparisons. These metrics, combined with high sensitivity and specificity at optimized cutoff values, support IL-35's diagnostic strength.

Comparable findings were reported by Hassan *et al.* in a 2024 study evaluating IL-35 levels in diabetic individuals. Although focused on periodontal inflammation, they noted that IL-35 had a diagnostic accuracy of 82.2% using ROC analysis in diabetes-related immune dysregulation, suggesting systemic immunoinflammatory alterations in diabetic patients influence IL-35 levels broadly [36]. Also, comparable diagnostic performance of IL-35 has been observed in other immune-mediated renal conditions. For instance, in patients with lupus nephritis, Nassif (2021) reported an AUC of 0.914 for serum IL-35, distinguishing nephritic from non-nephritic SLE patients, reinforcing its diagnostic relevance in inflammatory renal conditions [31]. Similarly, Zhang *et al.* evaluated IL-35 in idiopathic membranous nephropathy and found that serum IL-35 levels could predict remission duration, highlighting its prognostic as well as diagnostic potential [37]. Additionally, Farahat and Hassan (2024) demonstrated elevated diagnostic value of IL-35 in lupus nephritis with strong ROC metrics, reinforcing our conclusion that IL-35 performs robustly as a non-invasive biomarker across a spectrum of renal inflammatory diseases [38].

Although IL-35 has not been widely studied in the context of diabetic nephropathy, its role as an immunomodulatory cytokine positions it well for such clinical utility. Its ability to suppress pro-inflammatory signaling—especially pathways involving IL-6, IL-17, and TNF- α —may explain its depletion in advanced disease states, where inflammatory responses are heightened. Given its impressive AUC, sensitivity, and specificity in our study, IL-35 may aid in early detection of nephropathy in diabetic patients, potentially informing timely therapeutic interventions. These findings, in conjunction with our results, suggest that IL-35 could serve as an early and reliable marker for detecting the onset and progression of DN in diabetic individuals. Its immunosuppressive role in modulating inflammatory cytokines such as IL-6 and TNF- α likely underpins this diagnostic utility, reflecting the level of immune activation across disease states. However, further prospective studies are warranted to validate its clinical application and assess its performance in larger, diverse populations.

The study has several limitations that should be considered. Firstly, the cross-sectional design limits our ability to infer causality between serum IL-35 levels and diabetic kidney disease (DKD) progression, and longitudinal studies are needed to assess the temporal relationship. Secondly, the relatively small sample size of 30 participants per group may affect the generalizability of the findings, necessitating larger, multi-center studies to confirm these results. Additionally, the lack of data on renal tissue expression of IL-35 prevents a full understanding of its role in kidney inflammation, and future studies should incorporate tissue analysis. Furthermore, potential confounding factors, such as medication use, diabetes duration, and comorbidities, were not fully controlled, which could influence inflammatory markers. Finally, while several clinical and biochemical variables were assessed, the study did not evaluate other relevant inflammatory mediators that might interact with IL-35, suggesting that broader cytokine profiling could provide more comprehensive insights into its regulatory role in DKD.

Conclusion

In summary, the findings confirm that serum IL-35 levels are significantly reduced in diabetic nephropathy and inversely associated with disease progression. The cytokine's immune-modulatory properties, coupled with consistent findings in experimental and human studies, underscore its potential as a biomarker for DKD. Prospective and mechanistic studies are needed to validate IL-35's diagnostic and therapeutic utility in diabetes-associated renal disease.

Declarations

Conflict of Interest

The authors declare that they have no conflicts of interest relevant to this study.

Ethical Approval

This study was approved by the Ethics Committee of Al-Sader Educational Hospital. All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki declaration and its later amendments.

Informed Consent

Written informed consent was obtained from all individual participants included in the study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

Ammar K. Madlool: Study design, data collection, manuscript writing.

Author have read and approved the final manuscript.

Data Availability

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

References

1. Omoruyi F, Dilworth L, Facey A. Diabetes mellitus and its metabolic complications: the role of adipose tissues. 2021.
2. Xu Y, He Z, King GL. Introduction of hyperglycemia and dyslipidemia in the pathogenesis of diabetic vascular complications. *Curr Diabetes Rep.* 2005;5(2):91-97.
3. Dlodla PV, *et al.* Pancreatic β -cell dysfunction in type 2 diabetes: implications of inflammation and oxidative stress. *World J Diabetes.* 2023;14(3):130-146. doi:10.4239/wjd.v14.i3.130.
4. Jha R, Lopez-Trevino S, Kankanamalage HR, Jha JC. Diabetes and renal complications: an overview on pathophysiology, biomarkers and therapeutic interventions. *Biomedicines.* 2024;12(5):1098.
5. Lim A. Diabetic nephropathy—complications and treatment. *Int J Nephrol Renovasc Dis.* 2014;7:361-381. doi:10.2147/IJNRD.S40172.

6. Selby NM, Taal MW. An updated overview of diabetic nephropathy: diagnosis, prognosis, treatment goals and latest guidelines. *Diabetes Obes Metab.* 2020;22:3-15.
7. Ceriello A, Prattichizzo F, Phillip M, Hirsch IB, Mathieu C, Battelino T. Glycaemic management in diabetes: old and new approaches. *Lancet Diabetes Endocrinol.* 2022;10(1):75-84.
8. Persson F, Rossing P. Diagnosis of diabetic kidney disease: state of the art and future perspective. *Kidney Int Suppl.* 2018;8(1):2-7.
9. Afkarian M, *et al.* Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. *JAMA.* 2016;316(6):602-610. doi:10.1001/jama.2016.10924.
10. Pereira-Cespedes A, Jimenez-Morales A, Palomares-Bayo M, Martinez-Martinez F, Calleja-Hernandez MA. Medication review with follow-up for end-stage renal disease: drug-related problems and negative outcomes associated with medication—a systematic review. *J Clin Med.* 2023;12(15):5080.
11. Fu H, Liu S, Bastacky SI, Wang X, Tian XJ, Zhou D. Diabetic kidney diseases revisited: a new perspective for a new era. *Mol Metab.* 2019;30:250-263.
12. Khalid M, Petroianu G, Adem A. Advanced glycation end products and diabetes mellitus: mechanisms and perspectives. *Biomolecules.* 2022;12(4):542.
13. Jin Q, *et al.* Oxidative stress and inflammation in diabetic nephropathy: role of polyphenols. *Front Immunol.* 2023;14:1185317. doi:10.3389/fimmu.2023.1185317.
14. Opazo-Ríos L, *et al.* Lipotoxicity and diabetic nephropathy: novel mechanistic insights and therapeutic opportunities. *Int J Mol Sci.* 2020;21(7):2632. doi:10.3390/ijms21072632.
15. Diaz-Ricart M, *et al.* Endothelial damage, inflammation and immunity in chronic kidney disease. *Toxins.* 2020;12(6):361.
16. Mihai S, *et al.* Inflammation-related mechanisms in chronic kidney disease prediction, progression, and outcome. *J Immunol Res.* 2018;2018:2180373.
17. Ye C, Yano H, Workman CJ, Vignali DAA. Interleukin-35: structure, function and its impact on immune-related diseases. *J Interferon Cytokine Res.* 2021;41(11):391-406. doi:10.1089/jir.2021.0147.
18. Dambuza IM, *et al.* IL-12p35 induces expansion of IL-10 and IL-35-expressing regulatory B cells and ameliorates autoimmune disease. *Nat Commun.* 2017;8(1):719. doi:10.1038/s41467-017-00838-4.
19. Hildenbrand K, *et al.* Human interleukin-12 α and EB13 are cytokines with anti-inflammatory functions. *Sci Adv.* 2023;9(43):eadg6874.
20. Collison LW, *et al.* The composition and signaling of the IL-35 receptor are unconventional. *Nat Immunol.* 2012;13(3):290-299.
21. Murakami M, Kamimura D, Hirano T. Pleiotropy and specificity: insights from the interleukin 6 family of cytokines. *Immunity.* 2019;50(4):812-831.
22. Cai Z, *et al.* A novel potential target of IL-35-regulated JAK/STAT signaling pathway in lupus nephritis. *Clin Transl Med.* 2021;11(2):e309. doi:10.1002/ctm2.309.
23. Wang RX, *et al.* Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat Med.* 2014;20(6):633-641.
24. Wang D, Lei L. Interleukin-35 regulates the balance of Th17 and Treg responses during the pathogenesis of connective tissue diseases. *Int J Rheum Dis.* 2021;24(1):21-27. doi:10.1111/1756-185X.13962.
25. Olson BM, Sullivan JA, Burlingham WJ. Interleukin 35: a key mediator of suppression and the propagation of infectious tolerance. *Front Immunol.* 2013;4:315.
26. Teymouri M, Pirro M, Fallarino F, Gargaro M, Sahebkar A. IL-35, a hallmark of immune regulation in cancer progression, chronic infections and inflammatory diseases. *Int J Cancer.* 2018;143(9):2105-2115.
27. Wu D, *et al.* Interleukin 35 contributes to immunosuppression by regulating inflammatory cytokines and T cell populations in the acute phase of sepsis. *Clin Immunol.* 2022;235:108915.
28. Zhang SM, *et al.* Interleukin 35: protective role and mechanism in type 1 diabetes. *Cent Eur J Immunol.* 2023;48(1):48-53. doi:10.5114/ceji.2023.125043.
29. Jiang H, Zhang T, Yan MX, Wu W. IL-35 inhibits CD8 + T cells activity by suppressing expression of costimulatory molecule CD28 and Th1 cytokine production. *Transl Cancer Res.* 2019;8(4):1319-1326.
30. Abdel-Moneim A, Abd El-Twab SM, Nabil A, El Kazafy SA. Effect of antidiabetic therapy on TNF- α , IL-18, IL-23 and IL-35 levels in T2DM patients with coincidental *Helicobacter pylori* infection. *J Taibah Univ Sci.* 2020;14(1):1377-1385.
31. Nassif MA. Urine and serum interleukin-35 as potential biomarkers of lupus nephritis. *Cent Eur J Immunol.* 2021;46(3):351-359. doi:10.5114/ceji.2021.109151.
32. Yao F, *et al.* The renal level of a novel cytokine IL-35 is related to sepsis-associated acute kidney injury in mice. *Int J Clin Exp Pathol.* 2017;10(11):10998-11005.
33. Hu L, *et al.* IL-35 pretreatment alleviates lipopolysaccharide-induced acute kidney injury in mice by inhibiting NF- κ B activation. *Inflammation.* 2017;40(4):1393-1400. doi:10.1007/s10753-017-0582-9.
34. Chakraborty R, Mukherjee AK, Bala A. Breakthroughs in road mapping IL-35 mediated immunotherapy for type-1 and autoimmune diabetes mellitus. *Cytokine.* 2024;181:156692.
35. Neubauer-Geryk J, Wielicka M, Myśliwiec M, Zorena K, Bieniaszewski L. The relationship between TNF- α , IL-35, VEGF and cutaneous microvascular dysfunction in young patients with uncomplicated type 1 diabetes. *Biomedicines.* 2023;11(10):2857.
36. Hassan SS, Abdelkawy M, Shaker OG, Tarrad NAF. IL-39 and IL-35 gingival crevicular fluid levels in diabetic patients with generalized periodontitis. *Clin Oral Investig.* 2024;28(2):124.
37. Zhang N, *et al.* Level of interleukin-35 in patients with idiopathic membranous nephropathy and its predictive value for remission time. *Front Immunol.* 2022;13:926368.
38. Farahat MNR, Hassan MHA, Assem AA, El Nasherty MMA. Serum interleukin-35 as a non-invasive biomarker in lupus nephritis patients. *Al-Azhar Int Med J.* 2024;5(6):54.