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Research Article

Diagnostic value of Interleukin-40 and Interleukin-41 for Diabetic Kidney Disease and Diabetes

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Abstract

Background: Diabetic kidney disease (DKD) is a significant microvascular complication of diabetes and a leading cause of end-stage renal disease (ESRD) globally. Inflammatory mediators play a key role in the development of DKD. However, the impact of specific cytokines, such as interleukin-40 (IL-40) and interleukin-41 (IL-41), has not been sufficiently clarified, with limited published data available. This study aimed to evaluate IL-40 and IL-41 levels in individuals with DKD and diabetes mellitus (DM) patients without nephropathy and to investigate their diagnostic capability as biomarkers for distinguishing between these conditions.
Methods: This case-control study included 127 participants, divided into three groups: 42 patients with DKD, 43 diabetic patients without kidney disease, and 42 healthy controls. Serum levels of IL-40 and IL-41 were measured using an ELISA assay. Statistical analysis was performed using SPSS, and the diagnostic performance of these markers was evaluated using ROC curve analysis.
Results: The study demonstrated a significant decrease in IL-40 and IL-41 concentrations in DKD patients (IL-40: 15.791 ± 8.012 pg/ml, and IL-41: 0.525 ± 0.649 pg/ml) and those with DM (IL-40: 14.114 ± 3.686 pg/ml, and IL-41: 0.641 ± 0.534 pg/ml) compared to the control group (IL-40: 29.859 ± 8.742 pg/ml; IL-41: 3.013 ± 0.983 pg/ml) ($p < 0.001$). ROC curve analysis showed that IL-41 was more accurate in distinguishing between the control group and DKD patients (98.394%) and between the control group and DM patients (99.567%).
Conclusions: The ability of IL-40 and IL-41 cytokines was limited in differentiating between DKD patients and diabetes.
Keywords: Interleukin-40, interleukin-41, diabetes kidney disease, diabetes

Introduction

Diabetic kidney disease (DKD) represents a significant microvascular complication of diabetes mellitus (DM) and a leading cause of end-stage renal disease (ESRD) globally. This condition affects approximately 30-50% of individuals with type 1 diabetes mellitus (T1DM) and 20-40% of those with type 2 diabetes mellitus (T2DM) [1]. DKD is characterized by detrimental structural changes, including glomerular basement membrane hypertrophy, podocyte depletion, mesangial matrix thickening, and foot process fusion [2]. This disease's complex pathophysiological mechanisms encompass hyperglycemia-induced renal hemodynamic changes, oxidative stress, inflammatory responses, hypoxia, and activation of the renin-angiotensin-aldosterone system (RAAS) [3].

Recent studies have emphasized the importance of the immune system and inflammatory mediators in the pathogenesis of DKD, suggesting that cytokines may be pivotal in disease progression [4]. According to Donate-Correa *et al*. (2021), chronic hyperglycemia activates multiple inflammatory pathways, contributing to the development and progression of DKD [5]. Furthermore, another study by Donate-Correa *et al*. (2015) has highlighted that the imbalance between pro- and anti-inflammatory cytokines plays a significant role in determining the severity of diabetes and its complications [6]. However, the precise relationship between chronic inflammation and the development of DKD remains incompletely understood, particularly the role of some newly discovered cytokines.

Interleukin-40 (IL-40) and interleukin-41 (IL-41) have recently been identified as potential

modulators of immune responses. IL-40 has been linked to B-cell-mediated immune reactions [7], contributing to regulating B-cell responses in various contexts of chronic inflammation. A study by Navrátilová *et al*. (2021) revealed that IL-40 is increased in autoimmune diseases such as rheumatoid arthritis and decreases after B-cell-targeted therapy [8]. Conversely, IL-41 has demonstrated anti-inflammatory properties in multiple disease models. According to a study by Zhang *et al*. (2023), IL-41 can serve as a biomarker for anti-inflammatory response associated with hyperuricemia and other metabolic disorders [9]. Despite these discoveries, the roles of these cytokines in the context of diabetes and its complications, particularly DKD, remain largely unexplored.

Current research indicates that multiple biomarkers may be helpful in detecting DKD early and assessing its progression. Investigators have identified a variety of potential biomarkers, including kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), cystatin C, and several inflammatory cytokines [10]. As Alwahid *et al.* (2023) reported that emerging biomarkers like asprosin have shown promise in the early detection of nephropathy in T2DM, demonstrating significant diagnostic potential with high sensitivity and specificity [11]. Furthermore, a study by Al-Sudani and Al-Shammaa (2024) noted that inflammatory markers like urokinase-type plasminogen activator (UPA) could play significant roles in hemodialysis patients with T2DM [12]. However, the role of IL-40 and IL-41 in this context has not been adequately investigated, with a scarcity of published data, especially in Iraq.

Given the complex relationship between diabetes and DKD, it is essential to understand the differences in inflammatory profiles between diabetic patients with and without kidney complications. Donate-Correa *et al.* (2023) suggested that there are distinctive differences in cytokine dynamics between these two patient groups, which may help identify factors contributing to the development of DKD [13]. However, the role of IL-40 and IL-41 in this context has not been adequately explored.

This study aims to evaluate IL-40 and IL-41 concentrations in three groups: DKD patients, diabetic patients without kidney complications, and healthy individuals, and to investigate their potential as diagnostic biomarkers for distinguishing between these conditions. By including both diabetic patients with and without kidney complications, the study aims to elucidate whether changes in these cytokines are primarily associated with diabetes itself or with the subsequent development of kidney complications. The aim of this study is to enhance understanding of the inflammatory processes involved in diabetes and DKD, identifying potential new targets for diagnostic and therapeutic intervention.

Materials and Methods

Patients

This case-control study included 127 participants, divided into three groups: The diabetic kidney disease (DKD) group comprised 42 patients diagnosed with diabetic kidney disease based on estimated glomerular filtration rate (eGFR), diabetes history, glycated hemoglobin (HbA1c), and serum albumin levels. The diabetes mellitus (DM) group included 43 patients with diabetes without kidney complications, diagnosed based on elevated fasting blood glucose and HbA1c levels, with normal kidney function (eGFR > 90 ml/min/1.73m² and no proteinuria). The healthy control group consisted of 42 participants with no history of diabetes, hypertension, or kidney disorders, with normal fasting blood glucose (< 100 mg/dl) and HbA1c levels (< 5.7%). The study was conducted at Al-Imam AL-Hussein Medical City Hospital in Karbala City between November 2022 and January 2023. Demographic data (age, gender, weight) and clinical data (disease duration and medications) were recorded using standardized questionnaires. Exclusion criteria included participants with chronic liver diseases, acute infections, pregnancy, autoimmune diseases, and a history of nephrotoxic medication use.

Sample collection

Venous blood samples (5 mL) were collected using BD Vacutainer® serum tubes (model: 20231202; Becton Dickinson, USA). The Serum was separated by centrifugation (2,000 - 3,000 RBM, 20 min, room temperature) and stored at -80°C until analysis (within storage: 6 months). Target biomarker assay range for IL40 and IL41 was 1.5-96 ng/ml and 0.05-15 ng/ml, respectively. Serum samples were analyzed using an ELISA Kit model human protein IL-40 and IL-41 (BT LAB, China, Cat. No. E4654Hu for IL-40, and BT LAB, Cat. No. E3491Hu for IL-41).

Assay protocol

The ELISA assay was validated according to ICH Q2(R1) guidelines[14]. Precision was assessed by analyzing three serum samples in six replicates (intra-assay CV% = 8.5%) and over three consecutive days (inter-assay CV% =12.3%), all

within acceptable limits (<15%). Accuracy was determined through spike-and-recovery studies (n=5) at three concentration levels, showing mean recovery rates of 96-102% (acceptable range: 85-115%). Linearity was confirmed by serial dilutions (2-200 ng/mL) with R^2 = 0.998. The assay demonstrated a limit of detection (LOD) of 0.2 ng/mL (3×SD of blank). No significant cross-reactivity (<5%) was observed with analogous proteins, confirming high specificity.

Ethical approval

The Karbela Health Directorate's ethical committees approved the study with reference number 3773 on 3 November 2022. Prior to enrollment, all participants provided written informed consent. The study adhered to the ethical principles outlined in the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Data confidentiality was maintained using anonymized identifiers.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) (version 23). Data were expressed as mean ± standard deviation. Analysis of variance (ANOVA) and the Kruskal-Wallis test were used to determine statistically significant differences between multiple independent groups. Multiple comparison tests were conducted to identify specific differences between groups. Receiver operating characteristic (ROC) curves were plotted to evaluate the diagnostic capability of biomarkers, cutoff value, the area under the curve (AUC), sensitivity (%), specificity (%), positive predictive value (PPV), negative predictive value (NPV), and accuracy were determined. Statistical significance was set at $P < 0.05$.

Results

As illustrated in Table 1, the current study demonstrated statistically significant differences among the three study groups (control, DKD, DM) across all examined inflammatory and metabolic parameters. The results revealed a significant decrease in IL-40 levels in the DKD group (15.791 ± 8.012 pg/mL) and DM group (14.114 ± 3.686 pg/mL) compared to the control group (29.859 ± 8.742 pg/mL) (p=0.0002). Similarly, IL-41 levels were markedly decreased in the DKD group (0.525 ± 0.649 pg/mL) and DM group (0.641 ± 0.534 pg/mL) compared to the control group (3.013 ± 0.983 pg/mL) (p=0.0004). Additionally, a notable elevation in serum ferritin (S. ferritin) levels was recorded in the DKD group (315.586 ± 215.687 ng/mL) compared to the control group (78.209 ± 29.323 ng/mL) and DM group (122.079 ± 71.268 ng/mL)(p=0.0006). Regarding the unsaturated iron-binding capacity (UIBC), a significant increase was observed in the DKD group (247.781 ± 81.081 µg/dL) compared to the DM group (162.395 ± 11.632 µg/dL). At the same time, the difference was not significant compared to the control group (228.163 ± 34.868 µg/dL) (p=0.0004). Serum iron levels were significantly decreased in the DKD group (82.833 ± 50.662 µg/dL) and DM group (85.023 ± 9.580 µg/dL) compared to the control group (104.349 ± 14.832 µg/dL) (p=0.0023). The estimated glomerular filtration rate (eGFR) results demonstrated a severe reduction in the DKD group (8.888 ± 2.699) compared to the control group (132.226 ± 8.288) and DM group (133.488 ± 4.250) (p=0.0001). Furthermore, a significant elevation in serum phosphate (S. PO_4) levels was documented in the DKD group (5.055 ± 1.379 mg/dL) and DM group (4.079 ± 0.353 mg/dL) compared to the control group (3.228 ± 0.474 mg/dL) (p=0.0003). Values are presented as mean ± standard deviation SD). Different superscript letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$) based on a multiple comparison test.

DKD, Diabetic Kidney Disease; DM, Diabetes Mellitus; UIBC, Unsaturated Iron Binding Capacity; eGFR, Estimated Glomerular Filtration Rate; S.PO4, Serum Phosphate.

The ROC curve analysis, as shown in Table 2, demonstrated excellent diagnostic capability of IL-41 in differentiating between the DKD group and control group (AUC = 98.394%, 95% CI: 0.963-1.000, p=0.001) with 95.238% sensitivity, 95.349% specificity, and 95.294% accuracy at a cutoff value of 1.850 pg/mL, while IL-40 showed good diagnostic performance (AUC = 89.646%, 95% CI: 0.820-0.973, p = 0.003) with 83.333% sensitivity, 97.674% specificity, and 90.588% accuracy at a cutoff value of 17.960 pg/mL.

Regarding the differentiation between the DM group and control group, Table 3 showed that IL-41 achieved excellent diagnostic performance (AUC = 99.567%) with 97.674% sensitivity, 95.349% specificity, and 96.512% accuracy at a cutoff value of 1.807 pg/mL, and IL-40 also demonstrated excellent performance (AUC = 97.783%) with 90.698% sensitivity, 97.674% specificity, and 94.186% accuracy at a cutoff value of 17.953 pg/mL. However, both markers exhibited relatively poor performance in differentiating between the DKD group and DM group, as indicated in Table 4, with AUC values for IL-41 (62.652%) and IL-40 (52.658%),

accompanied by moderate to poor sensitivity and specificity. Collectively, these findings suggest that IL-41 and IL-40 could serve as valuable diagnostic tools in differentiating between diabetic and diabetic kidney disease patients and healthy individuals, with IL-41 demonstrating superior diagnostic performance. In contrast, the differentiation between diabetic and diabetic kidney disease patients remains challenging and requires additional markers or alternative diagnostic approaches.

Discussion

The current study provides valuable data on the potential diagnostic role of IL-40 and IL-41 in diabetic kidney disease (DKD) and diabetes mellitus (DM).

Table 1: Comparative analysis of interleukin-40 and interleukin-41, Iron metabolism, and renal function parameters (eGFR, and serum PO_4) among control, diabetic and diabetic kidney disease grou**ps.**

Parameters	Control Group		DKD Group		DM Group		p- value
	Mean	SD	Mean	SD	Mean	SD	
IL-40 (pg/mL)	29.859[a]	8.742	15.791[b]	8.012	14.114[bc]	3.686	0.0002
IL-41 (pg/mL)	3.013[a]	0.983	0.525[b]	0.649	0.641[bc]	0.534	0.0004
S.ferritin (ng/mL)	78.209[a]	29.323	315.586[b]	215.687	122.079[ac]	71.268	0.0006
UIBC (µg/dL)	228.163[a]	34.868	247.781[a]	81.081	162.395[c]	11.632	0.0004
Iron (µg/dL)	104.349[a]	14.832	82.833[b]	50.662	85.023[bc]	9.580	0.0023
eGFR	132.226[a]	8.288	8.888[b]	2.699	133.488 [ac]	4.250	0.0001
S.PO4 (mg/dL)	3.228[a]	0.474	5.055[b]	1.379	4.079[c]	0.353	0.0003

Table 2: Diagnostic performance of interleukin-40 and interleukin-41 in distinguishing between diabetic kidney disease patients and control group.

Metrics		Group	
		DKD	Control
		IL-40	IL-41
Standard error		0.039	0.011
Asymptotic significance		0.003	0.001
Asymptotic 95% Confidence Interval	Lower Bound	0.820	0.963
	Upper Bound	0.973	1.000
Cutoff value		17.960	1.850
Area Under Curve (AUC)		89.646%	98.394%
Sensitivity		83.333%	95.238%
Specificity		97.674%	95.349%
Accuracy		90.588%	95.294%
Positive Predictive Value		97.222%	95.238%
Negative Predictive Value		85.714%	95.349%
DKD: Diabetic Kidney Disease			

Table 3: Diagnostic performance of interleukin-40 and interleukin-41 in distinguishing between diabetes mellitus patients and control group

Metrics		Group	
		DM	Control
		IL-40	IL-41
Standard error		0.012	0.004
Asymptotic significance		0.002	0.003
Asymptotic 95% Confidence Interval	Lower Bound	0.954	0.988
	Upper Bound	1.000	1.000
Cutoff value		17.953	1.807
Area Under Curve (AUC)		97.783%	99.567%
Sensitivity		90.698%	97.674%
Specificity		97.674%	95.349%
Accuracy		94.186%	96.512%
Positive Predictive Value		94.186%	95.455%
Negative Predictive Value		94.186%	97.619%
DM: Diabetic mellitus			

Table 4: Diagnostic performance of interleukin-40 and interleukin-41 in distinguishing between diabetic kidney disease and diabetes mellitus patients.

Metrics		Group	
		DKD	DM
		IL-40	IL-41
Standard error		0.064	0.062
Asymptotic significance		0.673	0.045
Asymptotic 95% Confidence Interval	Lower Bound	0.401	0.505
	Upper Bound	0.652	0.748
Cutoff value		14.604	0.183
Area Under Curve (AUC)		52.658%	62.652%
Sensitivity		66.667%	42.857%
Specificity		48.837%	86.047%
Accuracy		42.353%	35.294%
Positive Predictive Value		44.000%	25.000%
Negative Predictive Value		40.000%	39.344%

DKD: Diabetic Kidney Disease, DM: Diabetic mellitus

Analysis of the data presented in the tables showed a significant decrease in both IL-40 and IL-41 levels in patients with diabetic kidney disease and diabetes compared to healthy individuals. The decrease is evident in IL-40 and IL-41 levels in the diabetic kidney disease group and the diabetes group compared to the control group.

These findings contradict some previous studies, such as Nussrat *et al.* (2023), which indicated elevated IL-40 levels in the serum of type 2 diabetes patients compared to healthy individuals, raising questions about the different mechanisms that may affect these cytokine levels in various pathological conditions or diverse population groups [15].

The data also reveal a significant relationship between cytokines and iron metabolism disorders in diabetic kidney disease patients, where a significant increase in serum ferritin levels was observed in diabetic kidney disease patients compared to the control group and diabetes group, along with a marked decrease in serum iron levels. These findings align with Wang *et al.* (2025) [16] study, which showed that biomarkers related to ferroptosis (iron-dependent cell death) can have predictive value in diabetic kidney disease. They also indicated a reciprocal relationship between ferroptosis and innate immunity in diabetic kidney disease, where iron metabolism disorders can exacerbate inflammation, and similarly, chronic inflammatory conditions can affect iron metabolism, forming a closed loop of damage. As Xie *et al.* (2024) highlighted, advances in understanding iron metabolism and oxidative stress in kidney diseases have revealed important mechanisms linking iron dysregulation to cellular dysfunction in both experimental models and human kidney diseases [17].

It is also interesting to note the sharp decline in estimated glomerular filtration rate (eGFR) in diabetic kidney disease patients compared to the control group and diabetes group, along with a significant increase in serum phosphate levels in diabetic kidney disease and diabetic patients. According to Yang *et al.* (2025), the stages of diabetic kidney disease affect calcium and phosphorus metabolism in the body, and with decreasing glomerular filtration rate, changes occur in mineral balance, including increased serum phosphate levels [18]. They also suggested that bone metabolism markers, including phosphate levels, can have predictive value in the development of diabetic kidney disease.

ROC curve analysis demonstrated high diagnostic value for IL-40 and IL-41 in distinguishing between healthy individuals and diabetic kidney disease or diabetic patients. From a biological perspective, IL-40 is associated with B cells and plays a vital role in humoral immune response, as indicated by Navrátilová *et al.* (2021) [8]. The decrease in IL-40 levels in diabetic and diabetic kidney disease patients may indicate a disturbance in B cell function and humoral immune response. On the other hand, IL-41 is known for its anti-inflammatory properties in various disease models, as mentioned by Shi *et al.* (2023) [7]. The significant decrease in IL-41 levels in diabetic and diabetic kidney disease patients may indicate a reduced anti-inflammatory response, which could contribute to low-grade chronic inflammation associated with diabetes. As Zhang *et al.* (2023) [9] demonstrated, IL-41 can serve as a biomarker of the anti-inflammatory response

associated with hyperuricemia and other metabolic disorders, further supporting its potential role in diabetes-related inflammatory processes.

Donate-Correa *et al*. (2015) have emphasized the importance of inflammatory cytokines in diabetic kidney disease, highlighting their pathophysiologic and therapeutic implications [6]. They suggest that distinctive differences in cytokine dynamics between diabetic patients with and without kidney complications may help identify factors contributing to the development of DKD. The current findings regarding IL-40 and IL-41 add to this understanding of inflammatory profiles in diabetic and diabetic kidney disease patients.

These findings suggest the potential use of IL-40 and IL-41, especially IL-41, as diagnostic markers to distinguish between healthy individuals and diabetic or diabetic kidney disease patients. These markers can be helpful in the early detection of diabetes and the regular monitoring of patients. Understanding their role in immune and inflammatory diseases may also open new horizons for therapeutic interventions, such as using stimulators or analogs of IL-40 and IL-41 or targeting regulatory pathways that affect their production or activity.

Despite the critical findings, the study has several limitations, including the moderate sample size and cross-sectional design that does not allow for determining causal relationships or tracking changes in cytokine levels with disease progression. Future research directions include longitudinal studies to track changes in IL-40 and IL-41 levels with the development of diabetes and its complications, mechanistic research to understand these cytokines' cellular and molecular effects, and evaluating the relationship between their levels and other clinical indicators.

Conclusions

The current study provides important evidence of decreased IL-40 and IL-41 levels in diabetic and diabetic kidney disease patients compared to healthy individuals, with high diagnostic value in distinguishing between these groups. These findings open new horizons for understanding the immune and inflammatory mechanisms in diabetes and diabetic kidney disease. It also suggests their potential function as immune markers associated with inflammatory responses in diabetes. These cytokines are valuable diagnostic tools for distinguishing between healthy individuals and those with diabetes, but they may be less effective in differentiating diabetic kidney complications from diabetes itself.

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