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Research Article

Correlation of Kidney Injury Molecule-1 and Nephrin Levels in Iraqi Patients with Diabetic Nephropathy

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Abstract

Background: Diabetic nephropathy is characterized by persistent microalbuminuria and metabolic changes that decline renal functions. Researchers have been prompted to explore new biomarkers such as KIM-1 and nephrin that may enhance the identification of disease. ***Objective***: To Evaluate biomarker levels of kidney injury molculre-1 (KIM-1) concentration and nephrin as early and sensitive markers of nephropathy in type 2 diabetic patients. ***Method***: One hundred T2DM patients were included in a cross-sectional study at the specialized center for endocrinology and diabetes, Baghdad. The first group includes 50 diabetic nephropathy (DN) patients, and the second group includes 50 T2DM patients without DN. Biochemical and clinical parameters were reported for participants, and serum and urine levels of KIM-1 and nephrin were analyzed by Enzyme-linked immunosorbent assay. ***Results***: The study showed a significant increase in serum and urinary levels of KIM-1 and nephrin in DN patients compared to the control group. Serum nephrin is positively correlated with urinary nephrin, serum creatinine, ACR ratio, serum and urine KIM-1, and negatively correlated with the estimated glomerular filtration rate. Urinary nephrin was positively correlated with urinary albumin/creatinine ratio, KIM-1 level in both serum and urine, and negatively correlated with estimated glomerular filtration rate. ***Conclusion***: KIM-1 and nephrin are specific and sensitive indicators of early-stage diabetic nephropathy-associated renal damage.

Keywords: T2DM, Diabetic nephropathy, KIM-1, Nephrin, Renal function.

ارتباط جزيء إصابة الكلى -1 ومستويات النفرين لدى المرضى العراقيين المصابين باعتلال الكلية بسبب داء السكري

الخلاصة

الخلفية: يتميز اعتلال الكلية السكري ببيلة زلالية دقيقة مستمرة وتغيرات أيضية تقلل من وظائف الكلى. تم حث الباحثين على استكشاف مؤشرات حيوية جديدة مثل KIM-1 والنيفرين التي قد تعزز تشخيص المرض. **الهدف**: تقييم مستويات المؤشرات الحيوية لتركيز إصابة الكلى (KIM-1) والنيفرين كعلامات مبكرة وحساسة لاعتلال الكلية في مرضى السكري من النوع 2. **الطريقة**: تم تضمين مائة مريض T2DM في دراسة مقطعية في المركز التخصصي للغدد الصماء والسكري، بغداد. تضم المجموعة الأولى 50 مريضا باعتلال الكلية السكري (DN) وتضم المجموعة الثانية 50 مريضا من T2DM بدون DN. تم قياس المعلمات البيوكيميائية والسريرية للمشاركين، وتم تحليل مستويات المصل والبول من KIM-1 والنيفرين بواسطة مقايسة الممتز المناعي المرتبط بالإنزيم. **النتائج**: أظهرت الدراسة زيادة كبيرة في مستويات المصل والبول من KIM-1 والنيفرين في مرضى DN مقارنة بالمجموعة الضابطة. يرتبط نيفرين المصل بشكل إيجابي مع النيفرين البولي، الكرياتينين في الدم، نسبة ACR، مستوى KIM-1 في المصل والبول، ويرتبط سلبا بمعدل الترشيح الكبيبي المقدر. ارتبط النفرين البولي بشكل إيجابي مع نسبة الألبومين/الكرياتينين البولية، ومستوى KIM-1 في كل من المصل والبول وارتبط سلبا بمعدل الترشيح الكبيبي المقدر. **الأستنتاج**: KIM-1 و nephrin هي مؤشرات محددة وحساسة للتلف الكلوي المرتبط باعتلال الكلية السكري في المرحلة المبكرة.

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INTRODUCTION

Diabetic nephropathy (DN) is a multifactorial illness that is one of the most prevalent diabetes complications and a leading cause of chronic kidney disease [1,2]. It is distinguished by increased urine albumin excretion, reduced glomerular filtration rate, or both. Predictions indicate that the prevalence of diabetic nephropathy will continue to rise, putting a considerable strain on the healthcare system and leading to greater rates of morbidity and mortality [3-5]. As a result, early diagnosis and adequate care are critical to limiting the progression of kidney injury [6,7]. This highlights the critical importance of evaluating novel biomarkers for the early identification of DN. New and more specific markers for early diagnosis and prediction of DN that appear in the urine prior to microalbuminuria are currently being studied. Because it is difficult to identify urinary podocytes directly, most of the studies have focused on the involvement of podocyte-specific proteins as tubular damage markers, such as nephrin and kidney injury molecule-1 (KIM-1) [8,9]. Nephrin is a transmembrane protein that has been found as an essential biomarker for predicting diabetic kidney disease and the severity of podocyte injury [10]. The current study aimed to assess the serum and urinary levels of KIM and nephrin, as well as their involvement in the early detection of nephropathy in T2DM Iraqi patients.

METHODS

One hundred T2DM patients are included in this cross-sectional study. The Ethical and Scientific Committee in the College of Pharmacy at the University of Baghdad, Iraq, gave its approval for this cross-sectional study (REAFUBCP-632022A). The overall population of 100 Iraqis, both males and females, was enrolled in the study, with an age range over 18 years divided into 2 groups. The first group consists of 50 diabetic nephropathy patients with microalbuminuria (ACR 30-300 mg/g), and the second group consists of 50 T2DM patients without DN with normoalbuminuria (ACR <30 mg/g). All diabetic patients were diagnosed with T2DM by the specialized endocrinologist according to the 2019 American Diabetes Association (ADA) guideline: "fasting blood sugar (FBS) ≥126 mg/dL (7.0 mmol/L) OR 2-h plasma glucose ≥ 200mg/dL (11.1 mmol/L) during OGTT OR HbA1C ≥ 6.5% or a random blood sugar (RBS) ≥ 200 mg/dL (11.1 mmol/L). Diabetic patients with DKD have been selected by the professional consultant nephrologist and diagnosed based on the urinary albumin creatinine ratio (ACR) [ACR> 30 mg\g] , From each participating category (patients and controls), a random spot urine sample was collected in a suitable urine container and divided into two parts: the first one was used immediately for measurement of urinary albumin and urinary creatinine for calculation of the albumin to creatinine ratio ACR; the second part was centrifuged for 20 min at 3000 rpm and then dispensed in Eppendorf to be stored refrigerated at -20 °C for determination of urine nephrin and urine KIM-1 level by the enzyme-linked immunosorbent assay test. Then a five-milliliter venous blood sample obtained by a vein puncture was collected using a 5-ml disposable syringe. 3 ml samples were collected in a gel tube and let to be clotted at room temperature for 5–10 minutes, then centrifuged to obtain the serum for 10 min at 3000 rpm. The serum has been divided into two parts: one for immediate enzymatic analysis for measurements of serum creatinine and lipid profile; the other part is stored in an Eppendorf tube to be kept frozen at a temperature of -20 ºC until the time of analysis, using specific ELISA kits for measurements of serum nephrin and serum KIM-1 levels.

Statistical analysis

The IBM SPSS software platform 26 was used to analyze the effect of different factors on the study parameters. A one-way ANOVA and t-test were used to compare the means. The chi-square test was used to compare percentages. A significant difference was considered at $p < 0.05$. The GraphPad Prism 9 program was used to formulate the figures for this study.

RESULTS

The demographic, anthropometric, clinical, and biochemical characteristics of all participants enrolled in this study are summarized in Table 1. There were no significant differences in age, gender, duration of disease, smoking, or drinking habits between the study groups (p>0.05). On the other hand, a significant difference was found in the serum levels of creatinine, ACR, and eGFR between the control group and the patient groups (p<0.05). The ACR and creatinine levels were higher in the DN patient group than in the control group, while eGFR showed the lowest levels in the DN group. Serum levels of HbA1c, FBG, and body mass index (BMI) when compared between both groups revealed a non-significant difference (p>0.05). The distribution of DN patients according to the KIDGO eGFR stages was considerably different between the groups, with the majority of control patients at stage 1 and only 10 at stage 2. while the distribution of DN patients was uneven, with most patients at stage 1 (Table 1). The results demonstrated show highly significant differences (p<0.01) in the means of KIM-1 levels measured in serum and urine between patients with DN and control group, as illustrated in Table 2. For both serum and urine levels, the DN group showed the highest levels of KIM-1 over the control group. All samples were assessed for the detection of the level of nephrin in serum and urine by using the ELISA technique. The levels of nephrin show highly significant differences (p=0.0001) between both groups.

Table 1: Sociodemographic parameters of the study groups

Character		Group 1 *n*=50	Group 2 *n*= 50	*p*-value
Age (year)		50.92±9.23	52.62±10.32	0.3
Gender *n*(%)	Female	22 (44)	26 (42)	0.4
	Male	28 (56)	24 (48)	
BMI (kg/m^2)		26.76±3.07	26.52±3.16	0.7
Smoking *n*(%)	Smoker	11(22)	13(26)	0.6
	Nonsmoker	39(78)	37(74)	
Alcohol Drink *n*(%)	Drinker	5 (10)	3(6)	0.2
	Non-drinker	45 (90)	47(94)	
T2DM duration (year)		8.7±4.33	8.08±4.3	0.4
HBa1c %		9.89±2.32	9.13±2.30	0.1
FSG mmol/L		12.84±2.44	11.95± 2.12	0.3
ACR 3-30mg/g		85.33±66.80	13.12±6.92	0.0001
S. Creatinine mmol/L		73.48±26.89	58.04±16.95	0.001
eGFR		98.16±31.49	123.92±49.90	0.003
CKD stages (according to eGFR ml/min/1.73m^2				
Stage 1 (≥90)		31	40	0.01
Stage 2 (60-90)		12	10	
Stage 3 (30-60)		7	0	
Stage 4 (15-29)		0	0	
Stage 5 (<15)		0	0	

Group 1: T2DM patients with DN, Group 2: T2DM patient without DN. BMI: body mass index, T2DM: type 2 diabetes mellitus, HbA1c: glycated hemoglobin, FBS: fasting blood sugar, eGFR: estimated glomerular filtration rate, ACR: urinary albumin-creatinine ratio, CKD: chronic kidney disease. Continuous were variable expressed as median±SD.

Table 2: Serum and urine levels of KIM-1 in T2DM patients with and without nephropathy

Groups	S.KIM-1	U.KIM-1
Group 1	3.61±1.26	2.91±0.71
Group 2	0.95±0.24	1.49±0.27
p-value	0.0001	0.0001

Values were expressed as mean±SD; Group 1: T2DM patients with DN; Group 2: T2DN without DN.

Higher concentrations were reported in patients with DN compared to the group without DN (Table 3). Table 4 shows Pearson's correlation analysis for the association of KIM-1 and nephrin with clinical parameters of DN. The serum nephrin level is positively correlated with urinary nephrin, S. creatinine, ACR ratio, and KIM-1 in serum and urine (r = 0.572, 0.236, 0.505, 0.731, and 0.703, respectively). However, it is negatively correlated with the estimated glomerular filtration rate (r= – 0.258).

Table 3: Serum and urine levels of nephrin in T2DM patients with and without nephropathy

Groups	S. Nephrin	U. Nephrin
Group 1	29.77±10.47	22.22±12.45
Group 2	6.03±3.02	7.37±2.49
p-value	0.0001	0.0001

Values were expressed as mean±SD; Group 1: T2DM patients with DN; Group 2: T2DN without DN.

Meanwhile, the urinary nephrin level was positively correlated with the urinary albumin/creatinine ratio and the KIM-1 level in both serum and urine (r= 0.453, 0.528, and 0.610, respectively) and negatively correlated with the estimated glomerular filtration rate (r= – 0.185). Additionally, the serum KIM-1 level was positively correlated with urinary KIM-1, S. creatinine, ACR ratio, and serum and urinary nephrin levels (r= 0.636, 0.274, 0.555, 0.731, and 0.528, respectively) and negatively correlated with estimated glomerular filtration rate (r= – 0.233). The result of urinary KIM-1 levels demonstrated a positive correlation with ACR, serum, and urinary nephrin levels (r = 0.423, 0.703, and 0.610, respectively) and a negative correlation with eGFR (r= – 0.208). The results for the diagnostic sensitivity and specificity of serum nephrin for nephropathy were 94% and 100%, respectively, and for urinary nephrin levels, they were 80% and 92%, respectively. For serum KIM-1, the sensitivity and specificity were 98%, and the sensitivity and specificity for urinary KIM-1 were 98% and 100%, respectively, as shown in Table 5 and Figures 1 (A and B), 2 (A and B).

DISCUSSION

Traditional markers such as estimated glomerular filtration rate (e-GFR) and albuminuria are used to diagnose DN; however, the gold standard for diagnosing DN is a renal biopsy, which is less widely utilized due to its intrusive nature [11]. Microalbuminuria, on the other hand, has been utilized as a diagnostic sign for kidney injury for over 30 years. However, new data show that changes in microalbuminuria cannot predict the course of nephropathy since renal impairment has already occurred in at least 33% of patients [12,13]. Aside from this, standard procedures such as BUN and creatinine testing are commonly utilized but are insensitive and vague, particularly in the context of acute kidney injury (AKI) from the perspective of DN.

Table 4: Pearson's correlation analysis of the association between KIM-1 levels, nephrin levels, and the markers of diabetic nephropathy

		HbA1c	FBG	S. creatinine	eGFR	ACR	S.KIM-1	U.KIM-1	S. Nephrin	U. Nephrin
HbA1c	*r*-value	1.0	0.58	-0.19	0.21	0.08	0.11	0.08	0.10	0.08
	p-value		0.00	0.06	0.04	0.44	0.28	0.42	0.31	0.43
FBG	*r*-value	0.58	1.0	-0.02	0.02	0.003	0.04	0.03	0.05	0.11
	p-value	0.00		0.83	0.87	0.98	0.66	0.79	0.59	0.30
S. creatinine	*r*-value	-0.19	-0.02	1.0	-0.80	0.25	0.27	0.16	0.24	0.06
	p-value	0.06	0.83		0.00	0.01	0.01	0.12	0.02	0.53
eGFR	*r*-value	0.21	0.02	-0.80	1.0	-0.24	-0.23	-0.21	-0.258	-0.19
	p-value	0.04	0.87	0.00		0.02	0.02	0.04	0.01	0.07
ACR	*r*-value	0.08	0.003	0.25	-0.024	1.0	0.56	0.42	0.51	0.45
	p-value	0.44	0.98	0.01	0.02		0.00	0.00	0.00	0.00
KIM.S	*r*-value	0.11	0.04	0.27	-0.23	0.56	1.0	0.64	0.73	0.53
	p-value	0.28	0.66	0.01	0.02	0.00		0.00	0.00	0.00
KIM.U	*r*-value	0.08	0.03	0.16	-0.021	0.42	0.64	1.0	0.70	0.61
	p-value	0.42	0.79	0.12	0.04	0.00	0.00		0.00	0.00
S.Nephrin	*r*-value	0.10	0.05	0.24	-0.26	0.51	0.73	0.70	1.0	0.57
	p-value	0.31	0.59	0.02	0.01	0.00	0.00	0.00		0.00
U.Nephrin	*r*-value	0.08	0.11	0.06	-0.19	0.45	0.53	0.61	0.57	1.0
	p-value	0.43	0.30	0.53	0.07	0.00	0.00	0.00	0.00	

ACR: albumin/creatinine ratio; eGFR: estimated glomerular filtration rate; HbA1c: glycated hemoglobin; KIM-1: kidney injury molecule-1; FBG: fasting blood glucose; U: urine; S: serum.

Table 5: Receiver Operating Characteristic curve data of the studied markers

Parameters	AUC	Explanation	*p*-value	The best Cutoff	Sensitivity %	Specificity %
S.KIM-1	0.997	Excellent	0.001	1.4215	98	98
U.KIM-1	0.999	Excellent	0.001	1.9810	98	100
S.Nephrin	0.991	Excellent	0.001	15.1655	94	100
U.Nephrin	0.852	Very good	0.001	10.2315	80	92

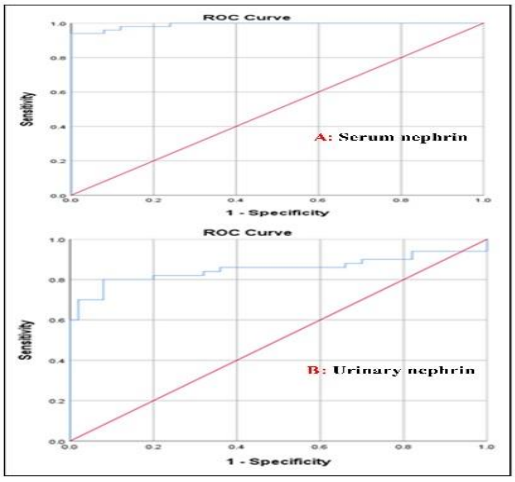

Figure 1: Receiver Operating Characteristic curve of the serum nephrin (A) and urinary nephrin (B) levels.

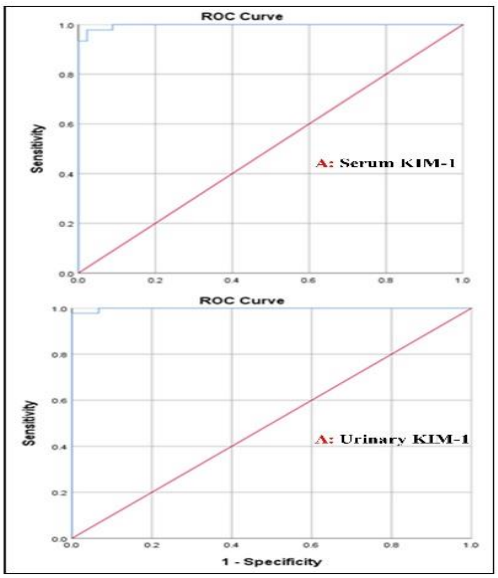

Figure 2: Receiver Operating Characteristic curve of the serum KIM-1 (A) and urinary KIM-1 (b) levels.

This highlights the critical need for novel biomarkers that can accurately predict AKI [14]. Such innovative biomarkers will not only improve the risk assessment of DKD patients, but will also reveal new insights into the disease's complicated pathogenesis as well as potential novel therapy targets. These indicators typically capture a single mechanism of the disease process, such as glomerular or tubular damage, inflammation, or

oxidative stress [7]. It is especially noteworthy that we discovered a statistically significant difference in blood and urine nephrin concentrations in diabetic nephropathic patients with microalbuminuria compared to normoalbuminuric T2DM subjects. These findings suggest that podocyte destruction may occur in T2DM patients prior to the development of microalbuminuria [8]. Despite the fact that there is a highly significant difference in urine nephrin levels between DM patients with normoalbuminuria and patients with DN with microalbuminuria, this protein still appears in both groups, indicating the presence of nephrin protein in the urine of T2DM patients with normoalbuminuria, demonstrating that nephrinuria may precede microalbuminuria [8]. Our findings were consistent with a research that found nephrinuria in 54% of DM patients with normoalbuminuria and 100% of DM patients with micro- and macroalbuminuria [15,16]. Similarly, higher urine nephrin levels were seen in 82% of patients with normoalbuminuria, 88% of patients with microalbuminuria, and 100% of patients with macroalbuminuria [8,17]. Because it is inversely connected with eGFR, the current study shown that it is possible to considerably enhance the prediction of eGFR decline using this biomarker. Several studies found that nephrinuria was associated with higher urine albumin concentrations and diabetes status. However, nephrinuria was found in a high proportion of diabetic patients with normoalbuminuria; thus, given that hyperglycemia is likely to further damage renal vasculature and the glomerular filtration barrier over time, nephrinuria may provide an early indicator of renal damage, even if not all diabetic patients with nephrinuria progress to kidney disease [17]. The current finding was consistent with a previous study that found significant nephrin loss and redistribution in the glomeruli of diabetic patients with microalbuminuria; this observation suggests that nephrin loss and redistribution may precede the development of glomerular lesions and be an early event in the progression of diabetic nephropathy [18]. It also revealed that patients with type 1 diabetes and nephropathy have structural changes to the glomerular filtration unit, such as increased width of podocyte foot processes and filtration slits [18]. In this regard, gene expression profiling studies revealed that the nephrin gene was one of those downregulated in DN; decreased nephrin mRNA and protein expression may be related with podocyte ultrastructural abnormalities, giving one potential explanation for proteinuria in DN [19]. Another study found that nephrin concentrations in the serum were nearly double those in the urine in patients with severe preeclampsia, and that nephrin levels in the serum were five times higher than in urine samples in normal pregnancies. As a result, one can infer that nephrin can be found in the systemic circulation or that nephrin secreted by podocytes while passing through the nephron can be reabsorbed in the renal tubular system and discovered in the serum. However, more research is needed to determine the source and clinical importance of serum nephrin in diabetic nephropathy patients [20]. The diagnostic accuracy of urine nephrin to predict glomerulopathy in the current investigation revealed extremely strong diagnostic sensitivity (80%) and specificity (92%), indicating that urinary nephrin may be a potential biomarker of glomerular damage. In terms of KIM-1 levels, we discovered a statistically significant difference in serum and urine KIM-1 concentrations in diabetic patients with DN and microalbuminuria compared to T2DM normoalbuminuric controls. These findings suggest that proximal tubule impairment may be present in people with T2DM prior to the emergence of microalbuminuria. There are numerous grounds to believe that KIM-1 is released into the circulation following kidney proximal tubule damage. Tubular cell polarity is lost after damage, and KIM-1 may be discharged directly into the interstitium. Furthermore, increased trans-epithelial permeability after tubular injury causes tubular contents to seep back into the circulation [21]. In addition, increased microvascular permeability contributes significantly to the pathogenesis of kidney damage [22]. In renal microvascular endothelial cells, the actin cytoskeleton architecture is disrupted, with loss of cell-cell and cell-matrix adhesion junctions, and endothelial cells are separated from the basement membrane, allowing KIM-1 to enter the circulation [9,23]. The current study found that elevated levels of KIM-1 can be detected in the blood and can be used as a biomarker of kidney injury. According to these findings, urine KIM-1 has a high sensitivity and specificity (98% and 100%, respectively) for predicting AKI in diabetic patients. However, more study and clinical studies are required to determine whether and how uKIM-1 can be employed in clinical diagnosis.

Conclusion

Because of the elevated levels of KIM-1 and nephrin in normoalbuminuric subjects, as well as the negative correlation between KIM-1 and nephrin concentration with eGFR and the high diagnostic sensitivity and specificity of these markers in patients with DN, serum and urinary levels of these markers could be very important markers for early detection of DN. Furthermore, these findings show that these indicators have a higher diagnostic value in the early identification of DN than microalbuminuria.

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Conflicts of interest

There are no conflicts of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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