Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Assessment of Some Biochemical Parameters, Neutrophil Gelatinase Lipocalin, And Kidney Injury Molecule-1 in Type 2 Diabetic Patients with Chronic Kidney Disease

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Received:22/06/2025
Accepted:09/09/2025
Published:31/12/2025

Keywords: Kidney disease, Chronic kidney disease, Diabetic kidney disease, Neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1(KIM-1)

DOI:10.62472/kjps.v16.i27.103-117

Abstract

Diabetic kidney disease (DKD), often referred to as diabetic nephropathy (DN), is a microvascular complication of diabetes that may progress to end-stage renal disease (ESRD), a condition associated with high rates of morbidity and mortality. The principal etiological factors contributing to the development of DKD include glomerulocapillary angiopathy and prolonged duration of diabetes, both of which are major contributors to morbidity and mortality in patients with type 2 diabetes mellitus (T2DM).

This study aimed to evaluate the diagnostic value of serum neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1) as early biochemical markers of DKD by determining their sensitivity and specificity in Iraqi patients with T2DM.

This cross-sectional study included 170 participants divided into three groups: Group I consisted of 50 healthy control subjects with an estimated glomerular filtration rate (eGFR) greater than 60 mL/min/1.73 m²; Group II included 60 patients with T2DM and chronic kidney disease (CKD); and Group III comprised 60 patients with T2DM without CKD. All participants underwent fasting blood sampling for the assessment of glycated hemoglobin (HbA1c), serum creatinine (SCr), blood urea nitrogen (BUN), fasting blood sugar (FBS), serum electrolytes, NGAL, and KIM-1.

The results demonstrated that patients with DKD had significantly elevated levels of urea, creatinine, HbA1c, and FBS, along with highly significant alterations in serum electrolytes, particularly calcium and phosphorus. Serum levels of NGAL and KIM-1 were significantly higher in the DKD group, with a strong positive correlation observed between the two biomarkers. Serum NGAL and KIM-1 levels increased progressively from the control group (168.68 ± 3.63 ng/mL; 79.39 ± 3.06 pg/mL) to diabetic patients without CKD (352.52 ± 6.56 ng/mL; 104.47 ± 1.88 pg/mL), and were highest in diabetic patients with CKD (935.50 ± 36.51 ng/mL; 309.15 ± 12.97 pg/mL). Receiver operating characteristic (ROC) curve analysis revealed a cutoff value of >421.76 ng/mL for NGAL and >134.91 pg/mL for KIM-1.

Conclusion: Serum NGAL and KIM-1 levels, in combination with eGFR and other renal function parameters indicative of declining kidney function, may be considered reliable early biomarkers for the detection of diabetic kidney disease.

دراسة كيموحيوية للمعدلات الجلاتينية المرتبطة بالليبوكالين والكانابيونيد جين في مرضى السكري النوع الثاني المصابين بمرضى الكلى المزمن

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الملخّص

يُعدّ مرض الكلى السكري(Diabetic Kidney Disease, DKD) ، والذي يُعرف غالبًا باسم الاعتلال الكلوي السكري (Diabetic Nephropathy, DN)، أحد المضاعفات الوعائية الدقيقة لمرض السكري، وقد يتطوّر إلى المرحلة النهائية من الفشل الكلوي(End-Stage Renal Disease, ESRD) ، التي ترتبط بمعدلات مرتفعة من المراضة والوفيات. وتشمل المسببات الرئيسة لتطوّر مرض الكلى في DKD اعتلال الأوعية الشعرية الكبيبية وطول مدة الإصابة بمرض السكري، وكلاهما يُعدّان من العوامل المهمة المساهمة في زيادة المراضة والوفيات لدى مرضى السكري من النوع الثاني (Type 2 Diabetes Mellitus, T2DM). هدفت هذه الدراسة إلى تقييم القيمة التشخيصية لكل من الليبوكالين المرتبط بجيلاتيناز العدلات في المصل (Neutrophil Gelatinase-Associated Lipocalin, NGAL)وجزيء إصابة الكلية-1 (Kidney Injury Molecule-1, KIM-1)بوصفهما واسمين حيويين كيميائيين مبكرين لمرض الكلى السكري، وذلك من خلال تحديد الحساسية والنوعية لهذين المؤشرين لدى مرضى السكري من النوع الثاني في العراق .شملت هذه الدراسة المقطعية 170 مريضًا، قُسّموا إلى ثلاث مجموعات: المجموعة الأولى ضمّت خمسين شخصًا سليمًا كمجموعة سيطرة مع معدل ترشيح كبيبي (eGFR) يزيد عن 60 مل/دقيقة/1.73 م²، والمجموعة الثانية ضمّت ستين مريضًا بالسكري من النوع الثاني المصحوب بمرض الكلى المزمن، بينما شملت المجموعة الثالثة ستين مريضًا بالسكري من النوع الثاني دون مرض كلوي مزمن. خضع جميع المشاركين لفحوصات دم صيامية، حيث تم قياس الخضاب السكري(HbA1c) ، والكرياتينين المصلي(SCr) ، ونيتروجين يوريا الدم(BUN) ، والكهارل، بالإضافة إلى NGAL و.KIM-1

أظهرت النتائج أن مجموعة مرضى DKD سجلت ارتفاعًا معنويًا في قيم اليوريا والكرياتينين و HbA1cوسكر الدم الصيامي (FBS)، إضافةً إلى فروق ذات دلالة إحصائية عالية في مستويات الكهارل المصلية، ولا سيما الكالسيوم والفوسفور. كما أظهرت هذه المجموعة ارتفاعًا معنويًا في المستويات المصلية لكل من KIM-1 وNGAL، مع وجود ارتباط إيجابي معنوي بين المؤشرين .لوحظ أن مستويات NGAL و KIM-1في المصل ارتفعت تدريجيًا من مجموعة السيطرة (168.68±3.63 نانوغرام/مل؛ 79.39±3.06 بيكوغرام/مل) إلى مجموعة مرضى السكري دون مرض كلوي مزمن (352.52±6.56 نانوغرام/مل؛ 104.47±1.88 بيكوغرام/مل)، ثم إلى مجموعة مرضى السكري المصحوبين بمرض الكلى المزمن (935.50±36.51 نانوغرام/مل؛ 309.15±12.97 بيكوغرام/مل). كما أظهر تحليل منحنى الخصائص التشغيلية للمستقبِل (ROC)أن قيمة القطع لـ NGAL كانت (421.76<) نانوغرام/مل، في حين بلغت قيمة القطع لـ (134.91<) KIM-1 بيكوغرام/مل.

الاستنتاج:

تشير النتائج إلى أن مستويات NGAL و KIM-1في المصل، بالاشتراك مع معدل الترشيح الكبيبي (eGFR) وبقية المؤشرات الكلوية الدالة على تدهور وظيفة الكلى، يمكن اعتمادها بوصفها واسمات حيوية مبكرة لمرض الكلى السكري.(DKD)

1. Introduction

Diabetes is a chronic, heterogeneous metabolic disease characterized by a complex pathogenesis. It is defined by elevated blood glucose levels, commonly referred to as hyperglycemia, resulting from abnormalities in insulin secretion, insulin action, or both. Hyperglycemia can appear in multiple types with diverse manifestations, including metabolic dysfunctions in carbohydrates, lipids, and proteins. (Banday et al., 2020). Diabetic kidney disease (DKD) is a microvascular complication of diabetes and a primary contributor to end-stage renal disease (ESRD), associated with elevated morbidity and death rates (Ahmed et al., 2022).The main etiologies of kidney disease progression in diabetic kidney disease (DKD) are glomerulocapillary angiopathy and prolonged diabetes duration, both of which significantly contribute to morbidity and mortality in type 2 diabetes mellitus (T2DM) (Yahya et al., 2023) .The disease develops silently, deteriorates, and results in irreversible harm(Quang et al., 2020) .The pathogenesis of disease frequently correlates with changes in the structure and function of renal cells resulting from continuous hyperglycemia, the activation of metabolic mechanisms linked to redox imbalance, and the inflammatory response (Ivanac-Janković et al., 2015). The estimated glomerular filtration rate (eGFR) utilising serum creatinine (SCr) may only be verified when the eGFR result is less than 60 mL/min/1.73 m². indicating that about 50% of renal function has already been lost (Bjornstad et al.,2015). Tubular-interstitial damage, characterized by complex changes in the structure, like glomerular and tubular hypertrophy ,was linked to all types of chronic kidney disease, including diabetic nephropathy (Yahya et al., 2023). Tubular involvement starts before glomerular involvement, evidenced by the detection of several tubular proteins and enzymes before the onset of microalbuminuria and an elevation in serum creatinine (SCr). (Chaudhary et al., 2010) Neutrophil gelatinase-associated lipocalin (NGAL) is a structural tubular marker that weighs 25 kDa and is part of the lipocalin superfamily. It significantly increases in the serum of patients within a few hours of after ischemia-reperfusion injury (He et al ,.2020). NGAL acts as a binder and transporter of small hydrophobic molecules, as well as a role in innate antibacterial responses. (Lin et al,. 2016). Neutrophil gelatinase-associated lipocalin (NGAL), a 25 kDa protein synthesized by damaged nephron epithelia, was a highly promising biomarker for renal epithelial injury. Unlike serum creatinine and urinary output, which measure kidney function, NGAL is particularly induced in injured nephrons and then released into the bloodstream and urine, allowing for easy measurement (Singer et al., 2013). Furthermore, NGAL has been documented to promote nephrogenesis and to facilitate the transformation of mesenchymal cells into renal epithelial cells. NGAL synthesis in kidney tubules increases in response to several harmful stimuli, including ischemia-reperfusion injury, indicating that this protein plays a crucial role in tubular regeneration and repair. (Greco et al., 2022). A type I transmembrane glycoprotein referred to as Kidney Injury Molecule 1 (KIM-1) , was significantly synthesized at the apical membranes of proximal tubular epithelial cells during tissue regeneration after toxic or ischemic acute kidney injury, as well as during tubular epithelial cell dedifferentiation (Bjornstad et al., 2015). The immunoglobulin superfamily protein also known kidney injury molecule (KIM)-1, or T cell immunoglobulin mucin domains (TIM)-1, is significantly elevated in the proximal tubule of an injured or diseased kidney; KIM-1 expression appears early in the course of the disease, and serum KIM-1 levels predict

the progression of DKD to end-stage kidney disease (ESKD) independent of the urinary albumin-to-creatinine ratio (ACR), hemoglobin A1C, and estimated glomerular filtration rate (eGFR) (Mori et al., 2021). This study aimed to evaluate the diagnostic value of serum NGAL and KIM-1 as early biochemical indicators of diabetic kidney disease (DKD) and to determine their sensitivities and specificities as nephropathy biomarkers in Iraqi patients with type 2 diabetes mellitus (T2DM).

2. Materials and Methods

2.1. Study Design, Sample, and Data Collection

A cross-sectional study was conducted at patients who had attended Baghdad Teaching Hospital and Ghazi al-Hariri Surgical Specialties Hospital "Kidney Diseases and Transplantation Center" The study was carried out on one hundred seventy control and patient with age from 35 to 80 years . Already-diagnosed T2DM. Patients collected from Hospital during the period from December 2024 to march 2025. The study included three groups divided to Group1: Control group fifty patient not receiving any medication and did not have a history of any acute or chronic kidney disease and history of diabetes. Group 2: sixty patients type 2 diabetes with chronic kidney disease, Group 3: sixty patients type 2 diabetes.

2.2. Sample Collection

Approximately 2 ml of venous blood was drawn from each participant in this study , put into an ethylene diamine tetra acetic acid (EDTA) tube to measure HbA1C ,the remaining blood 5 ml was placed into gel tube and allow to clot at room temperature about (18- 25 C) then centrifuged for 10 minutes at 3000 rpm to obtain the serum for measurement of fasting blood glucose (FBG), urea, creatinine, Uric acid, Albumin ,Ca ,phosphorus, and electrolyte using a fully automated device , also assay of ELISA technique used to measure serum NGAL and KIM-1 Kidney injury molecules . The target protein from the sample is added and adheres to antibodies coated on the wells. Subsequently, a biotinylated detection antibody specific for NGAL and KIM, was added and attached to the target protein in the sample. Subsequently -HRP Conjugated was added and bound to the biotinylated antibody. During the washing phase following incubation, any unbound Streptavidin-HRP was removed. The substrate solution was then added, and the color intensity correlated directly with the quantity of human protein present in the sample. The reaction was terminated by the addition of an acidic stop solution, and absorbance was measured at 450 nm, whereas the glomerular filtration rate can be estimated mathematically by modifying the CKD-EPI equation (Agrawal et al.,2018).

2.3. Inclusion Criteria

All diabetic patient were diagnosed with chronic kidney disease after duration with diabetes mellitus.

2.4. Exclusion Criteria

Patients with Cardiovascular disease, malignancy, cancer patients treatment with immunosuppressive drugs chemotherapy or radiotherapy ,pregnancy, patient with type 1 DM , patient with gestational diabetes, patient definitely diagnosed with other type of chronic renal disease such as IgA nephropathy ,patient with obstruction or injury in the urinary tract ,liver cirrhosis, the Presence of any sign or symptom of Autoimmune disorders.

2.5. Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and Least Significant Differences (LSD) post hoc test were performed to assess significant differences among means. Post hoc tests are an integral part of ANOVA. The ANOVA to test the equality of at least three group means using F test but this test do not identify which particular differences between pairs of means are significant. Hence, we should use post hoc tests to explore differences between each two means. Chi-square test was used to assess the significant difference between percentages, also correlation coefficients were estimated between all parameters. Receiver operation characteristic curve (ROC curve) was used to identify the validity of markers as an indicator of the disease. The markers were compared according to area under curve. The analysis was submitted by using MedCalc Software. $P < 0.05$ is considered statistically significant.

3. Result and Discussion

This study contributes to the discussion about the diagnostic role of NGAL, KIM -1 and other biochemical parameters in Diabetes mellitus with Chronic kidney diseases.

3.1. Description of The Studied Groups

In this study, one hundred seventy patients aged between (35-80) years were examined and divided into three groups: fifty healthy individuals (female 29, male 21) were represented as a control group who participated in this study, sixty type 2 diabetes patients with chronic kidney disease (female 16, male 44), sixty patients type 2 diabetes without chronic kidney disease (female 28, male 32).

3.2. Distribution of Studied Groups According to Age and Body Mass Index

Age was an important risk factor in the development of DKD, while older individuals were generally at higher risk due to age-related physiological changes and comorbidities (Kovesdy et al., 2018). The age distributions show in Table1 of the study group was significantly different ($p < 0.05$) among group the highest mean was detected in diabetic kidney disease DKD (59.18) years while is the lowest mean shown in control group (47.30) and (54.31) for DM without CKD group in addition BMI also A statistically significant difference between the Diabetic group and Diabetic kidney disease group.

Table1: Distribution of Age and BMI According to Study Groups

Variables	Control	DM without CKD	DM with CKD	P-value*
	Mean± SE	Mean± SE	Mean± SE	
Age	**47.30±0.61c**	**54.31±1.28b**	**59.18±1.60a**	**<0.001**
BMI	**25.99±0.48ab**	**27.50±0.55a**	**25.70±0.58b**	**0.004**

*: One-way ANOVA with LSD post-hoc test, different letters in the same row indicate statistically significant differences (P-value<0.05), while matching letters in the same row indicate no statistically significant differences (P-value >0.05).

This research demonstrated a strong link between chronic renal disease and aging. The elevated occurrence of chronic kidney disease was attributed to a decline in glomerular filtration associated with aging. Thus, a crucial strategy for improving results was to conduct screenings for chronic kidney disease in older adults, whose results are consistent with (Mihardja et al.,2018). According to Table1 similar results were reported by (Delanaye et al. (2019). due to a considerable decline in the GFR after the age of forty The result of Table1 found that DM patients had higher BMI levels than the control group which is similar to other studies such as Grant et al, (2021) who suggested that obesity plays a role in the pathogenesis of T2DM. Obesity is linked to a variety of metabolic dysfunctions, and diseases including insulin resistance, and chronic inflammation all of which are causally linked to the progression and development of diabetes obesity has arisen as a serious global public health issue, necessitating the implementation of intervention programs to address obesity to prevent DM(Yu et al., 2020).In the general population, obesity, as measured by an elevated body mass index (BMI), has been regarded as a cardiovascular risk factor. Obesity is linked to a higher risk of developing incident CKD and ESRD (Lu et al., 2014). Obesity and diabetes are significant contributors to chronic kidney disease (CKD) and end-stage renal disease (ESRD). Obesity and diabetes, along with other components of metabolic syndrome such as hypertension, are highly interrelated and facilitate the onset and advancement of renal disease. These factors collectively result in renal vasodilation, glomerular hyperfiltration, and albuminuria, contributing to the development of glomerulopathy. Hypertension contributes to disease development and may result in end-stage renal disease if inadequately managed.(Maric-Bilkan, 2012).

3.3. Distribution of Studied Groups According to Gender

The gender distribution appear in the Table2 of studied group differences was only significant in diabetic kidney disease group the male highest percentage (73.3) in patients with DKD compare with (53.3) for diabetic patient without CKD, while in female percentage was (26.7) for DKD and (46.7) for DM without CKD group.

Table2: Distribution of Sex According to Study Groups

Variables	Control	DM without CKD	DM with CKD
	No. (%)	No. (%)	No. (%)
Male	21(42)	32(53.3)	44(73.3)
Female	29(58)	28(46.7)	16(26.7)
P-value*	0.25	0.6	<0.001
*: Chi-square test			

In the Table2, both DM and CKD patients, the data illustrated the distribution of study groups according to gender indicates males are more affected than females, which is similar to a study in Iraq by (Kamil et al., 2021). who observed that males (60.8%)have a greater rate of chronic renal disease than females (39.20%). While a study by (Carrero et al., 2018) indicates that chronic kidney disease epidemiology varies by gender, affecting more women than men, the result disagrees with the present study. Gender differences in the duration, onset, and severity of specific risk variables, including albuminuria, cardiovascular disease,

diabetes, obesity, and socioeconomic status, can explain a portion of the higher risk observed in males (Weldegiorgis and Woodward et al., 2020).

3.4. Distribution of Some Biochemical Markers in Study Groups

The result of Table3 represents a comparison of various biomarkers among diabetic patients, diabetic kidney disease patients, and a control group. The parameters assessed include urea, creatinine, uric acid, and albumin. A highly significant rise in urea, creatinine, levels in the T2DM patient with CKD and without CKD groups in comparison to the control group, with mean urea value (113.98) for DKD and (38.95) for DM patient compared to mean value for control (35.95), While creatinine mean (3.71) for DKD and (0.93) for DM patient compared to mean value for control (0.90). While the result showed a significant decline in uric acid in the patient groups DKD compared to the control group and Significant different value between T2DM without CKD compared to control, in addition reduce of serum albumin in DKD compared to T2DM patient mean of ALB (4.58) for DM without CKD and control group (3.64).

Table3: Distribution of Uric Acid, Albumin, Urea, And Creatinine According to Study Groups

Variables	Control	DM without CKD	DM with CKD	P-value*
	Mean± SE	Mean± SE	Mean± SE	
Uric acid	6.23±0.23a	6.62±0.17a	5.43±0.35b	<0.001
Albumin	3.64±0.12b	4.58±0.06a	3.72±0.08b	<0.001
Urea	35.24±1.08b	38.95±1.13b	113.98±6.77a	<0.001
Creatinine	0.90±0.02b	0.93±0.02b	3.71±0.26a	<0.001
eGFR	86.30±2.92a	85.31±2.49a	24.53±1.92b	<0.001

*: One-way ANOVA with LSD post-hoc test, different letters in the same row indicate statistically significant differences (P-value<0.05), while matching letters in the same row indicate no statistically significant differences (P-value >0.05)

A result of eGFR that is highly significant between DM with and without CKD compared to the control group reduced GFR was the characteristic of CKD, which is also characterized by increased plasma concentrations of urea and creatinine. Both increased together in plasma levels as CKD progresses were highly significantly increased as compared to the healthy group, as seen in the Table3 and Table4 These results agreed with the results of the other studies (Kadhim et al., 2020), (Hassan, 2018). Diabetes causes hyperfiltration initially, where the kidneys filter the blood at a higher rate due to increased glucose levels. This puts extra pressure on the glomeruli. Over time, this increased pressure damages the glomeruli, leading to scarring and decreased filtration capacity (Fadem, 2022).

The kidneys remove two essential solutes eliminate from the body: blood urea and serum creatinine They also revealed that urea was the first organic solvent that was detected in the blood of individuals with CKD. If the kidneys in chronic renal disease is unable to filter nitrogenous wastes from the blood, these substances will accumulate and lead to a rise in both urea and creatinine levels. When renal function declines by twenty-five to fifty percent, blood urea levels increase, serving as a sensitive indicator of renal disease (Kadhim et al., 2020). Elevated serum uric acid (SUA) is independently correlated with obesity, hypertension, metabolic syndrome (MS), chronic kidney disease (CKD), and other conditions. Increased SUA levels have been connected to a heightened risk of developing diabetes mellitus (DM) in numerous observational studies., According to two meta-analyses, dose-response analysis revealed that the risk of DM raised by 6% and 17%

per 1 mg/dl increment in SUA. (Singh et al., 2023). The mean of albumin was decreased in the group with CKD compared to the healthy group in the current investigation, with statistically highly significant differences ($p \leq 0.001$), as shown in Table4. The current study's results agree with the results from the study by (Lang et al., 2018), patients with CKD exhibited reduced levels of serum albumin, which were significantly and independently related to a decline in renal function.

3.5. Distribution of Serum Electrolytes and Minerals According to Study Groups

Electrolytes, particularly sodium and potassium, are necessary for maintaining fluid balance and controlling blood pressure. In diabetic kidney disease, where kidney function is compromised, managing these electrolytes can help prevent hypertension and fluid overload (Cardiology et al., 2018). Proper management of calcium and phosphorus can improve overall mineral metabolism, which is often disrupted in patients with DKD. This can help maintain homeostasis and reduce complications(Block et al., 2004). The Table5 indicates to comparison of serum levels between study group (T2DM, T2DM with CKD, control group for minerals and electrolytes result show Phosphate highly significant result in T2DM patients with CKD in compare with control. The serum phosphate (4.80±0.20) for DKD was higher than control (4.41±0.61) additionally phosphate of T2DM (2.57±0.09) is lower than DKD and control the mean of serum potassium (5.14±0.14) for DKD was significant higher when compared to control (4.29±0.07) while T2DM (4.00±0.03). The serum calcium (8.61±0.12) in DKD and T2DM without Chronic kidney disease (8.93±0.07), (6.78±0.07) respectively. In addition, the serum sodium (136.36±0.53) for DKD while (139.83±0.56) for was lower than control (140.84±0.37) the serum chloride (105.81±0.59) for DKD was significantly higher than control (96.74±0.22) while serum chloride of T2DM (101.55±0.56) lower than DKD and higher than control group.

Table5: Distribution of Serum Electrolytes According to Study Groups

Variables	Control	DM without CKD	DM with CKD	P-value*
	Mean± SE	Mean± SE	Mean± SE	
Ca	8.93±0.07a	6.78±0.07c	8.61±0.12b	<0.001
PO4	4.41±0.61a	2.57±0.09b	4.80±0.20a	<0.001
K	4.29±0.07b	4.00±0.03c	5.14±0.14a	<0.001
Cl	96.74±0.22c	101.55±0.56b	105.81±0.59a	<0.001
Na	140.84±0.37a	139.83±0.56a	136.36±0.53b	<0.001

*: One-way ANOVA with LSD post-hoc test, different letters in the same row indicate statistically significant differences (P-value<0.05), while matching letters in the same row indicate no statistically significant differences (P-value >0.05).

The electrolyte parameters in this study group statistical analyzed to showed an increase in serum potassium while serum calcium and sodium decrease in DKD patient compared with control and T2DM as shown in Table5 ,this observation was in line with other studies (Lombardi et al., 2021).When serum potassium concentration is raised ,sodium and water reabsorption becomes decrease due to increase potassium reabsorption in the proximal tubule and thick ascending limb TAL ,when sodium is reabsorbed in collecting tubules due to an increase amount of sodium reaching the lumen ,potassium secretion is enhanced by promotion of exchangeable excretion of potassium by the same cation (Yamada and Inaba, 2021). Diabetic patients show a decrease in potassium concentration comparison to the control group as shown in

Table5which in agreement with study by (Coregliano-Ring et al., 2022).The prevalence of hypokalemia in adults over 55 years of age with diabetes mellitus ranges from 1.0% to 1.2%. The frequency was even greater in persons with associated chronic kidney disease (CKD) and increases with age (Jiménez-Marrero et al., 2020).The correlation between hyperglycemia and hypokalemia was dependent on the functionality of the potassium ATP channel (KATP) in islet cells. When glucose enters the beta cell through the glucose transporter 2 (GLUT2), it is converted to glucose-6-phosphate. An elevation in intracellular adenine nucleotides inhibits KATP, resulting in its closure, while hypokalemia is linked to the onset of hyperglycemia due to the reduction of potassium-dependent insulin production in response to elevated glucose levels (Coregliano-Ring et al., 2022). A comparison of the CKD group with the control group showed that the mean serum , calcium level was slightly decrease in the CKD group as seen in Table5. The research by (Janmaat et al., 2018)supports this study's findings that a decrease in serum calcium was associated with a faster reduction in renal function. This result is in line with previous studies by(Leaf and Christov, 2019) and Iraqi study by (Hassan, 2018) identified that was adecrease in the calcium concentration in serum when compared to the control group of patients with CKD. In contrast to the current study, earlier research by (Kovesdy et al., 2010) and (Lunn et al., 2010) revealed that significant increases in serum calcium were associated with CKD. These variations in results may be due to the sample size, and biological factors, in addition to age, gender, and ethnic differences in the normal ranges, which are frequently dynamic with meals or time of day (Horowitz, 2010).The mean of phosphorus (P) was raised in the Diabetic group with CKD compared to the healthy group in the current investigation with statistically highly significant differences as shown in Table5 . So, the study by (Fourtounas, 2011) concurs with the current study in that patients with CKD have a positive phosphorus balance, indicating that increase in Phosphorus results from the kidneys' failure to remove phosphorus. Also, current research has demonstrated that patients DM withCKD have a mean slightly reduced sodium level than their DM without CKD corresponding healthy group, as seen in Table5 . These results, as well as a previous study by (Kadhim et al., 2020) Whenshowed that hyponatremia is mostly increasing in dialysis patients as result of taking hypotonic fluids or excessive water. According to (Han et al., 2015) CKD patients with Na+ ≤135 mEq/l had an increased chance of developing End stage renal disease, whereas an increased risk of mortality was linked to both lower and higher Na+ levels, Clinicians should give greater attention to blood sodium levels while treating CKD patients because it may help to improve patient outcomes(Han et al., 2015).While the mean of phosphate for T2DM patient decline compare to mean of control group there was consistent with this study , it has been reported that low serum levels of phosphate indicate that diminished the serum levels of phosphorus correlated with heightened insulin resistance in healthy individuals. prior experimental study suggested that phosphorus depletion leads to Reduced insulin production from pancreatic beta cells, related to increased intracellular calcium and adenosine inhibition. Triphosphatase production. Thus, it has been suggested that decreased serum phosphate levels may interfere with serum glucose management in non-diabetics with obesity. However, epidemiological studies revealed that all-cause mortality was independently related with

elevated serum phosphorus in all populations, even those without chronic kidney disease and with serum phosphorus levels within the upper normal reference range (Raikou et al., 2020).

3.6. Estimation of Glucose and Hba1c In the Study Group

Regular monitoring of FBS and HbA1c provides insights into long-term glycemic control, which is critical for preventing the progression of DKD (Khan SE et al.,2020). Tracking FBS and HbA1c can help detect early signs of complications associated with DKD, allowing for timely intervention (Kovesdy et al., 2021). The blood sugar concentrations for HbA1C and fasting blood sugar (FBS) were measured by automated methods and indicated high significant levels to the patient groups when compared with the control group, the highest FBS mean were recorded at T2DM group (213.52±12.20) while HbA1C (8.95±0.41) followed by T2DM with CKD FBS concentrations were recorded (166.31±8.86) while HbA1C (6.89±0.21) in T2DM with CKD These findings explain significant different variations ($p < 0.001$) in HbA1C and serum FBS between the control and patient groups.

Table7: Distribution Of Glucose And Hba1c According To Study Groups

Variables	Control	DM without CKD	DM with CKD	P-value*
	Mean± SE	Mean± SE	Mean± SE	
Glucose	**84.94±0.70c**	**213.52±12.20a**	**166.31±8.86b**	**<0.001**
HbA1C	**4.79±0.04c**	**8.95±0.41a**	**6.89±0.21b**	**<0.001**
*One-way ANOVA with LSD post-hoc test, different letters in the same row indicate statistically significant differences (P-value<0.05), while matching letters in the same row indicate no statistically significant differences (P-value >0.05).				

The results were consistent with prior studies (Wallia et al., 2020); (Lee et al., 2016) indicating Patients with T2DM experience hyperglycemia primarily, Due to insulin resistance and decreased insulin secretion. This metabolic dysfunction causes decreased glucose uptake by cells, resulting in increased blood glucose levels compared to persons without T2DM, who maintain normal insulin function. These findings were in accordance with WHO (2011) which reviewed that HbA1c of 6.5% was recommended cut off for diabetes diagnosis was %. A value less than 6.5% does not exclude diabetes, which can be detected with glucose testing. As a result, it has been employed as a marker to measure long-term glycemic control (Sacks et al., 2023). The result in the present study showed significantly higher FBS and HbA1C in T2DM patients and CKD patients than in the control group, indicating diabetes was an Significant risk factor for the progression of CKD based on the decreased GFR, which agrees with previous study like (Tewari et al., 2021). who found that CKD was one of the major causes of morbidity, affecting 20%-40% of people with diabetes (Tewari et al., 2021). While (Nazzal et al., 2020) There was no relationship between HbA1C and CKD and People with T2DM who had a history of uncontrolled HbA1C levels did not have a higher risk of CKD.

3.7. Estimation of Serum NGAL and KIM-1 Concentration In The Study Group

Neutrophil gelatinase-associated lipocalin is one of the most promising tubular markers for diagnosing acute and chronic kidney diseases (Sueud et al., 2019).NGAL is a novel biomarker for the early diagnosis of acute renal failure due to its tiny molecular size and resistance to degradation. NGAL excretion in blood and urine has been identified as an early predictive biomarker of kidney injury (DyabAllawi et al. 2017). Kidney injury molecule-1 was a type 1 transmembrane protein, with an immunoglobulin and mucin domain. KIM-1 was

found to appear early in acute renal tubular injury (Zhao et al., 2019, Khawaja et al., 2019). Numerous studies have enhanced their effectiveness as markers and indicators of injury in chronic renal disease.(Quang et al., 2020). Table8 show that they are a highly significant increase in the mean of NGAL (935.50±36.51) in T2DM with CKD Patient groups and T2DM patient groups (352.52±6.56)as compared to control group (168.68±3.63), additionally also show a highly significant increase in mean of KIM (309.15±12.97) in T2DM with CKD patient groups and T2DM (104.47±1.88)patient groups as compared to control group (79.39±3.06).

Table8: Distribution of Kim-1 And NGAL According to Study Groups

Variables	Control	DM without CKD	DM with CKD	P-value*
	Mean± SE	Mean± SE	Mean± SE	
Kim-1	79.39±3.06c	104.47±1.88b	309.15±12.97a	<0.001
NGAL	168.68±3.63c	352.52±6.56b	935.50±36.51a	<0.001
*One-way ANOVA with LSD post-hoc test, different letters in the same row indicate statistically significant differences (P-value<0.05), while matching letters in the same row indicate no statistically significant differences (P-value >0.05).				

In the Table8 result show the mean of both NGAL and KIM in different study group was significant different (<0.001) the mean of NGAL is higher in stage 3 of CKD (690.12±18.07), in stage 4 of chronic kidney disease (1149.31±72.49) , in addition (967.08±34.81) for stage 5 of CKD compared to T2DM patient groups and control groups (168.68±3.63). Also, kidney injury molecules show higher significance in stages 3 of chronic kidney disease (199.86±6.79) and mean of KIM (405.37±15.26) in stage 4 of CKD and (341.29±14.36) mean of KIM in stage 5 of CKD versus T2DM patient groups and control groups (79.39±3.06) respectively.

Table10: Distribution of Kim-1 And NGAL According to Stage

Group	Control	DM without CKD	DM with stage 3 CKD	DM with stage 3 4KD	DM with stage 5 CKD	P-value*
	Mean± SE	Mean± SE	Mean± SE	Mean± SE	Mean± SE	
Kim-1	79.39±3.06e	104.47±1.88d	199.86±6.79c	405.37±15.26a	341.29±14.36b	<0.001
NGAL	168.68±3.63e	352.52±6.56d	690.12±18.07c	1149.31±72.49a	967.08±34.81b	<0.001
*One-way ANOVA with LSD post-hoc test, different letters in the same row indicate statistically significant differences (P-value<0.05), while matching letters in the same row indicate no statistically significant differences (P-value >0.05).						

These result presented in the Table8 show a higher NGAL levels in T2DM with CKD patient compared to T2DM patient without CKD and control group this consistency with the results of (Nazzal et al., 2020) who found T2DM patients with CKD had a considerably higher serum NGAL level, Additionally, Diabetes patients have elevated NGAL levels compared to the control group, corroborating the findings of (Najafi et al.,2021) on evaluating the role of NGAL in diabetic individuals. Increased levels of NGAL can be found in plasma starting 2-4 hours after a kidney injury, resulting from changes in tubular reabsorption, glomerular filtration, and increased secretion in tubular epithelial cell. (Firu et al., 2015). Serum KIM-1 has served as a marker for the rapid deterioration of renal function in type 2 diabetes (Ding et al., 2011; Satirapoj et al.,

2019). In stage 5 chronic kidney disease (CKD), the levels of biomarkers like Kidney Injury Molecule-1 (KIM-1) and Neutrophil Gelatinase-associated Lipocalin (NGAL) may decrease due to several factors, including loss of Functional Nephrons. As CKD progresses to stage 5, there is a significant loss of functional nephrons. KIM-1 and NGAL was primarily produced by renal tubular cells in response to injury. With fewer functional nephrons, the overall production of these markers may decline(Vaidya et al., 2008). Dialysis impacts many patients with stage 5 CKD who are on dialysis, which can remove circulating levels of these biomarkers from the blood. The dialysis process itself may lower the levels of KIM-1 and NGAL (Kirkland, S. W., et al.2013).

3.8. Receiver Operating Characteristic (ROC) Curve Analysis for NGAL And KIM1 In the Studied Groups

According to the Analysis of the receiver operating characteristic (ROC) curve, Fig.1 the optimal cut-off points for NGAL in DM Vs DKD were (>421.76) At these cut-off values, the sensitivity of KIM-1 was 100%%, with showing a specificity of 100%, if patient have NGAL more than 421.76 it has a 100 % chance of kidney damage.

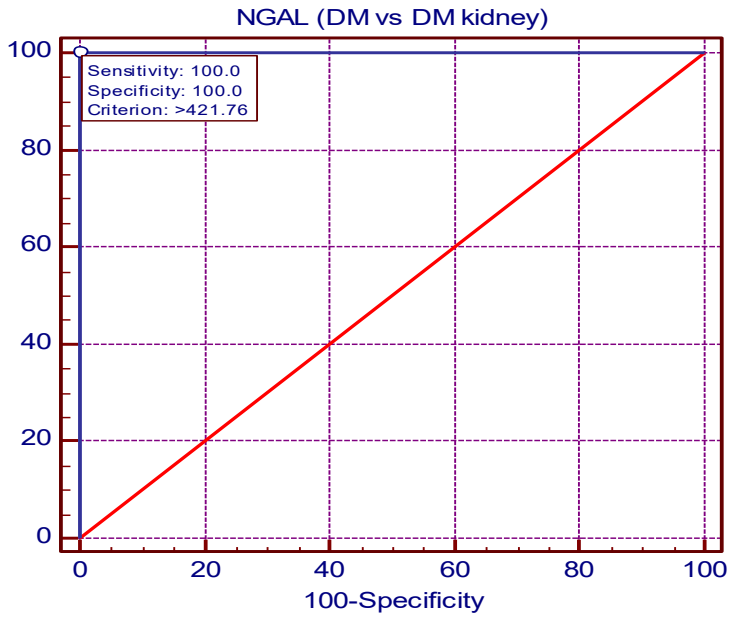

Figure1: ROC analysis of NGAL between DM with and without CKD groups

3.9. Receiver Operating Characteristic (ROC) Curve Analysis for NGAL And KIM1 In the Studied Groups

According to the Analysis of the receiver operating characteristic (ROC) curve, Fig.2 the ideal cut-off points for KIM-1 in DM Vs DKD were (>134.91). At these cut-off values, the sensitivity of KIM-1 was 100%% %, with showing a specificity of 100%, if a patient has a KIM-1 value more than 134.91, it has a 100 % chance of kidney damage.

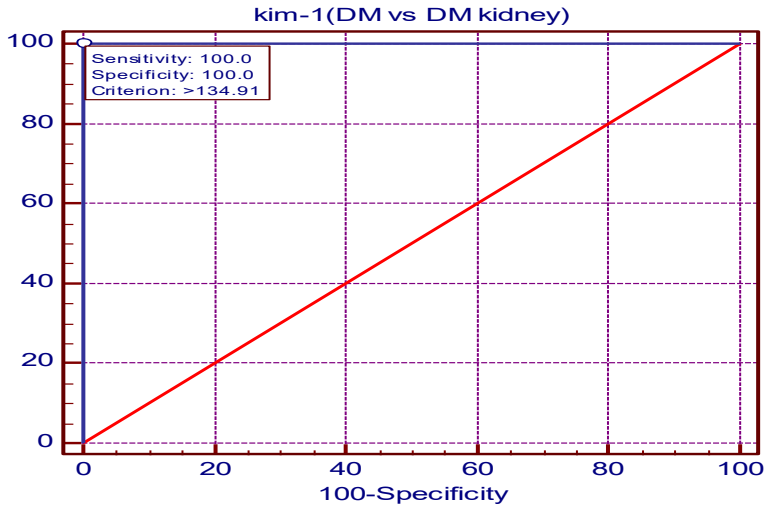

Figure2: ROC analysis of Kim-1 between DM with and without CKD groups

4. Conclusion

Finally, we can conclude that it can be used both NGAL AND KIM as a prediction marker for the detection and diagnosis the renal damage before reaching to end stage of CKD or HD and kidney transplant. Increase NGAL and KIM in diabetic patients compared to the control group. This early indicator of renal failure and, group of diabetes with CKD increases, compared to diabetic patients, because the kidneys are injured and are unable to filter or reabsorb proteins from diabetes.

5. Ethical Statement

Patients provided verbal informed consent prior to specimen collection. The local ethics committee reviewed and approved the research protocol, subject information, and permission form. The study obtains approval for research ethics. in the ministry of health of Iraq on Medical city, Baghdad teaching Hospital and Ghazi al-Hariri Surgical Specialties Hospital Kidney Diseases and Transplantation Centre. The study objectives were described to all volunteers.

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