ISSN: 0067-2904

The Prognostic Value for Tissue Inhibitor of Metalloproteinase-2 and Fatty Acid-Binding Protein-1 as Biomarkers for Chronic Kidney Disease

Fatima Gh. Talab, Hind S. Ahmed*
Department of Chemistry/ College of Education for Pure Science (Ibn Al Haitham)/ University of Baghdad, Baghdad-Iraq

Received: 26/6/2024 Accepted: 17/10/2024 Published: 30/12/2025

Abstract

Globally, chronic kidney disease (CKD) has emerged as a significant public health concern, characterized by high rates of morbidity and mortality. To assess the risk of kidney damage, researchers have identified tissue inhibitor of matrix metalloproteinase-2 (TIMP-2) and fatty acid-binding protein-1 (FABP-1) as valuable biomarkers. This study aims to analyse the effectiveness of specific biomarkers in assessing CKD and its associated mechanisms in Iraqi patients. The study was conducted from December 2023 to May 2024. Ninety subjects, aged 48–65 years; including 60 patients with CKD (38 male and 22 female) attended the Baghdad Teaching Hospital/ Medical City/ Dialysis Unit- Baghdad, Iraq. In addition, 30 healthy people (15 male and 15 female) were selected as a control group. The findings reveal a significant (p= 0.001) in serum TIMP-2 and FABP-1 levels among CKD patients as compared to the control group (6.30±2.19 vs. 0.62±0.21) ng/mL and (9.38±3.14 vs. 1.80±0.01) ng/mL respectively. In addition, the present results revealed a significant positive correlation ($p < 0.05$) between serum TIMP-2 and FABP-1 and levels of various parameters, including fasting glucose, lipid profile, and renal function tests. Conversely, a significant negative correlation ($p < 0.05$) was observed between serum TIMP-2 and FABP-1 levels with serum total protein, albumin, and estimated glomerular filtration rate in the CKD group. In conclusion, the study concludes that elevated serum levels of TIMP-2 and FAB-1 in CKD patients underscore their critical role in renal dysfunction. Therefore, these factors may serve as biomarkers of kidney injury progression among patients with CKD.

Keywords: Chronic kidney disease, Diabetic nephropathy, Fatty acid-binding protein-1, Glomerular filtration rate, Tissue inhibitor of metalloproteinase-2.

القيمة التنبؤية للمثبط النسيجي للميتالوبروتيناز-2 والبروتين المرتبط بالحامض الدهني-1 كدالات حيوية لمرض الكلى المزمن

فاطمة غازي طلب، هند شاكر أحمد*
قسم الكيمياء، كلية التربية للعلوم الصرفة (ابن الهيثم)، جامعة بغداد، بغداد، العراق

*Email: hind.sh.a@ihcoedu.uobaghdad.edu.iq

الخلاصة

برز مرض الكلى المزمن (CKD) كمشكلة صحية عامة كبيرة، تتميز بارتفاع معدلات الإصابة والوفيات على مستوى العالم. لتقييم خطر تلف الكلى، حدد الباحثون المثبط النسيجي للميتالوبروتيناز-2 والبروتين المرتبط بالحامض الدهني-1 كعلامات حيوية قيمة. تهدف هذه الدراسة إلى تحليل فعالية الدالات الحيوية المحددة في تقييم مرض الكلى المزمن والآليات المرتبطة به لدى المرضى العراقيين. أجريت الدراسة من كانون الأول 2023 إلى ايار 2024. شارك في الدراسة تسعون شخصًا تتراوح أعمارهم بين 48 و 65 عامًا؛ بما في ذلك 60 مريضًا يعانون من مرض الكلى المزمن (38 ذكرًا و 22 أنثى) من الذين يراجعون مستشفى بغداد التعليمي/ مدينة الطب/ وحدة غسيل الكلى- بغداد، العراق. بالإضافة إلى ذلك، تم اختيار 30 شخصًا سليمًا (15 ذكرًا و 15 أنثى) كمجموعة سيطرة. كشفت النتائج عن وجود ارتباط إيجابي معنوي ($p < 0.05$) بين مستويات المثبط النسيجي للميتالوبروتيناز-2 والبروتين المرتبط بالحامض الدهني-1 في المصل لدى مرضى الكلى المزمن مقارنة بمجموعة السيطرة (6.30 ± 2.19 مقابل 0.62 ± 0.21) نانوغرام/ مل و (9.38 ± 3.14 مقابل 1.80 ± 0.01) نانوغرام/ مل على التوالي. بالإضافة إلى ذلك، كشفت النتائج الحالية عن وجود ارتباط إيجابي معنوي ($p < 0.05$) بين مستويات المثبط النسيجي للميتالوبروتيناز-2 والبروتين المرتبط بالحامض الدهني-1 في المصل ومستويات المعلمات المختلفة، بما في ذلك كلوكوز الصائم وصورة الدهون واختبارات وظائف الكلى. وعلى العكس من ذلك، لوحظ وجود ارتباط سلبي معنوي ($p < 0.05$) بين مستويات المثبط النسيجي للميتالوبروتيناز-2 والبروتين المرتبط بالحامض الدهني-1 في المصل مع البروتين الكلي في المصل والألبومين ومعدل الترشيح الكبيبي المقدر لدى مرضى الكلى المزمن. خلصت الدراسة إلى أن ارتفاع مستويات المثبط النسيجي للميتالوبروتيناز-2 والبروتين المرتبط بالحامض الدهني-1 في مصل مرضى الفشل الكلوي المزمن يؤكد على دورهما الحاسم في اختلال وظائف الكلى. وبالتالي، قد تعمل هذه العوامل كمؤشرات حيوية لتطور إصابة الكلى بين مرضى الكلى المزمن.

1. Introduction

Chronic kidney disease (CKD) is a condition characterized by gradual and irreversible damage to the kidneys, leading to loss of kidney function over time. It is defined by abnormalities in kidney structure or function, lasting up to 3 months, with health implications. The primary diagnostic criteria include a urinary albumin to creatinine ratio ≥ 30 mg/g or estimated glomerular filtration rate (eGFR) < 60 mL/min/ 1.73 m^2; CKD has emerged as a major public health issue, affecting 8-16% worldwide, leading to end-stage renal disease (ESRD). It is the 12th primary cause of death and the 7th primary cause of disability worldwide [1]. Various factors contribute to the development of CKD, including diabetes mellitus (DM), hypertension (HTN), obesity, chronic kidney inflammation, and genetic disorders, i.e., polycystic kidney disease. Common risk features comprise progressive age, family history of kidney disease, and high blood cholesterol concentrations [2]. Early detection through blood and urine tests is essential to managing CKD. Additionally, there is a pressing need for more sensitive and specific biomarkers are needed for early diagnosis and prediction of disease severity, which can help in early treatment and produce more effective drugs for management [3]. Since the incidence of diabetics in the ESRD population is rising along with the overall number of diabetics, diabetic nephropathy (DN) is a significant concern, leading to higher rates of morbidity and death. Therefore, it is essential to categorize DN patients as soon as possible to assist early treatments and improve diagnosis [4].

Tissue inhibitor of metalloproteinase-2 (TIMP-2) is a protein that regulates matrix metalloproteinase (MMP) activity, which are enzymes involved in the degradation and remodeling of proteins within the human body [5]. Also, TIMP-2 inhibits MMPs by binding to the catalytic zinc cofactor. Its expression can be stimulated by various cytokines, chemokines, and factors intricate in proliferation and differentiation can stimulate its expression. It contributes to G1 cell cycle arrest in the early phase of cell injury, thus

donating to maintaining the protein balance in the body, reducing tissue damage, and its association with kidney injury [6]. Imbalances between MMPs/TIMPS have been linked to the pathogenesis, clinical manifestations, and prognosis of arterial and venous diseases. Research indicates that the CKD condition contributes to supporting the risk pathways oriented from MMPs toward cardiovascular (CV). Numerous MMP molecules are likely responsible for CV, kidney damage, and related clinical manifestations. Hence, such biomarkers warrant further investigation, suggesting that higher levels of these markers may be related to deteriorating CKD and tissue abnormalities and may indicate kidney tissue damage and failure. Deteriorating CKD can lead to severe health problems such as kidney failure and the need for a kidney transplant [7]. While TIMP-2's anti-fibrotic properties and its role in cell cycle regulation has been identified for some time. However, its presence in distal tubule cells has been elucidated in vitro. It is a classic marker of acute kidney injury (AKI), but its role in assessing kidney damage during the progression of CKD is uncertain [8].

Fatty acid binding protein-1 (FABP-1), also known as liver-type FABP-1 (LFABP), is a 14-kDa molecule expressed in the kidney's proximal tubules. It plays a crucial role in fatty acid (FA) metabolism; their function is to transport non-esterified FA (unbound FAs) into cells and facilitate their use as an energy source. This protein is expressed in kidney cells and can be revealed as a kidney injury biomarker. Upregulation of FABPs, especially FABP-1, has been associated with renal damage in CKD [9]. Therefore, this study aims to analyze the usefulness of TIMP-2 and FABP-1 in evaluating the mechanisms related to CKD in Iraqi patients.

2. Materials and Methods

2.1 Subjects

The study was conducted from December 2023 to May 2024, involving ninety subjects, aged between 48–65 years; 60 patients with CKD (38 male and 22 female) attended the Baghdad Teaching Hospital/ Medical City/ Dialysis Unit- Baghdad, Iraq. These patients had been undergoing dialysis for a duration of 2-10 years, between 2-3 once weekly. Additionally, a control group of 30 healthy individuals (15 males and 15 females) was included. A questionnaire containing age, height, weight, duration of CKD, family history, smoking, and treatment was discussed; CKD was diagnosed according to the Kidney Disease Outcomes Quality Initiative (KDOQI)/ 2002 criteria [10].

2.2 Inclusion and exclusion criteria

The inclusion criteria for the study specified that participants must be aged between, advanced CKD (stages 4–5), and participation in a hemodialysis program at least during the 6 months before recruitment. Exclusion criteria were AKI, pregnancy, type 1 DM, liver disease, thyroid dysfunction, and malignancy.

2.3 Biochemical analysis

For each participant, measurements were taken for sex, age, weight, height, body mass index (BMI), systolic and diastolic blood pressure (SBP and DBP, respectively). Additional anthropometric and clinical data were also collected. A biochemical automated analyzer (Cobas e411) was used to test the levels of FSG, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) in blood samples obtained from each individual while they were fasting for a laboratory examination. Low-density lipoproteins (LDL) were estimated using Friedewald's equation, and very LDL (VLDL) was calculated by dividing TG by 5 using the following formula: LDL-C (mg/dL)= TC- HDL-C – (TG/5)

Glycated hemoglobin (HbA1c) is separated using automated and accurate ion-exchange high-performance liquid chromatography (HPLC) techniques by the Bio-Rad VARIANT hemoglobin A1C. Non HDL-C was calculated by subtracting HDL-C from TC [11]. Serum urea, creatinine, total protein, and albumin levels were measured to determine renal function. The eGFR was calculated according to the CKD-EPI formula [12].

$eGFR = 144 \times (SCr/0.7)^{-1.209} \times (0.993)^{Age}$ [For women and serum creatinine > 0.7 mg/dL]

$eGFR = 141 \times (SCr/0.9)^{-1.209} \times (0.993)^{Age}$ [For men and serum creatinine > 0.9 mg/dL]

Additionally, serum TIMP-2 and FABP-1 levels were evaluated using a sandwich enzyme immunoassay (Cat No. E-EL-H1453 and E-EL-H6153; Elabscience, USA), respectively, with sensitivity: 0.1 ng/mL and detection range: 0.16-10 ng/mL for TIMP-2 and sensitivity: 0.28 ng/mL with detection range: 0.47-30 ng/mL for FABP-1. Figure 1 illustrates a flow chart of the study.

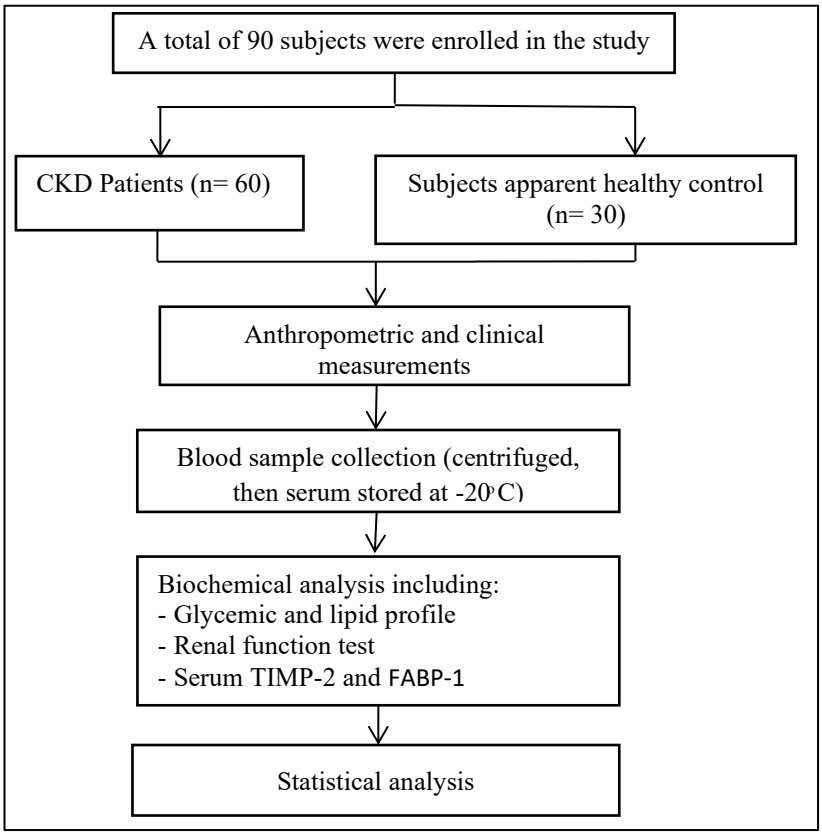

Figure 1: Flow-chart of the study

2.4 Statistical analysis

The Statistical Package for the Social Sciences (SPSS)—IBM 29, Chicago, USA—was used to analyze the data. All results were presented as mean ± standard deviation (SD). The chi-square test was employed to compare percentage data. Numerical variables with normally distributed data were compared between two groups using the independent samples t-test. Also, Pearson's correlation (r) was used to investigate the relationships between TIMP-2 and FABP-1 and other CKD group variables. The p-values below 0.05 were regarded as significant. The receiver operating characteristic (ROC) curve was used to analyze the possibility of using TIMP-2 and FABP-1 in the prognostic of CKD by measuring the area under the curve (AUC) with the cut-off values and their sensitivity and specificity.

3. Results

Table 1 reveals no statistically significant (p= 0.06) in age between the studied groups. However, there were considerable (p= 0.001) increases in BMI and SBP, as well as in DBP (p= 0.03) in CKD patients compared to a control group.

Table 1: Demographic, anthropometric and clinical manifestations of the studied groups

Parameters	Mean ± SD		*p*-value
	CKD	Control	
Number	60	30	-
Male sex	38(63%)	15(50%)	0.001
Age (Years)	55.60± 10.22	53.43± 5.46	0.06
BMI (kg/m^2)	30.52± 2.14	24.85± 1.12	0.001
SBP (mmHg)	152.30± 2.41	120.60± 0.21	0.001
DBP (mmHg)	90.21± 1.87	82.15± 1.02	0.030

The difference is significant at $p < 0.05$ and highly significant at $p < 0.01$.

Table 2 shows significant increases (p= 0.001) in FSG, serum TC, TG, VLDL, and non HDL-C in the CKD group compared with controls. A significant decrease (p= 0.001) in serum HDL-C level was observed in the CKD group compared to the controls. However, no significant (p= 0.06) difference was found in HbA1c between the two groups.

Table 2: Glycemic and lipid profiles of the studied groups

Parameters	Mean ± SD		*p*- value
	CKD (n= 60)	Control (n= 30)	
FSG (mg/dL)	158.44±17.26	98.20±5.31	0.001
HbA1c (%)	8.25±1.12	5.67±0.08	0.06
TC (mg/dL)	215.60 ± 10.45	142.50 ± 10.22	0.001
TG (mg/dL)	250.20 ± 10.53	106.35 ± 8.68	0.001
VLDL (mg/dL)	50.04 ± 2.08	21.27 ± 1.73	0.001
HDL-C (mg/dL)	35.20 ± 5.06	58.34 ± 5.86	0.001
LDL-C (mg/dL)	130.36 ± 3.28	62.89 ± 2.62	0.001
Non HDL-C (mg/dL)	180.40 ± 5.36	84.15 ± 4.30	0.001

The difference is highly significant at $p < 0.01$ and not significant at $p > 0.05$.

The CKD group had significant (p= 0.001) increases in serum urea and creatinine levels compared to the controls. Moreover, serum total protein, albumin levels, and eGFR decreased considerably (p= 0.001) in the CKD group compared to the controls, as shown in Table 3.

Table 3: Serum renal function test and total protein for the studied groups

Parameters	Mean ± SD		*p*-value
	CKD (n= 60)	Control (n= 30)	
Urea (mg/dL)	135.40±8.45	20.05±1.12	0.001
Creatinine (mg/dL)	10.13±0.82	0.62 ±0.01	0.001
Total protein (g/L)	50.72±6.40	68.80±4.25	0.001
Albumin (g/L)	32.80±5.24	45.0±3.12	0.001
eGFR (mL/min/1.73m^2)	34.72±16.70	105.80±17.06	0.001

The difference is highly significant at $p < 0.01$.

Table 4 reveals significant (p= 0.001) increases in TIMP-2 and FABP-1 levels in CKD patients compared to the controls.

Table 4: Serum TIMP-2 and FABP-1 for the studied groups

Parameters	Mean ± SD		*p*-value
	CKD (n= 60)	Control (n= 30)	
TIMP-2 (ng/mL)	6.30±2.19	0.62±0.21	0.001
FABP-1 (ng/mL)	9.38±3.14	1.80±0.01	0.001

The difference is highly significant at $p < 0.01$.

The current findings demonstrate a significant positive correlation ($p < 0.05$) between the levels of TIMP-2 and FABP-1 and various parameter, including FSG, TC, TG, LDL-C, urea, and creatinine. Furthermore, there is a significant negative correlation ($p < 0.05$) between TIMP-2 and FABP-1 levels with serum total protein, albumin, and eGFR. Additionally, no significant association was observed between the serum levels TIMP-2 and FABP-1 with HbA1c, as shown in Table 5.

Table 5: Correlation coefficient between TIMP-2 and FABP-1 with other study parameters in CKD group

Parameters	TIMP-2 (ng/mL)		FABP-1 (ng/mL)	
	r	*p*	r	*p*
FSG (mg/dL)	0.450	0.001	0.510	0.001
HbA1c (%)	0.153	0.06	0.148	0.08
Urea (mg/dL)	0.346	0.005	0.278	0.006
Creatinine (mg/dL)	0.630	0.001	0.530	0.001
Total protein (g/L)	-0.425	0.001	-0.382	0.001
Albumin (g/L)	-0.352	0.030	-0.345	0.005
eGFR (mL/min/1.73m^2)	-0.507	0.001	-0.460	0.001
TIMP-2 (ng/mL)	-	-	0.651	0.001
FABP-1 (ng/mL)	0.540	0.001	-	-

The correlation is significant at $p < 0.05$.

The ROC, sensitivity, specificity, and AUC in the studied groups are displayed in Table 6. The ROC curve indicates that TIMP-2 is an excellent, sensitive marker in the diagnosis of CKD, and it exhibits an AUC of 1.000 with p= 0.0001 when tested in the control group, as shown in Figure 2. Moreover, the ROC curve analysis indicates that FABP-1 is an excellent and sensitive marker for diagnosing CKD. It has exhibited an AUC of 1.000 with p= 0.0001 when tested in the control group, as shown in Figure 3.

Table 6: Sensitivity, specificity, and AUC of TIMP-2 and FABP-1 in the studied groups

ROC	SD	*p*-value	Sensitivity	Specificity	Cut-off value	AUC	
						Lower Bound	Upper Bound
TIMP-2 (ng/mL)	0.023	0.0001	93%	96%	0.32	0.932	1.000
FABP-1 (ng/mL)	0.010	0.0001	98%	93%	0.67	0.971	1.000

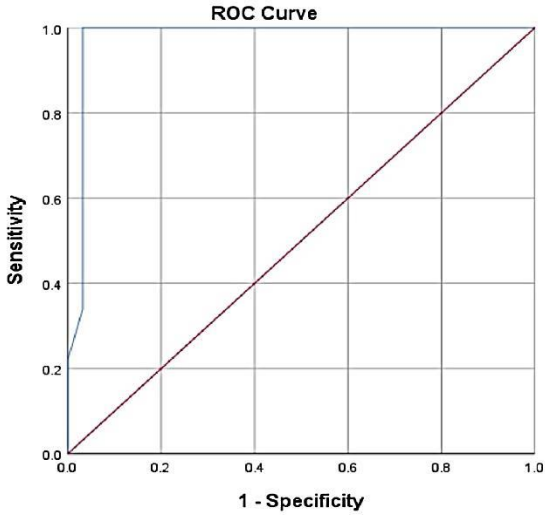

Figure 2: The ROC curve for TIMP-2 in patients and control

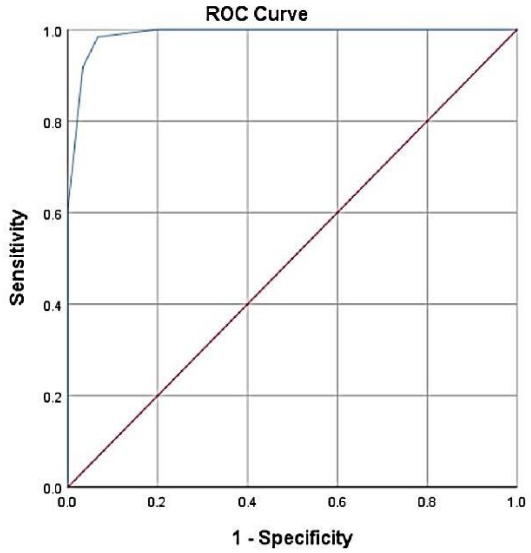

Figure 3: The ROC curve for FABP-1 in patients and control

4. Discussion

CKD activates the kidneys' defensive and protective mechanisms in response to immune cell activity, inflammation, or apoptosis [13]. The incidence of CKD varies by state, often falling between 10% and 20%. However, it progressively increases, primarily in developed states. This trend is especially evident among older people, as shown in this study. Furthermore, DM, obesity, HTN, age, and primary renal diseases are associated with CKD. The GFR declines with age, and the prevalence of CKD is highest in older adults [14].

Additionally, CKD is associated with an elevated risk of cardiovascular disease (CVD) and all-cause mortality due to accelerated aging, with the highest absolute risk increase in those over 75. Indeed, premature death is a more common outcome than CKD progression to kidney failure requiring kidney replacement therapy. The progressive aging of the world population contributes to the projection that CKD will become the second most common cause of death before the end of the century in countries with long life expectancy. According to present data, CKD patients had an age range from 55-65 years, which agrees with a

previous study [15]. The kidneys control blood glucose levels by filtering the blood and selectively reabsorbing glucose to maintain glucose homeostasis. Nevertheless, the complex control of glucose management may be jeopardized in CKD. Glucosuria is a medical disorder when glucose is found in the urine and is caused by defects in renal glucose reabsorption. This phenomenon pushes the person into DM by raising blood glucose levels [16]. The CKD-related metabolic abnormalities can have a significant effect on glucose metabolism. Individuals in this study who were diagnosed with CKD may have abnormalities in their glucose metabolism, including decreased insulin sensitivity and impaired glucose tolerance. The changes in glucose metabolism may increase the risk of acquiring DM. Diabetic kidney disease (DKD) is a common and severely incapacitating consequence of DM. It contributes significantly to the worldwide CKD burden [17].

Regarding the lipid profile of CKD patients in this study, declining renal function appears to drastically alter the lipid level outcomes as a side effect of CKD, documented in previous data [18]. The quantity and quality of circulating lipoproteins vary depending on the uremic condition. CKD patients with dyslipidemia are typically characterized by high TG and low HDL-C values, along with additional abnormalities in lipoprotein composition. These alterations are generally believed to encourage atherosclerosis, which raises the CV burden in CKD patients [19]. The density and atherogenic potential of cholesterol are more critical its total concentration. However, patients with CKD do not see a substantial increase in LDL or TC values [20]; LDL-C exhibits a distinct structural alteration with a preponderance of dense and tiny LDL particles. Small, dense LDL particles are known to be more atherogenic than other LDL subfractions because of their enhanced susceptibility to oxidation and increased capacity to permeate the artery intima [21]. Under uremic circumstances, HDL production and maturation are impaired. First, there is a notable decrease in the production of hepatic lipoprotein A-I, a crucial HDL lipoprotein. Second, down-regulation of the transporters impairs the maturation of nascent HDL by preventing the outflow of free cholesterol molecules from tissue macrophages via ATP-binding cassette (ABC) transporters, such as ABCA1 and ABCR1. Third, there is a decrease in plasma levels and the activity of lecithin-cholesterol acyltransferase, an enzyme that changes free cholesterol into cholesteryl ester (CE) and mature HDL-C.

Moreover, CKD patients exhibit increased activity of CE transport protein (CETP) activity [22]. Elevated CETP activity is associated with lower levels of HDL-C because it mediates the exchange of TG and CE between HDL lipoproteins and ApoB. Consequently, patients with CKD have significantly lower plasma levels and maturation of HDL-C, which may lead to poor antioxidant and anti-inflammatory properties of HDL particles when uremic. Non-HDL-C may be a better predictor of CVD risk in patients with non-dialysis CKD. However, previous study indicates that HLD-C levels were positively related to risk in the presence of inflammation [23].

Hypertriglyceridemia (HTG) is an early indicator of CKD and is one of the most common features of dyslipidemia in patients with CKD, particularly those with diabetes and those undergoing peritoneal dialysis [24]. Insulin resistance (IR)-induced disruption of intracellular glucose transport routes also increases the development of metabolic syndrome [25]. The primary reason for increased plasma TG levels is the delayed breakdown of lipoproteins enriched-TG. Lipoprotein lipase (LPL) hydrolyzes TG contained in VLDL or chylomicrons to release free fatty acids. In uremic circumstances, LPL expression is increased [26]. Furthermore, parathyroid hormone-induced IR and over-expression of its competitive inhibitor, such as apolipoprotein C-III, decrease LPL activity in individuals with CKD. As VLDL receptors in adipocytes and muscle cells are down-regulated in uremia, there is also a deficiency in the clearance of circulating VLDL particles [27]. Anemia, hypervolemia, HTN,

and low levels of serum total protein and albumin are common comorbidities in patients with ESRD [28].

In the present study, mean levels of urea and creatinine were significantly elevated in patients with CKD compared to healthy individuals. These results are consistent with several previous studies [29, 30]. The rise in urea and creatinine levels occurs due to a decrease in the number of nephrons in patients with kidney failure, which causes a reduction in the GFR and is responsible for a significantly lower level of water and solutes. As a result, the kidneys lose their efficiency in eliminating nitrogenous wastes from the blood, which leads to the accumulation of these components in the bloodstream. The development of the disease is mainly caused by high levels of urea in the blood. However, it is strongly affected by either a catabolic state or excessive protein intake, leading to increased production of protein catabolic wastes [31].

Furthermore, it should be noted that in observational studies, CKD patients were defined as those with GFR < 60 mL/min/1.73 m2, and some studies used GFR as an index for renal function. However, in this study, CKD patients had eGFR= 34.72±16.70 mL/min/1.73 m2. The disease spectrum of CKD varies between different populations; i.e., in the Asian population, chronic glomerulonephritis predominates. In contrast, in European populations, DN and hypertensive nephrosclerosis are more common; this may be another important reason for the inconsistency in the results of observational studies that should not be ignored [32].

It has been proposed that kidney disease may promote the development of ESRD through multiple mechanisms, for example, the critical role of the renin-angiotensin-aldosterone system (RAAS) in the development of renal damage in proteinuric kidney disease. Upregulation of RAAS can lead to endothelial dysfunction, inflammation, and oxidative stress. Additionally, RAAS affects blood flow in the body by changing the balance between aqueous humor production and its flow through physiological pathways, as the glomerulus has filtration barriers regulated by RAAS [33].

A tenfold increase in serum TIMP-2 levels was accompanied by a fourfold increase in serum FABP-1 in patients with CKD compared with controls. This pattern is specific to this study. The negative association between serum TIMP-2 levels and decreased eGFR suggests an associated systemic generation. This elevation could be caused by over-activation of MMP-2, which can indeed be observed for inflammation and fibrosis, especially in the development of kidney fibrosis, a prominent CKD feature consistent with previous data [34]. Thus, there was a potential mechanism to protect against excessive loss of TIMP-2 via urine and maintain homeostasis of serum levels. According to previous data, a decrease in serum TIMP-2 level in stage I CKD indicates increased tubular reabsorption of TIMP-2. In contrast, the expression and secretion of TIMP-2 are found to be strongly correlated with the distal tubules. This hypothesis seemed feasible until stage IV CKD, when serum and urine TIMP-2 values increased concomitantly, ending the previous period of stability. These increases could also signify a protective anti-fibrotic reaction, distanced over time from pro-inflammatory and migratory stimuli. Also, these elevations among CKD cases are associated with proteinuria, increasing their role as markers of tubular damage in CKD patients [35].
Consequently, the comprehensive assessment of serum, one of the parameters examined, made a thorough examination of the kidney injury stage linked to CKD possible. The cross-sectional approach and relatively small study group also raise the possibility of bias. Nonetheless, the study design and the number of patients examined were modified to account for the total CKD population [36]. However, increased mRNA expression of both markers is

described in several kidney diseases, i.e., focal segmental glomerulosclerosis, immunoglobulin A nephropathy, lupus nephritis, DN, vasculitis, and CKD. Hyperglycemia is associated with increased levels of transforming growth factor-β, which is reported to be able to influence the MMP promoter. At the transcriptional level, MMP-2 mRNA was found to be increased despite decreased MMP-2 activity [37]. In clinical data, the reduced level of TIMPs was further demonstrated by decreased TIMP-2 concentrations, which had previously been shown in diabetic patients [38]. Contrary to the present results, only circulating TIMP-1 levels, not TIMP-2, increased in CKD patients [39]. The present study includes diabetic patients with comorbid CKD, and the effect of DM, not only related to its longer duration, appears to prevail over the expected impact of CKD on the MMPs/TIMPs axis, which may differ in diverse tissues.

In addition, MMP activity is likely to be affected by drugs in human studies, such as angiotensin-converting enzyme inhibitors. The reduced activity of the axis of MMPs and TIMPs is consistent with the decreased MMP-2/TIMP-2 ratio in DM alone and chronic renal failure (CRF) alone [40]. However, it provides evidence of elevated MMP activity in the DN and putatively enhanced extracellular matrix turnover, which may further differentiate the effects of DN on MMP activity from those observed with DM or CRF alone. However, increased MMP/TIMP ratio in DN may only show inappropriate tendency and alternation with increased MMP activity [41].

Furthermore, high levels of FABP1 in urine or blood may indicate kidney dysfunction. Monitoring FABPs can be valuable in assessing the severity of renal injury and may have implications for diagnostic and therapeutic strategies in managing CKD. The glomeruli filter the circulating fraction of FABP-1 and subsequently reabsorbed in the proximal renal tubules. This explains its increased concentration in urine when proximal tubular cells are injured. It has shown that the human FABP-1 gene is overexpressed in the kidney and that stress can lead to increased excretion of the human FABP-1 gene in the urine due to conditions such as urinary protein excess, tubular dilatation, ischemia, and toxins, HTN and hyperglycemia [42]. Moreover, a clinical investigation discovered associations between urinary FABP1 excretion, tubulointerstitial damage degree, and CKD progress rate among non-diabetic patients. These outcomes suggest that urinary FABP-1 can be used as a clinical marker to monitor for renal impairment and categorize individuals in case of renal function decline over time [43].

Numerous conditions, including hyperglycemia, HTN, proteinuria, and toxin-induced injury to proximal tubule cells, directly or via gene expression regulation that increases urinary-derived FABP-1 secretion [44]. Furthermore, it has been revealed that FABP1 may be essential to kidney damage and repair and that it may predict the onset and severity of diverse kidney diseases by determining the level of FABP-1 in urine [45,46]. The FABP-1 levels were linked to DN in this investigation, and an increase in plasma FABP-1 levels coincided with an eGFR decline. Massive proteinuria and FA overload in the proximal tubules are symptoms of CKD, and FA overload can also be brought on by HTG [47]. Furthermore, compared to individuals with mild change nephrotic syndrome, patients with CKD had considerably increased urine excretions of linoleic acid and arachidonic acids. Following peroxidation, non-oxidized FAs seem cytotoxic and may cause macrophage infiltration, releasing inflammatory mediators and accelerating tubulointerstitial damage [48]. This could be because DN patients have greater plasma FABP-1 levels. Additionally, independent of the stage of the disease, Panduru et al. demonstrated that FABP-1 was a predictor of DKD progression in humans. After the onset of microalbuminuria, the increase in FA binding to albumin may lead to increased FA load in the proximal tubules, leading to up-regulation of the L-FABP gene to increase FFA export to mitochondria [49]. Moreover, it was found that the level of FABP was significantly increased in the group of people with diabetes with CKD compared to people with diabetes without CKD; this may be due to hypoxia and oxidative

stress, which possibly cooperate with increased albuminuria excretion, leading to elevated serum FABP and urinary FABP [50]. Serum FABP shows a strong relationship with eGFR in the present analyses, reflecting its role in renal deterioration.

Conclusions

Serum levels of TIMP-2 and FABP-1 may serve as valuable tools in the comprehensive assessment of kidney injury associated with CKD, encompassing apoptosis, fibrosis, inflammation, and cell migration. Elevated serum concentrations of TIMP-2 indicate the delayed regulation of protective anti-apoptotic and anti-fibrotic mechanisms, which emerge only after fully sustained tubular damage. Thus, there is currently enough data to support the idea that CKD, eGFR, TIMP-2, and FABP-1 are causally related. These findings underscore the importance of eye exams on individuals diagnosed with different CKD-related conditions. Furthermore, this study emphasizes the importance of using more extensive and diverse populations to examine these associations in more detail. These findings imply that serum FABP-1 can be used as a clinical marker to screen for renal impairment and identify individuals most likely to check their renal function decline over time.

Acknowledgements

The authors thank the Department of Chemistry, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad for approving the research.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Clearance

The Institutional Scientific Committee at the University of Baghdad approved this study according to the Declaration of Helsinki for human studies, which is consistent with the instructions of the Iraqi Ministry of Health and Environment according to the code number (85954) on (22/11/2023).

References

[1] K. Kalantar-Zadeh, T.H. Jafar, D. Nitsch, B.L. Neuen, and V. Perkovic, "Chronic Kidney Disease," *The lancet*, vol. 398, no. 10302, pp. 786-802, 2021.

[2] A.J. Abdulrahman, M.A-H Jabarah, and S.A. Najjar, "Effects of Liraglutide on Weight Control and Blood Pressure in Type 2 Diabetes Mellitus Iraqi Patients," *Journal of The Faculty of Medicine Baghdad*, vol. 64, no. 4, pp. 227-232, 2022.

[3] C. Heybeli, A. Bentall, J. Wen, M.P. Alexander, F.K. Buadi, F. Cosio, P.G. Dean, A. Dispenzieri, D. Dingli, M. El Ters, M.A. Gertz, A. Hatem, P. Kapoor, H. Khamash, T. Kourelis, S. Kumar, E.C. Lorenz, M. Mai, E. Muchtar, D.L. Murray, and N. Leung, "A Study from the Mayo Clinic Evaluated Long-Term Outcomes of kidney Transplantation in Patients with AL Amyloidosis from The Mayo Clinic," *Kidney International*, vol. 99, no. 3, pp. 707, 2021.

[4] Z. Abood, M. Rasheed, and H. Omran, "Significance of Cystatin C for Early Diagnosis of Contrast-Induced Nephropathy in Iraqi Patients Undergoing Coronary Angiography," *Medical Journal of Babylon*, vol. 18, no. 3, pp. 191-194, 2021.

[5] L. Costanzo, B. Soto, R. Meier, and P. Geraghty, "The Biology and Function of Tissue Inhibitor of Metalloproteinase 2 in the Lungs," *Pulmonary Medicine*. Vol. 2022, no. 3632764, pp. 1-12, 2022.

[6] H. Wang, L. Huang, L. Wu, J. Lan, X. Feng, P. Li, and Y. Peng, "The MMP-2/TIMP-2 System in Alzheimer Disease," *CNS & Neurological Disorders-Drug Targets*, vol. 19, no. 6, pp. 402-416, 2020.

[7] M. Provenzano, M. Andreucci, C. Garofalo, T. Faga, A. Michael, N. Ielapi, R. Grande, P. Sapienza, S. de Franciscis, P. Mastroroberto, and R. Serra, "The Association of Matrix

Metalloproteinases with Chronic Kidney Disease and Peripheral Vascular Disease: A light at the End of the Tunnel?," *Biomolecules*, vol. 10, no. 1, pp. 154, 2020.

[8] M. Kobusiak-Prokopowicz, J. Krzysztofik, K. Kaaz, B. Jolda-Mydlowska, and A. Mysiak, "MMP-2 and TIMP-2 in Patients with Heart Failure and Chronic Kidney Disease," *Open Medicine,* vol. 13, no. 1, pp. 237-246, 2018.

[9] I.T. Tsai, C.C. Wu, W.C. Hung, T.L. Lee, C.F. Hsuan, C.T. Wei, Y.C. Lu, T.H. Yu, F.M. Chung, Y.J. Lee, and C.P. Wang, "FABP1 And FABP2 as Markers of Diabetic Nephropathy," *International Journal of Medical Sciences*, vol. 17, no. 15, pp. 2338, 2022.

[10] National Kidney Foundation, "K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification," *American Journal of Kidney Disease*, vol. 39, no. 2 (Suppl 1), pp. S1–266, 2002.

[11] M.R. Langlois and A.D. Sniderman, "Non-HDL Cholesterol or Apo B: Which to Prefer as a Target for the Prevention of Atherosclerotic Cardiovascular Disease?," *Current Cardiology Reports*, vol. 22, no. 8, pp. 67, 2020.

[12] N. Lameire, K. Eckardt, B. Kasiske, D. Wheeler, A. Levin, P. E. Stevens, R. W. Bilous, E. J. Lamb, and J. J. K. I. Coresh, "KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease," *Kidney International Supplements*, vol. 3, no. 1, pp. 1-150, 2013.

[13] M.M. Cusick, R.L. Tisdale, G.M. Chertow, D.K. Owens, and J.D. Goldhaber-Fiebert, "Population-Wide Screening for Chronic Kidney Disease: A Cost-Effectiveness Analysis," *Annals of Internal Medicine,* vol. 176, no. 6, pp.788–97, 2023.

[14] A. Ortiz, F. Mattace-Raso, M.J. Soler, and D. Fouque, "Ageing Meets Kidney Disease," *Nephrology Dialysis Transplantation,* vol. 38, no. 3, pp. 523-526, 2023.

[15] A.H. Ad'hiah, Z.A. Ahmed, M.A. Al-Naseri, E.D. Salman, O.B. Al-saffar, H.S. Ahmed, T.A. Hussain, E.M. Idan, I.B. Hassan, B.A. Shihab, L.O. Mohammed, and F.S. Al-Nasiri, "Cytokine Gene Polymorphisms in Iraqi Arabs," *Human Immunology*, vol. 79, pp. 91–92**,** 2018**.**

[16] M.S. Mohammed and H.S. Ahmed, "Plasminogen Activator Urokinase Receptor as a Diagnostic and Prognostic Biomarker in Type 2 Diabetic Patients with Cardiovascular Disease," *Journal of Cardiovascular and Thoracic Research*, Vol. 15, no. 3, pp.154-160, 2023.

[17] H.T. AlAni and M.Q. Al-Lami**,** "Evaluation of some Biochemical and Hematological Parameters in Patients with Chronic Kidney Disease," *Journal of The Faculty of Medicine Baghdad*, vol. 66, no. 2, pp. 154-161, 2024.

[18] E.M.M. Raoof and H.S. Ahmed, "Circulating Adipsin as Biomarker and its Implication in Type 2 Diabetes Mellitus," *International Journal of Drug Delivery Technology*, vol. 12, no. 2, pp. 588-593, 2022.

[19] A.A. Obeed, T.A. AL-Kinani, and H.I. AL-Qadhi, "Effect of Dapagliflozin on Hemoglobin Level in Heart Failure Patients with Chronic Kidney Disease and/or Diabetes," *Journal of The Faculty of Medicine Baghdad*, vol. 64, no. 24, 203-207, 2022.

[20] B.H. Zhang, F. Yin, Y.N. Qiao, and S.D. Guo,"Triglyceride and Triglyceride-Rich Lipoproteins in Atherosclerosis," *Frontiers in Molecular Biosciences,* vol. 9, no. 909151, pp. 1-21, 2022.

[21] J.H. Moon, K. Kim, and S.H. Choi, "Lipoprotein Lipase: Is It a Magic Target for the Treatment of Hypertriglyceridemia," *Endocrinology and Metabolism (Seoul)*, vol. 37, no. 4, pp. 575-586, 2022.

[22] N. Mishra, K.A. Manik, and M. Ahmad, "Lipid Profile Alteration in CKD – (A Case – Control Study)," *African Journal of Biological Sciences*, vol. 6, no. 6, pp. 2340-2348, 2024.

[23] A.A. Khesbak, "The Effect of Some Biochemical and Physiological Parameters in Iraqi Patients with Renal Failure," *Journal* of *Biology*, *Agriculture* and *Healthcare*, vol. 10, no. 14, pp. 2018–2021, 2020.

[24] Z.S. Abdulrahman, M.Q. Alatrakji, A.A. Al-Maliky, K.I. Hussein, and S.A. Hussain, "Influence of Metformin dose and Treatment Adherence on Glycemic Control, Adiposity, and Cardiovascular Risk Markers in Iraqi Patients with T2DM," *Journal of The Faculty of Medicine Baghdad*, vol. 64, no. 4, 218-226, 2022.

[25] M.A. Arif, M.K. Rasheed, and A.A. Ismaeel, "Association between Vitamin D Levels and Insulin Resistance in Patients with Type 2 Diabetes Mellitus," *Advancements in Life Sciences*, vol. 11, no. 2, pp. 419-424, 2024.

[26] A.M. Abd Al Ridha, D.J. Kadhim, and A.H.A. Alkhazrajy, "A Cross-Sectional Study of Urinary Neutrophil Gelatinase-Associated Lipocalin and its Association with Steroid Responsiveness in Iraqi Children with Idiopathic Nephrotic Syndrome," *Journal of The Faculty of Medicine Baghdad*, Vol. 65, No.3, pp. 163-172, 2023.

[27] H.S. Azzal, M.J. Majeed, A.A.D. Allawi, and F.A. Hammoudi, "Correlation of Soluble Klotho with Progress Stages of Diabetic Nephropathy**,"** *Biochemical and Cellular Archives*, vol. 21, no.1, pp. 1585-1588, 2021.

[28] S. Singh and S. Bhatta, "Biochemical and Hematological Parameters in Chronic Kidney Disease," *Journal of Manmohan Memorial Institute of Health Sciences*, vol. 4, no. 1, pp. 4–11, 2018.

[29] N. Singhal, D.R.D. Mathur, D.A.K. Bhargava, and D.D. Gupta, "Comparative Study of Renal Function Test and Thyroid Hormone in Chronic Kidney Disease," *International Journal of Medical Research and Review,* vol. 8, no. 6, pp. 427–431, 2020.

[30] S.M. Hussein, "Nutritional status for Patients with Hypertension in Hemodialysis Units at Baghdad Teaching Hospital," *Medico-Legal Update***,** vol. 20, no. 1, pp. 1386-1392, 2020.

[31] Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group, "KDIGO 2022 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease," *Kidney International*, vol. 102, no. 4S, pp. S1–S123, 2022.

[32] United States Renal Data System, "2020 USRDS Annual Data Report: Epidemiology of Kidney Disease in the United States. Bethesda, MD, National Institutes of Health," *National Institute of Diabetes and Digestive and Kidney Diseases*, 2020.

[33] S. Lai, A.M. Perrotta, D. Bagordo, S. Mazzaferro, P. Menè, F. Errigo, F. Tinti, S. Rotondi, A. Molfino, M. Simeoni, A.P. Mitterhofer, and R. Cianci, "Literature Review on the Cross-Link between Ocular and Renal Disease: Renin Angiotensin Aldosterone System Is a Main Actor," *European Review for Medical and Pharmacological Sciences*, vol. 26, no. 13, pp. 4774-4788, 2022.

[34] D.R. Emlet, N. Pastor-Soler, A. Marciszyn, X. Wen, H. Gomez, W.H. Humphries, S. Morrisroe, J.K. Volpe, and J.A. Kellum,"Insulin-Like Growth Factor Binding Protein 7 and Tissue Inhibitor of Metalloproteinases-2: Differential Expression and Secretion in Human Kidney Tubule Cells." *American Journal of Physiology-Renal Physiology*, vol 312, no. 2, pp. F284–F296, 2017.

[35] S. Chen, L. Chen, and H. Jiang, "Prognosis and Risk Factors of Chronic Kidney Disease Progression in Patients with Diabetic Kidney Disease and Non-Diabetic Kidney Disease: A Prospective Cohort CKD-ROUTE Study," *Renal Failure*, vol. 44, no. 1, pp. 1309–1318, 2022.

[36] H.N. Reich, D. Tritchler, D.C. Cattran, A.M. Herzenberg, F. Eichinger, A. Boucherot, A. Henger, C.C. Berthier, V. Nair, C.D. Cohen, J.W. Scholey, and M. Kretzler. "A Molecular Signature of Proteinuria in Glomerulonephritis." *PLOS One*, vol. 5, no. e13451, 2010.

[37] I.K. Salim and A.R. Diajil, "Assessment of Salivary Immunoglobulin A, Interleukin-6 and C-Reactive Protein in Chronic Kidney Disease Patients on Hemodialysis and on Conservative Treatment," *Journal of Baghdad College of Dentistry*, vol. 34, no.2 , pp. 62-73, 2022.

[38] A.K. Death, E.J. Fisher, K.C. McGrath, and D.K. Yue, "High Glucose Alters Matrix Metalloproteinase Expression in Two Key Vascular Cells: Potential Impact on Atherosclerosis in Diabetes." *Atherosclerosis*, vol. 168, no. 2, pp. 263-269, 2003.

[39] S.V. McLennan, X.Y. Wang, V. Moreno, D.K. Yue, and S.M. Twigg. "Connective Tissue Growth Factor Mediates High Glucose Effects on Matrix Degradation Through Tissue Inhibitor of Matrix Metalloproteinase Type 1: Implications for Diabetic Nephropathy." *Endocrinology* vol. 145, no. 12, pp. 5646-5655, 2004.

[40] R. Lobmann, A. Ambrosch, G. Schultz, K. Waldmann, S. Schiweck, and H. Lehnert, "Expression of Matrix-Metalloproteinases and Their Inhibitors in the Wounds of Diabetic and Non-Diabetic Patients," *Diabetologia*, vol. 45, pp. 1011-1016, 2002.

[41] J.H. Horstrup, M. Gehrmann, B. Schneider, A. Ploger, P. Froese, T. Schirop, D. Kampf, U. Frei, R. Neumann, and K.U. Eckardt, "Elevation of Serum and Urine Levels of TIMP-1 and Tenascin in Patients with Renal Disease," *Nephrology Dialysis Transplantation*, vol. 17, no. 6, pp. 1005-1013, 2002.

[42] T. Nakamura, C. Ushiyama, S. Suzuki, M. Hara, N. Shimada, I. Ebihara, and H. Koide, "Urinary Excretion of Podocytes in Patients with Diabetic Nephropathy," *Nephrology Dialysis Transplantation*, vol. 15, no. 9, pp. 1379-1383, 2000.

[43] Y. Xu, Y. Xie, X. Shao, Z. Ni, and S. Mou, "L-FABP: A Novel Biomarker of Kidney Disease," *Clinical Chimica Acta,* vol. 445, pp. 85-90, 2015.

[44] S.K. Parr, A.J. Clark, A. Bian, A.K. Shintani, N.E. Wickersham, L.B. Ware, T.A. Ikizler, and E.D. Siew, "Urinary L-FABP Predicts Poor Outcomes in Critically Ill Patients with Early Acute Kidney Injury," *Kidney International,* vol. 87, no. 3, pp. 640-648, 2015.

[45] J. Yang, H.M. Choi, M.Y. Seo, J.Y. Lee, K. Kim, H. Jun, C.W Jung, K.T. Park, M-G. Kim, S-K. Jo, W. Cho, and H.K. Kim, "Urine Liver-Type Fatty Acid-Binding Protein Predicts Graft Outcome Up to 2 Years after Kidney Transplantation," *Transplantation Proceedings*, vol. 46, no. 2, pp. 376-380, 2014.

[46] K. Jin, K. Norris, and N.D. Vaziri, "Dysregulation of Hepatic Fatty Acid Metabolism in Chronic Kidney Disease," *Nephrology Dialysis Transplantation*, vol. 28, no. 2, pp. 313-320, 2013.

[47] N. Zuo, Y. Suzuki, T. Sugaya, K. Osaki, Y. Kanaguchi, L. Wang, and Y. Tomino, "Protective Effects of Tubular Liver-Type Fatty Acid-Binding Protein against Glomerular Damage in Murine IgA Nephropathy," *Nephrology Dialysis Transplantation*, vol. 26, no. 7, pp. 2127-2137, 2011.

[48] H. Sasaki, A. Kamijo-Ikemori, T. Sugaya, K. Yamashita, T. Yokoyama, J. Koike, T. Sato, T. Yasuda, and K. Kimura, "Urinary Fatty Acids and Liver-Type Fatty Acid Binding Protein in Diabetic Nephropathy," *Nephron Clinical Practice*, vol. 112, no. 3, pp. c148-c156, 2009.

[49] N.M. Panduru, C. Forsblom, M. Saraheimo, L. Thorn, A. Bierhaus, P.M. Humpert PM, P-H. Groop, and on behalf of the FinnDiane Study Group. "Urinary Liver-Type Fatty Acid-Binding Protein and Progression of Diabetic Nephropathy in Type 1 Diabetes," *Diabetes Care,* vol. 36, no. 7, pp. 2077–83, 2013.

[50] L. Zhang, Sh. Xue, M. Wu, and D. Dong, "Performance of Urinary Liver-Type Fatty Acid-Binding Protein in Diabetic Nephropathy: A Meta-Analysis," *Frontiers in Medicine*, vol. 9, no. 914587, pp. 1-11, 2022.