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Estimation of TIMP-2 in Patients with Chronic Kidney Disease

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Abstract:

Background: *Chronic kidney disease (CKD) is a progressive condition with high morbidity and mortality, often associated with hypertension and diabetes. Early detection is essential for effective management. TIMP-2 has shown promise as a biomarker due to its role in extracellular matrix regulation and renal fibrosis, potentially offering benefits over traditional kidney function markers.*

Objective: *Estimating TIMP-2 levels in patients with chronic kidney disease and assessing its correlation with clinical and demographic factors.*

Methods: *A case-control study was conducted involving 90 participants individuals aged 30–70 years were enrolled, including 60 CKD patients (30 dialysis and 30 pre-dialysis stages) and 30 age- and sex-matched healthy controls. Serum levels of TIMP-2 were, creatinine, and (eGFR) were assessed.*

Results: *TIMP-2 levels were significantly elevated in CKD patients compared to healthy controls with a stepwise increase across disease stages. Dialysis patients exhibited the highest concentrations. TIMP-2 was significantly associated with declining renal function, correlating with elevated creatinine and reduced eGFR. While TIMP-2 levels showed no significant differences by age, sex, BMI, or hypertension, they were markedly higher in diabetic patients.*

Conclusion: *TIMP-2 shows strong potential as a biomarker for detecting and monitoring CKD, particularly in advanced stages. Its progressive increase across CKD severity and its association with renal function support its use in disease assessment. Further research may establish its clinical utility.*

Keywords: *TIMP-2; Chronic Kidney Disease ; Dialysis; Pre-dialysis; Renal Function.*

INTRODUCTION:

A progressive and irreversible deterioration in kidney function denotes chronic kidney disease (CKD), a global public health concern. Over Eight hundred and fifty million people, making it a significant global health concern. and it is linked to a high risk of hospitalization, cardiovascular disease, and early mortality **[1].** There are significant financial and societal costs significant associated with dialysis or kidney transplantation when chronic kidney disease (CKD) progresses to end-stage renal disease (ESRD) **[2].** A low estimated glomerular filtration rate (eGFR) and/or an increase in albuminuria are signs of chronic kidney disease (CKD), which is diagnosed when kidney structural or functional abnormalities in the kidney last more than three months **[3]**. based on estimated glomerular filtration rate (eGFR) levels, the disease is classified into five stages, with renal replacement therapy required at stage 5. Chronic kidney disease (CKD) is disproportionately prevalent in developing countries, where a lack of early diagnostic resources leads to delayed detection and accelerated disease progression.

The burden of CKD is disproportionately high in developing countries, where limited access to early diagnostic tools leads to delayed detection and accelerated progression **[4].** Epidemiological data from the Middle East show a high prevalence of chronic kidney disease (CKD) in the region due to high rates of obesity, diabetes, and hypertension. These conditions typically affect people at a younger age compared to Western populations **[5].** Because conventional markers like serum creatinine and urea are not sensitive to early structural damage, early detection of chronic kidney disease (CKD) remains suboptimal despite its substantial clinical impact **[6].**

In order to enhance risk stratification and early detection of chronic kidney disease (CKD), studies are currently underway on emerging biomarkers. One such biomarker, tissue inhibitor of metalloproteinase-2 (TIMP-2), is an increasingly promising inhibitor. TIMP-2 is of 21-kDa glycoprotein that inhibits the breakdown and remodeling of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs) **[7].** TIMP-2 regulates extracellular matrix turnover, which is critical for tubular epithelial damage and renal fibrogenesis, two major pathological aspects of chronic kidney disease **[8].** Increased TIMP-2 expression has been found in fibrotic kidney tissue, indicating chronic tubular stress and interstitial matrix accumulation **[9].**

Although TIMP-2 has been extensively studied and validated as a biomarker in acute kidney injury (AKI), especially when combined with IGFBP7 to predict tubular stress and early renal damage **[10].** Its role in chronic kidney disease is less clear. According to emerging evidence, TIMP-2 may also reflect ongoing subclinical injury and fibrotic changes in CKD, making it useful for assessing disease progression **[11].** However, further research and validation are needed in large, well-characterized CKD cohorts to clarify its clinical utility across different stages and comorbid conditions such as diabetes and hypertension **[12].**

This study aims to estimate serum TIMP-2 levels in patients with CKD across various stages and to evaluate its potential diagnostic and prognostic value. Furthermore, the study seeks to explore correlations between TIMP-2 concentrations and clinical variables including renal function parameters, metabolic status, and comorbidities. Establishing the role of TIMP-2 in CKD may contribute to more effective monitoring and early intervention strategies.

MATERIALS AND METHODS:

Study Design and Ethical Considerations

This case-control study was conducted between November 2024 and March 2025 at Al-Hussein Teaching Hospital in Karbala, Iraq. A total of 90 participants aged 30–70 years were recruited. The study population included three groups the first group (Dialysis CKD) involved 30 patients with confirmed end-stage renal disease undergoing regular hemodialysis. The second group (Pre-dialysis stages CKD) include 30 patients in CKD stages 2–4 not on dialysis and the third group (Controls) individuals 30 age- and sex-matched apparently healthy individuals with no history of renal disease.

Inclusion Criteria: Iraqi Males and female patients with chronic kidney disease diagnosed specialized physician according to KDIGO Diagnostic criteria the clinical medical and history examination.

Exclusion Criteria: Patients had suffering acute kidney injury, cardiovascular diseases, Patients with COVID-19 and recent cardiac, Patients kidney recent surgery and renal cancer, lung cancer or colon cancer were excluded from current in this study.

Data Collection and Clinical Evaluation

Demographic data, smoking status, comorbidities (diabetes, hypertension), medication use, and family history of CKD were collected via structured questionnaires. Physical examinations included height, weight, and blood pressure (systolic and diastolic). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) and categorized according to WHO guidelines.

Sample Collection and Storage

Venous blood samples (5 mL) were collected using gel tubes. After clotting, the samples were centrifuged at 3000 rpm for 20 minutes. The separated serum was aliquoted into three 1.5 mL Eppendorf tubes and stored

at −20°C until analysis. One aliquot was designated for biomarker assessment (TIMP-2), and others for routine kidney function and electrolyte testing and 25-OH Vitamin D and PTH.

2.5 Biomarker and Biochemical Analyses

- TIMP-2 level were determined using human-specific sandwich ELISA kits (BT LAB, China), following the manufacturer's protocols. Absorbance was measured at 450 nm, and concentrations were calculated from standard curves. Creatinine was measured by kinetic alkaline picrate method (Jaffe reaction) using Architect c4000 autoanalyzer (Abbott, USA). eGFR was calculated using the CKD-EPI equation. Urea was determined enzymatically via urease and GLDH reactions. Electrolytes (Na^+, K^+): Sodium was measured via sodium-dependent β-galactosidase method; potassium via pyruvate kinase-lactate dehydrogenase coupled assay. Calcium: Determined colorimetrically using Arsenazo-III dye. 25-OH Vitamin D and PTH: Quantified using CMIA (Chemiluminescent Microparticle Immunoassay) on the Architect i1000SR system (Abbott, USA).

Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM, USA). Categorical variables were presented as numbers and percentages, while continuous data were expressed as median (interquartile range). Normality was assessed via the Shapiro-Wilk test. Between-group comparisons were performed using the Mann-Whitney U test or Kruskal-Wallis test, depending on the number of groups. Spearman's rank correlation coefficient was used to assess relationships between continuous variables. Logistic regression was applied to evaluate associations with CKD status. ROC curve analysis was conducted to determine diagnostic performance. A p-value < 0.05 was considered statistically significant.

Results: Serum TIMP-2 levels were significantly higher in CKD patients than in healthy controls (p = 0.004), indicating potential diagnostic relevance (Table 1). When comparing disease stages, TIMP-2 levels increased progressively across the groups, being lowest in controls, slightly higher in pre-dialysis patients, and highest in dialysis patients. This trend was statistically significant (p = 0.001), as shown in Table 1. Further subgroup analysis demonstrated that CKD patients with diabetes had significantly elevated TIMP-2 levels compared to non-diabetic patients (p = 0.027) (Table 1).

Table 1. Serum TIMP-2 Levels and Their Clinical and Biochemical Associations Across CKD Groups

Biomarker	Control n=30		Patients n=60		P-value
	Median (min-max)	IQR	Median (min-max)	IQR	
TIMP-2 ng/mL	0.199 (0.14-1.45)	0.273	0.233 (0.14 – 1.45)	0.23	0.004

	Control n=30		Pre-dialysis stages n=30		On dialysis n=30		P-value
TIMP-2 ng/mL	0.199 (0.14-1.45)	0.273	0.222 (0.12-0.77)	0.260	0.379 (0.14-1.57)	0.610	0.001

Diabetic Status				
Yes n=37		No n=23		P-value
TIMP-2	0.267 (0.12-1.57)	0.26	0.205 (0.14-1.10)	0.28	0.027

Data are presented as median (IQR). Kruskal-Wallis and Mann–Whitney U tests were used to assess group differences. Spearman's rank correlation was used to evaluate associations between TIMP-2 and continuous variables.

Correlation of Serum TIMP-2 with Clinical and Biochemical Parameters

Correlation analysis revealed a significant negative association between TIMP-2 and eGFR, and a positive correlation with serum creatinine ($p \leq 0.001$), indicating that higher TIMP-2 levels reflect worse renal function (Table 2). Additionally, TIMP-2 showed a moderate positive correlation with PTH ($p = 0.020$), while correlations with other electrolytes and vitamin D3 were not statistically significant (Table 2).

Table 2. Correlation of Serum TIMP-2 with Clinical and Biochemical Parameters

Parameter	Correlation (r)	P-value
eGFR (mL/min/1.73 m²)	-0.418	0.001
Creatinine (mg/dL)	0.115	0.287
Urea (mg/dL)	0.400	<0.001
Sodium (Na) mmol/L	-0.109	0.313
Potassium (K) mmol/L	0.094	0.383
Calcium (Ca) mg/dL	-0.113	0.295
Vitamin D3 (ng/mL)	-0.053	0.623
PTH (pg/mL)	0.305	0.020

Statistical significance was considered at $p \leq 0.05$; r: Spearman correlation coefficient; +: positive correlation; N: number of participants; Min: minimum; Max: maximum.

The Odd Ratio of TIMP-2 in Patients with CKD.

Logistic regression analysis showed that TIMP-2 was not a statistically significant independent predictor of CKD status ($p = 0.090$) (Table 3).

Table3. Odds Ratio for Serum KIM-1 in CKD Patients

Marker	Odds Ratio	95% CI (Lower–Upper)	P-value
TIMP-2	1.907	0.741 – 61.233	0.090

OR: Odds Ratio, CI; Confidence Interval, P-value

Receiver Operating Characteristic (ROC) .

ROC curve analysis revealed a moderate diagnostic value of TIMP-2:

For differentiating pre-dialysis patients from controls, the AUC was 0.634 with 65% sensitivity and 60% specificity (Table 4, Figure 1).For dialysis patients versus controls, the AUC increased to 0.715, with 76% sensitivity and 60% specificity (Figure 2).For distinguishing between pre-dialysis and dialysis patients, the AUC was 0.715, with 71% sensitivity and 63% specificity (Table 4, Figure 3)

Table4. Diagnostic Performance of Serum KIM-1 Based on ROC Analysis Across CKD Stages

Groups	AUC	P-value	Cut-off (ng/L)	Sensitivity	Specificity
Control vs. Pre-dialysis	0.634	0.077	0.207	0.65	0.60

Control vs. Dialysis	0.715	0.002	0.208	0.76	0.60
Pre-dialysis vs. Dialysis	0.715	0.005	0.230	0.71	0.63

ROC: Receiver operating characteristic; significant at p ≤ 0.05 ; AUC; Area under

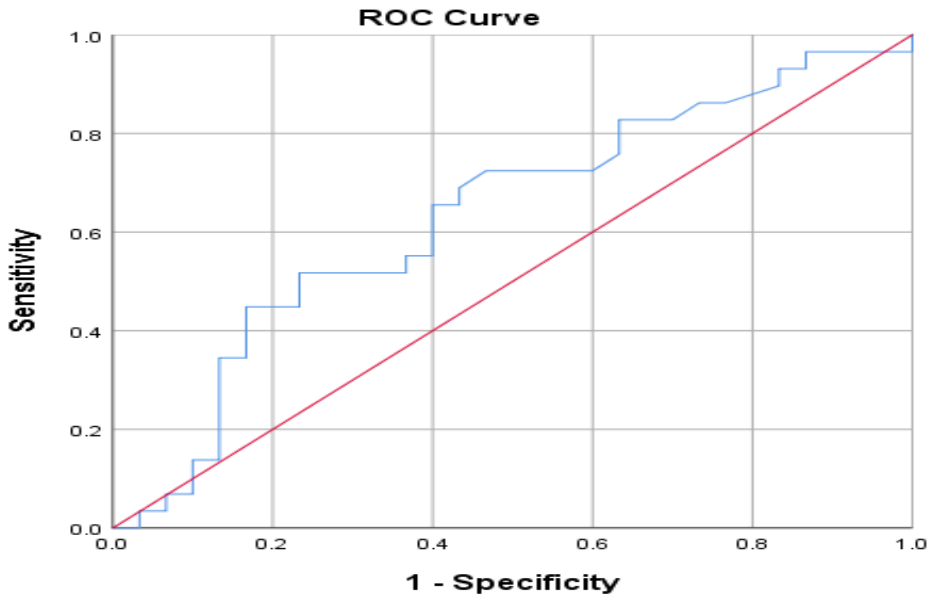

Figure 1: Receiver Operating Characteristic (ROC) of TIMP-2 in Pre-dialysis CKD Patients Compared to Control Group.

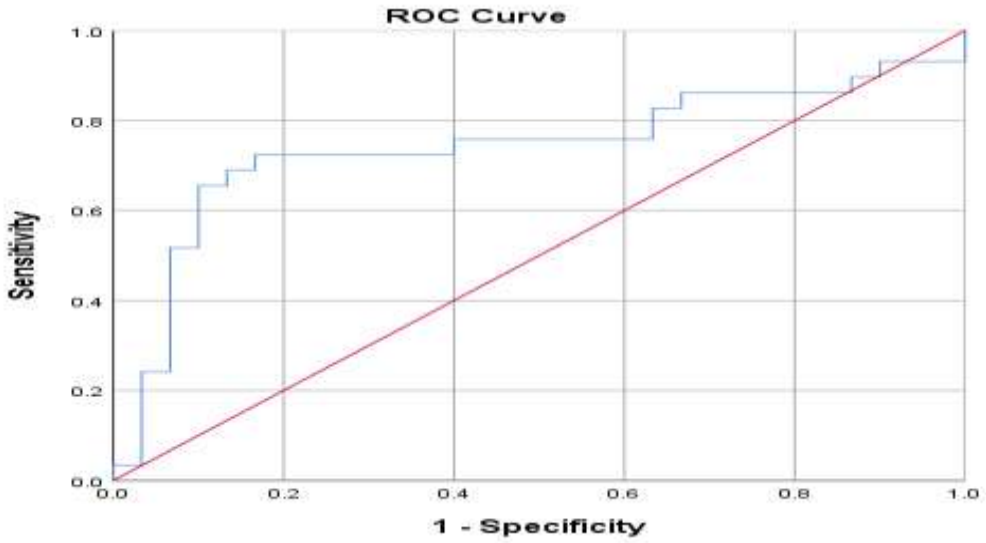

Figure 2: Receiver Operating Characteristic (ROC) of TIMP-2 In-dialysis Patients Compared to Control Group.

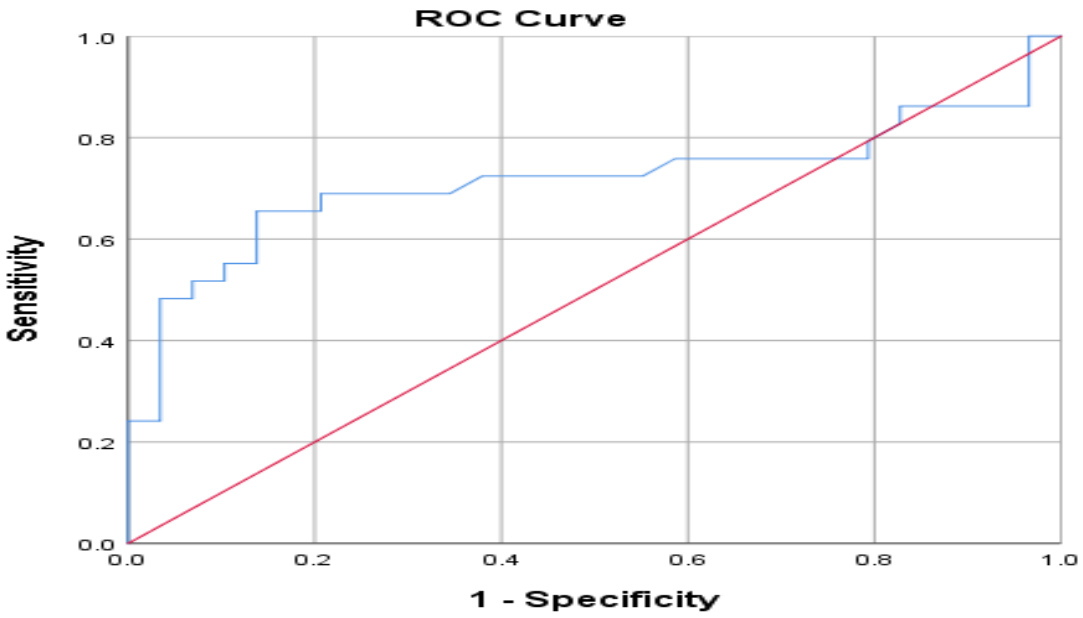

Figure 3: Receiver Operating Characteristic (ROC) of TIMP-2 In-dialysis Patients Compared to pre-Dialysis Patients group

DISCUSSION:

The current study provides compelling evidence to support Tissue Inhibitor of Metalloproteinases-2 (TIMP-2) as a dynamic and stage-sensitive biomarker in chronic kidney disease (CKD). High TIMP-2 levels in CKD patients, especially those on dialysis, indicate a progressive tubular stress response that worsens with worsening renal function ($p < 0.001$). This graded increase across disease stages provides diagnostic clarity as well as insight into the pathophysiology of CKD progression.

Unlike currently used conventional kidney markers, such as serum creatinine, which often demonstrate irreversible damage, TIMP-2 levels demonstrate ongoing damage and active remodeling of the extracellular matrix, which is consistent with its mechanism of action in kidney fibrosis **[13].** Its biological accuracy as a marker is enhanced by its independence from age, gender, and body mass index, eliminating any potential confounding demographic factors. This is a critical advantage in personalized medicine.

Crucially, TIMP-2 did not significantly associated with BMI, age, or gender, suggesting that it is a biologically specific biomarker of renal injury. This independence from confounding demographic variables points to that TIMP-2 expression is directly and tightly controlled by localized tubular stress and matrix remodeling within the kidney, rather than systemic physiological factors. Traditional markers like serum creatinine, are heavily influenced by muscle mass, which varies greatly depending on gender, age, and body type. There may be diagnostic ambiguity when people with low muscle mass such as elderly or female patients present with have falsely low creatinine levels despite underlying renal impairment. The stability of TIMP-2 across these variables increases its potential as a universal marker in precision nephrology and improve its dependability in a variety of populations. **[14,15].**

Unexpectedly, compared to non-diabetic controls, diabetic CKD patients had noticeably higher levels of TIMP-2. This finding highlights how sensitive the biomarker is to the distinct pathological setting of diabetic nephropathy, which is marked by inflammation, oxidative stress brought on by chronic hyperglycemia, and an increase of profibrotic cytokines such as TGF-β1. These factors enhance extracellular matrix accumulation and tubular-interstitial fibrosis, in which TIMP- 2 regulates matrix degradation and inhibits metalloproteinase activity. This biological interaction highlights TIMP-2's potential as a diagnostic and treatment target for metabolic renal injury, as well as why its expression is elevated in diabetic kidneys. **[16,17].**

On the other hand, neither smoking status nor hypertension were significantly correlation with TIMP-2 levels. This finding suggests that sustained or high-intensity tubular injury is necessary directly for TIMP-2 to become elevated, rather than hemodynamic stress or low-grade chronic nephrotoxic exposure.

It is believed that cellular stress pathways like advanced fibrotic signaling and G2/M cell cycle arrest are the major causes of TIMP-2 expression. When renal damage is severe or progressive, these mechanisms are usually activated, which limits the body's reaction to harmless stimuli like smoking or high blood pressure. **[18,19].**

The potential role of TIMP-2 as a biomarker in chronic kidney disease (CKD) is supported by its strong positive correlation with serum creatinine and negative correlation with estimated glomerular filtration rate (eGFR). Its moderate correlation with parathyroid hormone (PTH) may also suggest a potential interaction between mineral metabolism abnormalities and the fibrosis pathways observed in CKD **[20].**

Conclusion:

TIMP-2 is a promising biomarker for tubular damage identification and disease progression assessment in chronic kidney disease, according to this study. Its biological significance is highlighted by its gradual rise across CKD stages, robust correlation with renal function indicators, and specific sensitivity to diabetic pathology. TIMP-2's diagnostic consistency is improved by the fact that, unlike traditional biomarkers, it seems to be unaffected by minor nephrotoxic exposures and stable across demographic differences. Combining TIMP-2 with other markers, like Kidney Injury Molecule-1(KIM-1) and Insulin-like Growth Factor-Binding Protein (IGFBP7), may enhance early detection techniques and facilitate more individualized CKD management, despite its limited predictive performance when used alone. For its prognostic significance in a variety of clinical contexts, more longitudinal research is required.

Ethical Approval:

An ethical certificate was obtained from the relevant committees at the University of Karbala, No. 24-31, dated 29/6/2025. All research participants were informed and allowed to give their consent before sample collection. By document number 2690,22/10/2024, a local college and hospital ethics committee reviewed and approved the protocol, subject information, and consent form.

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Conflict of Interest:

- The researcher advertises no potential conflict of interest respecting to the research, compilation and publication of the present articl
- The authors declare that the content of this article has not been published previously
- and will not be submitted for publication elsewhere while the manuscript is under review.

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