Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbala /College of Medicine

Department of Chemistry and Biochemistry

Assessing Serum Kidney Injury Molecule-1 and Tissue Inhibitor of Metalloproteinase-2 as Diagnostic Markers in Chronic Kidney Disease in Kerbala Governorate

A Thesis Submitted to the Council of the College of Medicine, University of Kerbala, in Partial Fulfillment of the Requirements for the Master Degree in

[Clinical Biochemistry]

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

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We certify that this thesis

Assessing Serum Kidney Injury Molecule-1 and Tissue Inhibitor of Metalloproteinase-2 as Diagnostic Markers in Chronic Kidney Disease

was carried under our supervision at the College of Medicine, University of Kerbala, as partial fulfillment for the requirement of the degree of Master of Science in "Clinical Biochemistry"

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Dedication

With love, I dedicate this work to my biggest supporter (my father),

and the one who alleviated my difficulties with her prayers (my mother)

To my support after God,

To my companion, my dear husband,

who bore many burdens for me and supported me with silence, patience, and love.

To my little girl, the heartbeat of my life,

who endured my absence and preoccupation, and who was my light during the darkest days of this journey.

And to myself,

for my patience, resilience, and perseverance despite exhaustion.

I dedicate the fruits of this humble effort to you all, with deep gratitude and love.

Acknowledgments

First, and above all, thanks to the Great Merciful Almighty (Allah) Who gave me health, strength, patience, and perseverance and facilitated the ways for me to accomplish this work.

I am grateful to my master, Abi Al-Fadl Al-Abbas, peace be upon him, who is a man of generosity and compassion. I wouldn't find myself here if it wasn't for you, sir.

I would like to express my gratitude to all the patients for their cooperation, as well as all the individuals working in the research laboratory of the Department of Chemistry and Biochemistry at the College of Medicine, University of Karbala. I am also thankful to the dedicated medical staff at AL Hussein Hospital, Their invaluable assistance was crucial in making this thesis a reality.

I would like to express my sincere gratitude to my advisor Asst. Prof. Dr . Maher Abbood Mukheef, College of Medicine, University of Kerbala for his continuous support, patience, motivation, and immense knowledge

Thanks are due to Prof. Dr. Khaled Khalil Al-Araji, Dean of the College of Medicine, University of Kerbala, and to Asst. Prof. Dr. Rana Mohammed Hameed, Head of the Department of Chemistry and Biochemistry

Finally, to everyone who helped me with advice or work and stood by me and gave me a helping hand when I needed it, you have all my thanks and appreciation.

Fatima

Summary

Chronic Kidney Disease (CKD) is a progressive condition characterized by irreversible loss of renal function, driven by multiple etiologies including diabetes, hypertension, ischemic injury, and glomerulonephritis. Early detection of tubular injury is critical to delay progression to end-stage renal disease (ESRD) requiring dialysis or transplantation.

The current study was a case-control study involving 88 subjects: 58 CKD patients (29 pre-dialysis stages, 29 on dialysis) and 30 healthy controls, (aged 30–70 years) was conducted. Blood samples were analyzed using ELISA to measure Serum Kidney Injury Molecule-1 (KIM-1), Tissue Inhibitor of Metalloproteinases-2 (TIMP-2), other biochemical markers such as serum creatinine, serum urea, sodium and potassium concentration, calcium, vitamin D3 and parathyroid hormone (PTH) levels. Ethical approval and informed consent were obtained. There were no statistically significant differences in age or BMI among the three groups (p value > 0.05). Males represented 60% and females 40% of all participants. The most affected age group was ≥ 60 years (58.1%). Additionally, a majority of CKD patients had comorbid hypertension (72.3%) and type 2 diabetes mellitus (53%). A significant proportion of patients (over 66%) lacked regular physical activity, highlighting the need for lifestyle interventions. KIM-1 (is a kidney biomarker in the current study) levels were significantly elevated on dialysis patients compared to pre-dialysis and controls (p-value was 0.001)., but showed no significant difference between pre-dialysis patients and controls (p = 0.897). KIM-1 was higher in smokers, correlated positively with serum creatinine, and negatively with eGFR, but was not influenced by age, sex, BMI, or comorbidities such as diabetes and hypertension. ROC analysis

demonstrated fair diagnostic accuracy for dialysis patients versus controls (AUC = 0.751), with a cut-off of 0.48 ng/mL yielding 79% sensitivity and

67% specificity. Serum TIMP-2 levels were significantly higher in CKD patients than in healthy controls ($p = 0.004$), These findings indicate the potential diagnostic relevance of TIMP-2 in distinguishing CKD patients from healthy individuals. 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 ($p = 0.001$). Further subgroup analysis demonstrated that CKD patients with diabetes had significantly elevated TIMP-2 levels compared to non-diabetic patients ($p = 0.027$). TIMP-2 showed a stronger correlation with renal dysfunction than KIM-1. ROC analysis indicated moderate discriminatory power for dialysis patients versus controls, the AUC increased to 0.715, with 76% sensitivity and 60% specificity. For differentiating pre-dialysis patients from controls, the AUC was 0.634 with 65% sensitivity and 60% specificity.

In conclusion, KIM-1 and TIMP-2 may serve as useful indicators of kidney damage, especially in advanced stages of CKD. The findings suggest that the combined use of KIM-1 an TIMP-2 represents a promising approach for improving the evaluation and diagnosis of chronic kidney disease (CKD). KIM-1's specificity for proximal tubule injury and TIMP-2's ability to track disease progression across CKD stages highlight their complementary roles. Integrating these biomarkers, particularly in combination with others, may enhance early detection. Further longitudinal studies are warranted to validate their prognostic value and clinical applicability in diverse patient groups.

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List of Abbreviations

ATF-4	Activating Transcription Factor 4
ATF-4	Activating Transcription Factor 4
ATF-6	Activating Transcription Factor 6
BIM	Body Mass Index
BiP	Binding Immunoglobulin Protein
BUN	Blood Urea Nitrogen
Ca^{2+}	Calcium Ion
cAMP	Cyclic Adenosine Monophosphate
CHOP	C/EBP Homologous Protein
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CKD-HD	Chronic Kidney Disease – Hemodialysis
CKD-NT	Chronic Kidney Disease – Non-Dialysis Treatment
CR	Creatinine
CyclD-CDK4	Cyclin D - Cyclin Dependent Kinase 4 Complex

CyclE-CDK2	Cyclin E - Cyclin Dependent Kinase 2 Complex
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
eGFR	Estimated Glomerular Filtration Rate
EGFR	Epidermal Growth Factor Receptor
eIF2α	Eukaryotic Initiation Factor 2 Alpha
ER	Endoplasmic Reticulum
Gasdermin D	GSMD
GFR	Glomerular Filtration Rate
GLN	Glomerulonephritis
HTN	Hypertension
IGFBP-7	Insulin-Like Growth Factor Binding Protein 7
IHD	Ischemic Heart Disease
IRE1	Inositol-Requiring Enzyme 1
K^+	Potassium Ion
KIM-1	Kidney Injury Molecule-1
MMP2	Matrix Metalloproteinase-2
MT1-MMP	Membrane-Type 1 Matrix Metalloproteinase
Na^+	Sodium Ion

NF-κB	Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells
ng/ml	Nanogram per milliliter
NLRP3	NOD-like Receptor Protein 3
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NT-GSMD	N-Terminal Gasdermin D
PERK	Protein Kinase RNA-like ER Kinase
pro-MMP2	Pro-Matrix Metalloproteinase-2
PTH	Parathyroid Hormone
ROC	Receiver Operating Characteristic
SBP	Systolic Blood Pressure
SPSS	Statistical Package for the Social Sciences
TGF-β	Transforming Growth Factor Beta
TIMP-2	Tissue Inhibitor of Metalloproteinase-2
USA	United States of America
Vit D	Vitamin D3
XBP-1	X-box Binding Protein 1

Chapter One

Introduction

1. Introduction

1.1. Chronic Kidney Disease

Chronic kidney disease is a progressive condition characterized by structural and functional changes to the kidney due to various causes. It is a long-term condition that damages the kidneys and decreases their ability to filter waste from the blood**(Elendu et al., 2023)**. CKD can progress over time and eventually lead to kidney failure. It is typically defined as a reduction in kidney function, an estimated glomerular filtration rate (eGFR) of less than 60 mL/min per 1·73m²,or markers of kidney damage, such as albuminuria, hematuria, or abnormalities detected through laboratory testing or imaging and that are present for at least 3 months **(Caravaca-Fontán et al., 2018)**

The prevalence of different aetiologies of chronic kidney disease varies considerably by region. There are many causes of chronic kidney disease, including those that are common and well researched, such as diabetes, glomerulonephritis, and cystic kidney diseases, but causation in chronic kidney disease is not yet fully understood**(Lv et al., 2019)**.

It is contentious whether hypertension induces or results from chronic renal disease, despite the close association between the two conditions **(Pugh et al., 2019).**

Kidney failure occurs when eGFR decreases to less than 15 mL/min per 1·73 m². In general, the prevalence of chronic kidney disease increases with age and, in high-income countries, is more common in people with obesity, diabetes, and hypertension**(Webster et al., 2017)**.

Cells in the body produce waste products such as urea, creatinine, and ammonia, which must be removed from the blood before they accumulate to

harmful levels. The kidneys play a role in this process by producing urine to excrete these waste products. Additionally, the kidneys perform essential functions; (i) the body regulates blood volume by either excreting or conserving water, (ii) electrolyte content, in the blood is regulated by excretion or storage of minerals, (iii) the balance of acidity and alkalinity in the blood is regulated through the excretion or preservation of ions like H^+ or HCO3 and (iv)Tissue fluid plays a supportive role in kidney function which also contributes to the regulation of all the above parameters **(Ajiboye et al., 2022**). Kidneys are important for maintaining the blood's composition in order for the body to function properly. It prevents the accumulation of waste products and extra water in the body, maintains stable levels of electrolytes such as sodium, potassium, and phosphate, and makes hormones that help regulate blood pressure, produce red blood cells, and maintain bone strength **(Madhavan Unny et al., 2023)**.

1.2. Classification of Chronic Kidney Disease

is based on the severity of kidney function decline, measured by the glomerular filtration rate (GFR), and the extent of albuminuria (protein in the urine). The most widely used classification system is from the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. CKD is categorized into five-stages, according to the GFR, and in three stages, according to the albuminuria, as shown in the (Table 1.1) and (Table 1.2) **(Simpson 2022).**

Table 1.1: The Classification of Chronic Kidney Disease (CKD) Based On Glomerular Filtration Rate (GFR)

	GFR value ml/min/1.73m²	**Classification**
1	≥ 90	Normal or High With Kidney Damage
2	60-89	Mildly Decreased
3A	45-59	Moderately Decreased
3B	30-44	Moderately to Severely Decreased
4	15-29	Severely Decreased
5	<15	Kidney Failure/End-Stage Kidney Disease - ESRD

In addition to eGFR results, the diagnosis of stage 1 or 2 CKD requires a structural abnormality of the kidneys (such as polycystic kidney disease) or afunctional abnormality (such as persistent proteinuria or haematuria). In the absence of these factors an eGFR between 60 and 89 is not considered abnormal.Consequently, an adult patient with diabetic nephropathy, GFR estimated equal 42 ml/min, and albuminuria of 200 mg/24 h for more than three months was therefore categorized as having CKD stage IIIB A2. It's worth noting that albuminuria between 30-300 mg/g was formerly referred to as microalbuminuria and albuminuria larger than 300 mg/g as macroalbuminuria The inclusion of albumin severity .Albuminuria, particularly when severe (A3 category: >300 mg/g), is a strong predictor of CKD progression and adverse outcomes.

Table 1.2: Classification of Albuminuria and Albumin Creatinine Ratio ACR.

Category	**Albuminuria mg/24 h**	**A/C Ratio mg/g**	**Classification**
A1	<30	<30	Normal to Mild Increase)
A2	30-300	30-300	Moderate Increase - Microalbuminuria
A3	>300	>300	Severe Increase- Macroalbuminuria

In the CKD classification is justified as a means of evaluating the risk of renal dysfunction development, as demonstrated in the (Table 1.3). (Silveiro 2022).

Table 1.3: Risk of Renal Outcomes According to the GFR and Albuminuria; GFR in ml/min/1.73m².

Stages	**GFR**	**<30mg/g**	**30 g-300mg/g**	**>300mg/g**
1	>90	Low risk	Moderate risk	High risk
2	60-89	Low risk	Moderate risk	High risk

3A	45-59	Moderate risk	High risk	Very high risk
3B	30-44	High risk	Very high risk	Very high risk
4	15-29	Very high risk	Very high risk	Very high risk
5	<15	Very high risk	Very high risk	Very high risk

The above-mentioned staging system helps physicians in determining which method and intensity of monitoring for CKD patients. The development of risk prediction tools can lead to a more accurate risk prediction for each patient. In addition to the GFR and albuminuria, the cause of the kidney disease, as well as other factors (such as age, gender, ethnicity, cholesterol levels, smoking, Hypertension and Diabetes should also be considered. (Shih, Chen et al. 2021).

1.3. Epidemiology

Chronic Kidney Disease (CKD) is a major global public health issue, affecting approximately 9–15% of the adult population worldwide. The prevalence varies by region, with higher rates in low- and middle-income countries due to risk factors like diabetes, hypertension, and poor healthcare access.

United States: ~14% of adults have CKD.

Europe: Prevalence ranges from 3% to 17% depending on the country.

Asia: High prevalence, particularly in China (~10.8%) and India (~17.2%).

Africa & Latin America: Limited data, but studies suggest increasing prevalence. **(Xasanova and Integrity 2024).**

In the United States, CKD affected an estimated 26 million people in 2016 **(Vaidya, Aeddula et al. 2021)**Chronic kidney disease (CKD) poses a growing global health challenge, with around 10% of adults affected by some form of the disease. This high prevalence contributes significantly to mortality and disability worldwide. Each year, CKD is responsible for an estimated 1.2 million deaths, making it a major cause of global mortality. Additionally, it leads to 28 million years of life lost (YLL) annually, which reflects the number of years individuals lose due to premature death caused by CKD-related complications, such as cardiovascular disease and kidney failure.Looking ahead, the burden of CKD is expected to increase substantially, with projections indicating that by 2040, CKD could become the fifth leading cause of death globally.**(Lv, Zhang et al. 2019).**

1.4. Causes of Chronic Kidney Disease

Anywhere in the globe, chronic kidney disorder, or CKD, and its eventual end-stage renal disease, also called ESRD, can be caused by several main factors Prevention approaches for CKD and ESRD need to focus on the causes leading to kidney disease **(Kalantar-Zadeh, Jafar et al. 2021).**

Table 1.4: Causes of CKD along with their Respective Percentages.

Cause	Percentage (%)
Type 2 Diabetes	**30% - 50%**
Type 1 Diabetes	**3.9%**
Hypertension	**27.2%**

Primary Glomerulonephritis	**8.2%**
Chronic Tubulointerstitial Nephritis	**3.6%**
Hereditary or Cystic Diseases	**3.1%**
Secondary Glomerulonephritis or Vasculitis	**2.1%**
Plasma Cell Dyscrasias or Neoplasm	**2.1%**

Several factors can contribute to CKD, including medical conditions and genetic predispositions.

1.4.1 Prerenal Disease

Patients with chronic prerenal disease are more likely to develop acute tubular necrosis and other forms of intrinsic kidney injury as a result of chronically diminished renal perfusion, which occurs in those with chronic heart failure or cirrhosis. This may cause a steady deterioration in renal function over time **(Xasanova and Integrity 2024).**

Intrinsic Renal Disease

Nephrosclerosis is the most frequent kind of chronic renal vascular disease, causing progressive damage to the tubulointerstitium, glomeruli, and blood vessels over time. Ischaemic nephropathy can develop over months or years as a result of renal artery stenosis caused by atherosclerosis or fibromuscular dysplasia, two prevalent kidney vascular disorders. The condition is defined by glandular and tubulointerstitial dysfunction.**(Vasiliu, Diaconu et al. 2024)**.

1.4.2 Intrinsic Glomerular disease (Nephritic or Nephrotic):

Changes in urine cytology that imply a nephritic pattern involve the presence of red blood cell (RBC) splints dysmorphic red cells, and, in rare cases, white blood cells (WBCs). Proteinuria can occur at different levels..**(Goldfarb-Rumyantzev and Schulman 2023)**. The most common causes are post-infectious glomerulonephritis, infective endocarditis, IgA nephropathy, lupus nephritis, Goodpasture syndrome, and vasculitis**(Ninan 2024).**

Nephrotic characteristic is determined by dormant urine microscopic examination having tiny cells or extends, and additionally, nephropathy in the nephrotic range (>3.5 g/24 hours). The most prevalent causes of nephrotic are diabetic nephropathy , focal segmental glomeruloscleroses, membranous nephropathy and basic change syndrome **(Moiseyenko, Psarova et al. 2023)**.

1.4.3 Intrinsic Tubular and Interstitial Disease:

Polycystic kidney disease ranks among the most common chronic tubulointerstitial disorders. Other possibilities include a condition called reflux nephropathy in children and young adults, kidney damage (which is usually caused through excessive calcium levels and hypercalciuria), and Sjögren syndrome **(O'Callaghan 2022).**

Obstructive Nephropathy is a condition in which chronic occlusion may be caused by a form of prostatic illness, or an abdomen or pelvic tumours pressing on the ureter(s). Birthmarks may prevent the ureteropelvic and ureterovesical junctions from flowing properly. "Retro Fibrosis and neurogenic bladder are both rare manifestations of persistent ureteral obstacle **(Xasanova and Integrity, 2024).** It is also significant to consider the underlying factors and development of CKD. The primary root cause of

ESKD remains unclear in many cases.If the source of chronic renal disease, or CKD, is unknown, an inquiry to renal disease may be necessary. When haematuria and/or proteinuria indicate a kidney issue, a renal biopsy may be recommended. Despite research, the main cause of ESKD in approximately 15% of patients continues unknown. In such cases the focus continues on prevention of prog whose cases is unknown in approximately 15% of patients approaching ESKD, determining the metabolic effects of renal failure, and preparing for renal replacement treatment if needed.**(Hull, Adenwalla et al. 2022).**

1.5. Signs and Symptoms of Chronic Kidney Disease

Chronic Kidney Disease (CKD) often develops slowly and may not show symptoms until it's more advanced. Here are the common signs and symptoms:

Table1.5 Progression of Signs and Symptoms Across Stages of Chronic Kidney Disease (CKD). (Webster et al., 2017)

CKD Stage	**Signs & Symptoms**
Early Stages (1–2)	- Fatigue or weakness - Poor appetite - Trouble concentrating - Sleep issues - Mild increase in urination (especially at night)
Middle Stages (3–4)	- Swelling in hands, feet, or ankles - High blood pressure - Back pain (side of affected kidney) - Foamy or dark urine - Itching - Nausea

Late Stage (5 - ESRD)	- Little to no urine output - Severe fatigue - Confusion or memory issues - Shortness of breath - Metallic taste in mouth - Seizures or coma (in rare, severe cases)

Chronic Kidney Disease (CKD) is often insidious, with many affected individuals remaining asymptomatic until the disease progresses to advanced stages, typically when eGFR falls below 30 mL/min/1.73 m². **(Kidney Disease: Improving Global Outcomes [KDIGO] et al., 2021)**

The progression rate of CKD varies depending on its etiology, environmental exposures, and medical interventions. In many cases, progression to end-stage renal disease (ESRD) may occur over months to decades. **(Levey et al., Kidney Int. 2020)** Symptoms of kidney failure are largely due to progressive uremia, anemia, fluid retention, electrolyte disturbances, bone-mineral disorders, and metabolic acidosis, which may ultimately be life-threatening **(NKF Guidelines, 2020).** Patients with kidney failure may have their survival prolonged through renal replacement therapy, including chronic dialysis and kidney transplantation. However, due to the scarcity of organ donors and age-related comorbidities, transplantation may not always be feasible, leaving dialysis as the primary treatment option **(GBD 2019 Chronic Kidney Disease Collaborators).** Renal failure is associated with high mortality and a significantly impaired quality of life, particularly in the first year of dialysis. These findings underscore the importance of early detection and strategies to preserve kidney function in individuals at risk**. (Fletcher, Damery et al. 2022; Jha et al., Lancet 2013).**

1.6. Diagnosis of Chronic Kidney Disease

Chronic kidney disease (CKD) is often diagnosed by detecting proteins in the blood and urine, although it may also be diagnosed in individuals with typical excretory renal activity if there are other signs of kidney damage. As stated by Cheo, Low, and colleagues in 2022, Some of these symptoms include urination of hematuria related to the kidneys, structural abnormalities of the the urinary tract, and genetic variations. Various diagnoses can affect the kidneys, such as polycystic kidney disease, electrolyte disturbances resulting from tubular damage, and pathological abnormalities identified through renal biopsy.

1.6.1 Blood Tests: Urea and Electrolytes / Estimated Glomerular Filtration Rate (eGFR)

Initial screening tests that are appropriate for use include the creatinine level and an eGFR. If an abnormal blood test is found (eGFR < 60 ml/min/1.73 m^2), further investigations must be done within 14 days to rule out a serious kidney condition (AKI). To diagnose chronic kidney disease (CKD) based on a reduced eGFR, two eGFR tests taken at least 90 days apart must show values below 60 ml/min/1.73 m^2. **(Bhat et al., 2024).**

1.6.2 Urine Dip (Routine Urine Examination)

1.6.2.1 Proteinuria

Proteinuria is the most important indicator of renal risk and has a strong correlation with adverse disease outcomes. Its significance in individuals with kidney disease cannot be overstated, as it is linked to an increased risk of death, stroke, and the development of kidney problems **(Chang et al., 2021).**

The glomeruli and tubules are damaged in many ways by the excessive protein level leading to chronic kidney disease (CKD) and increased nephropathy. As mentioned earlier, this raises the possibility of heart attack and mortality **(Lim et al., 2021).**

Individuals suspected of having nephrotic syndrome or chronic kidney disease should formally measure their protein levels with an ACR or PCR. These ratios are calculated by dividing the millimoles per litre of creatinine by the milligrams per litre of immunoglobulin or protein in the urine. This allows one to account for the urine protein levels accurately. Collecting urine at regular intervals of a single day has very little advantage, as such collections are often cumbersome for patients and usually performed poorly. Low-level proteinuria should be repeated to confirm, and though levels should be monitored regularly. ACR is generally used for assessment in diabetic kidney disease. The predictive accuracy of ACR and PCR is comparable to that of PCR, even at low dosages **(Fulton 2023).**

1.6.2.1.1 Assessment of Proteinuria

The KDIGO guidelines suggest assessing for nephritis by early morning urine sample and then measuring the arterial blood pressure (ACR). Older terminology has been replaced with an index from A1 to A3 to define albuminuria **(Chagnac and Friedman 2024).**

A variety of proteins can be observed in a healthy person's urine. Among these kinds of proteins, albumin comprises around 20%, immunoglobulins about 5%. Chronic kidney disease is characterized by normochromic, normocytic, and hypoproliferative anemia. A lack of erythropoietin (EPO), a protein that activates erythropoiesis and is produced in the renal cortex, may lead to

anemia in people with chronic renal disease (CKD). That, which accounts for around half of all proteins, is known as the Tamm-Horsfall protein. A few examples of possible beneficial characterization and quantification techniques are electrophoresis, mass spectrometry, chromatography, immunoassays, fluorescence spectroscopy, infrared spectroscopy, and Raman spectroscopy. However, dipstick assays and protein-specific immunochemistry are the most time- and money-saving methods. Albuminuria is more common in proteinuric illnesses than in others; nevertheless, the most used protein section on urine dip sticks detect albumin, and not be detecting other types of urinary proteins **(Bastos 2019).** More accurate strategies are required. When higher-quality proteinuria strategies are unavailable, the KDIGO guidelines state that "trace to +" and "+ or greater" protein readings on dipsticks can be utilized to denote albuminuria levels between 30 and 299 mg/g and above 300 mg/g, respectively **(Sumida, Nadkarni et al. 2020).**

1.6.2.2 Hematuria

A urological assessment, including physical examination, should be conducted in individuals than forty-five years if they present with unexplained visible hemorrhaging, unless there is significant kidney injury or other evidence of intrinsic renal disorder **(Rai, Escrig et al. 2022).**

1.6.3 Imaging Techniques

Ultrasound is the imaging modality of choice for evaluating the kidneys and can be extremely informative **(Caroli, Remuzzi et al. 2021).** It can help confirm the following:

1.Presence of two kidneys: One kidney may be congenitally absent, atrophied, or surgically removed.

2.Renal size: Small kidneys on ultrasound suggest chronicity of renal disease, while nephromegaly is associated with diabetes, polycystic kidney disease, and infiltrative disorders.

3.Cortical thickness: cortical thinning is a sign of chronicity. Obstruction of the renal tract: hydronephrosis ± hydroureter is suggestive of an underlying urological disorder and the level of the obstruction may be apparent on the scan.

4.Cysts: most commonly simple cysts are noted that are of no consequence; however, a complex cyst may be malignant and require further urological assessment. Multiple, usually bilateral cysts in enlarged kidneys are suggestive of polycystic kidney disease **(Colquhoun and Surgery 2023).**

Reduced cortex thickness, increased resonance, scars, or many cysts on an ultrasound of the urinary tract are all signs of a chronic disease. Potentially more precisely diagnosed conditions include chronic hydronephrosis due to obstructive uropathy, and cystic enlargement of the kidney from ADPKD. If a constriction of the renal arteries is suspected, Doppler ultrasound will measure the blood flow via the renal vessels **(Petrucci, Clementi et al. 2018).**

Centralized tomography offers a more precise visualisation of the collecting duct system, ultrasound, kidney size. The diagnostic utility of renal angiography increases in the presence of systemic vasculitis, stenosis of the kidney arteries, or multiple aneurysms, as it can reveal irregular constrictions. Voiding cystourethrography is mainly used when chronic vesicourethral

reflux is suspected of causing CKD, which confirms the diagnosis and estimates the severity of reflux **(Wymer at al. 2023).**

1.6.3.1 Establishing Chronicity

Acute kidney damage, or AKI, must be considered as an issue when an individual's medical history, urine and blood tests results, and estimated glomerular filtration rate, or eGFR, drops below 60 mL/min/1.73 m². Here are a few variables that could be relevant: This condition, microhematuria, or an indication of enduring chronic hypertension are clinical signs that may indicate recurrence **(Patel, Raman et al. 2024).** present hypertensive fundal changes, darkening of the skin, scratch marks, left ventricular hypertrophy, and other physical manifestations that could suggest persistency. The presence of different diseases, such as multiple myeloma of the skin or systemic vasculitis, may assist in the diagnostic process. In contrast to CKD, AKI is associated with normal parathyroid hormone (PTH) levels, even if low levels of serum calcium and elevated phosphorus levels may not distinguish between the two. Patients with chronic renal disease or similarly increased blood urea nitrogen/creatinine levels frequently get sudden onset of AKI symptoms **(Vaidya, Aeddula et al. 2024).**

1.6.3.2 Assessment of Glomerular Filtration Rate

The estimated glomerular filtration rate (eGFR) is a widely used calculation for assessing kidney function by estimating how effectively the kidneys filter waste from the blood. It is essential for diagnosing chronic kidney disease (CKD), monitoring disease progression, and informing treatment decisions

(KDIGO 2021 Guidelines). In addition to classical markers like serum creatinine and cystatin C, several emerging biomarkers such as kidney injury molecule-1 (KIM-1) are being investigated for early detection of both acute and chronic kidney injury. **(Levey et al., Kidney Int 2020; Vaidya et al., Nat Rev Nephrol 2008).** Other potential biomarkers under research include β2-microglobulin, retinol-binding protein (RBP), neutrophil gelatinase-associated lipocalin (NGAL), L-type fatty acid-binding protein (L-FABP), fibroblast growth factor 23 (FGF-23), and beta-trace protein. These have shown varying degrees of association with CKD progression (**Matsushita et al., JASN 2012; Devarajan, NEJM 2008).** Additionally, findings from the Chronic Renal Insufficiency Cohort (CRIC) study demonstrated significant associations between CKD progression and cardiac biomarkers such as high-sensitivity troponin T, NT-proBNP, the plasma chemokine CXCL12, and urine NGAL. (**Anandkumar, Dheerendra et al. 2023; CRIC Study Group, 2015).**

1.7 Complications of Chronic Kidney Disease

The interaction among many issues related to ongoing renal disease becomes more apparent and prevalent at lower levels of kidney function. Substandard quality of life, elevated mortality, and morbidity are all outcomes of these issues. Prescribing erythropoiesis-stimulating agents may be essential for treating certain complications that can be readily identified and quantified. Among these complications are cardiovascular disease, hypertension, anemia, mineral bone disorder, volume overload, electrolyte imbalances, and acid-base disturbances. Anorexia, fatigue, cachexia, itching, nausea, and sexual dysfunction are among the less clearly distinguished effects that may manifest as complex symptoms commonly observed in advanced chronic

kidney disease. The panel identified these subsequent CKD-related issues as substantial contributors to the global epidemic of CKD-related illnesses **(Ammirati 2020).**

1.7.1 Hypertension

Hypertension remains one of the most damaging complications of CKD and is thought to contribute to the acceleration of progressive decline in kidney function, cardiovascular diseases (CVD), and related mortality **(Ku, Lee et al. 2019).**

1.7.2 Cardiovascular Complications:

As chronic kidney disease (CKD) progresses in severity, the risk of cardiovascular disease (CVD) escalates. Epicardial adipose tissue thickness is highly correlated with the incidence of cardiovascular disease events in patients with chronic kidney disease, as supported by solid data. Thus, the assessment of epicardial adipose tissue may serve as a reliable marker of cardiovascular risk in individuals with chronic renal disease. CVD represents the leading cause of mortality in CKD patients, and the prevalence and burden of this complication increases with declining kidney function For example, the risk of mortality from CVD is 8.1- fold greater in a patient with CKD stage G5 A3 (eGFR < 15 ml/min per 1.73m² and urinary albumin- creatinine ratio > 300 mg/g) than in a reference population without kidney disease**(Vallianou, Mitesh et al. 2019)**.

Although the presence of chronic kidney disease (CKD) elevates the risk of conventional atherosclerotic cardiovascular events, a significant portion of this heightened risk is attributable to non-atherosclerotic conditions such as

valvular disease, arterial calcification, and left ventricular hypertrophy accompanied by diastolic and systolic dysfunction, among others. Sudden cardiac arrest, heart failure, and atrial and ventricular dysrhythmias may all appear as signs of these conditions. Individuals with chronic renal failure (CKD) understand that mitigating conventional cardiovascular risk indicators, such as cholesterol and blood pressure, is beneficial, particularly in patients with CKD stages 1-3. Nevertheless, additional risk factors must be considered in individuals with chronic kidney disease, most of which pertain to complications associated with CKD. Complication of chronic kidney disease include (Hypertension, Proteinuria, Anemia, also known Dyslipidemia, mineral and bone disorder, electro imbalance, particularly high potassium levels, metabolic acidosis, volume overload). **(Alkhaqani 2022)**.

1.7.3 Anemia:

Chronic kidney dysfunction (CKD) was initially linked to anemia in 1836 by Richard Bright, who defined it as hemoglobin (Hb) levels of ≤12 g/dL in women and ≤13 g/dL in males.

This condition is highly prevalent, although treatable, with increasing frequency in the more advanced stages of the disease. It can be found in more than 50% of individuals with CKD in stages 4 and 5, and symptoms may appear earlier than in those with metabolic syndrome (MS). Hypoproliferative, normocytic, and normochromic anemia is typical in CKD. Anemia in CKD patients can occur from insufficient erythropoietin (EPO), a chemical produced in the renal cortex which promotes erythropoiesis **(Fishbane S, Spinowitz B. 2018).**

In addition to anemia, other factors play a significant role in CKD-related complications. Patients receiving dialysis may lose about 1–3 grams of iron annually, exacerbating the prevalent issue of iron deficiency. Non-dialysis patients also often exhibit low iron levels. This finding may be attributed to regular phlebotomies, blood loss through the hemodialysis system, or reduced iron absorption. The incidence of iron deficiency in individuals with persistent kidney disease has been demonstrated with erythropoietin analogues **(Kaplan J. Roxadustat 2019).**

Anemia in CKD is typically normocytic and normochromic, resulting from reduced erythropoietin production due to decreased renal mass function.

abnormal iron metabolism, and reduced red blood cell survival. Hemoglobin levels should be checked annually in stage 3 of CKD, every 6 months in stages 4 and 5, and monthly in dialysis patients. Erythropoiesis-stimulating agents should be considered when hemoglobin levels fall bellow 10 g/dL, with iron saturation at least 20% to 30% and ferritin greater than 200 ng/mL in dialysis patients. the target hemoglobin concentration is 10 to 11.5 g/dL. Anemia complications in CKD patients has been well characterized and worldwide with iron and erythropoiesis-stimulating agents (ESAs). However, the optimal doses of ESAs and parenteral iron have not been established **(Daniel W Coyne, MD, et al. 2019).**

1.7.4 Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD): is a systemic disorder of mineral and bone metabolism caused by chronic kidney disease (CKD). It results from the kidneys' reduced ability to maintain the normal levels of minerals, hormones, and bone turnover. CKD-MBD affects the bones, blood vessels, and soft tissues

1.7.5 Salt and Water Retention:

In stages 4 to 5 of chronic kidney disease, and possibly in stage 3, there is loss of defense against both sodium excess and sodium depletion. In clinical practice, sodium excess with fluid retention is by far the most common, although the exact prevalence has not been determined. While the extracellular fluid volume may be expanded, the sodium balance appears to be relatively well-maintained until end-stage renal disease**(Naber and Purohit 2021)**.Excess sodium and fluid contribute to not only edema, which may negatively affect quality of life, but also hypertension and thereby CVD (specifically concentric left ventricular hypertrophy, which can result in diastolic dysfunction **(Deferrari, Cipriani et al. 2021)**.

1.7.6 Metabolic Acidosis and Electrolyte Disorders: Metabolic acidosis is common in CKD and is caused when the acid intake and generation exceed the renal acid excretion. In early stages, it may be manifest as "acid excess with normal bicarbonate," a state of positive acid balance without low plasma bicarbonate due to buffering and renal adaptation. Chronic metabolic acidosis contributes to skeletal muscle catabolism, insensitivity to endocrine hormones, and bone disease and may accelerate the progression of CKD **(Kim, E et al. 2021).**

1.8. Progression of Chronic Kidney Disease

Most patients do not progress from CKD Stage 3 to 5, but ~17% of CKD Stage 4 patients will progress to Stage 5 and ~1% of CKD Stage 3 patients will. However, the transition to CKD Stage 4 is often insidious and underrecognized. Importantly, this transition represents a "clinical event" similar to a stroke or acute myocardial infarction because CKD Stage 4 is

marked by a major increase in cardiovascular mortality and progression to CKD Stage 5. During CKD Stage 4, death is a competing risk for progression to ESRD. Comprehensive systems targeting early recognition, prevention and management, and treatment by primary care physicians and physician extenders are required at this critical stage in collaboration with nephrologists**(Kampmann, Heaf et al. 2023)**.

Experimental studies suggest that KIM-1 may be an indicator of AKI to CKD transition. Studies in the human indicated that urinary KIM-1 was sensitive and specific marker of injury as well as predictors of outcome. Two systematic reviews reported that KIM-1 was an efficient novel urinary biomarker in diagnosis of AKI within 24 hours after kidney injury. especially in the diagnosis of ischemic acute tubular necrosis**(Wen and Parikh 2021)**. Although the extensive analyses have been carried out, owing to the limitation of relatively small population settings, heterogeneous patient type, less clinical trial and different detection time, the application of KIM-1 in early diagnosis of AKI still needs to be validated and thoroughly investigated in larger studies. KIM-lit is expressed in proximal tubule cells and is thought to promote apoptotic and necrotic cell clearance. Upon injury, KIM-1 is upregulated and shed into the urine and extracellular space. It is thought to activate immune cells in injury-induced immune response**(Tao, Chen et al. 2021)**

1.9. Kidney Injury Molecule-1

Is a transmembrane glycoprotein serving as an adhesion molecule, and was kidney injury molecule-1 identified in the S3 segment of the proximal tubule. While its expression remains minimal in normal kidneys, it experiences significant upregulation in response to ischemic-reperfusion injury

(Agarwal, Sudhini et al. 2021).The presence of the soluble, released form of urinary KIM-1 has been confirmed to be associated with acute tubular necrosis, thus establishing it as a marker of proximal tubular injury. It is worth noting that the genes that code for KIM-1, as well as NGAL, were found to be significantly upregulated late after the injury. This could potentially function as an indicator of persistent renal damage following the initial acute insult and may, therefore, serve as a prognostic factor for the development of subsequent chronic kidney disease (CKD) **(Karmakova, Sergeeva et al. 2021).**

Together with TIM-1 and HAVcr-1, Kidney Injury Molecule 1 (KIM-1) belongs to the T-cell Immunoglobulin and Mucin Domain Family (TIM). TIM glycoproteins are involved in regulating immune responses and are present on immune cells. Unlike other members of the KIM-1 family, epithelial cells, along with immunocompetent cells, express the protein. The cellular and humoral effects mediated by KIM-1 influence various physiological and pathological processes **(Alekseev B.Ya., Kaprin A.D.et al. 2021)**.

We have defined the current understanding of the mechanisms back KIM-1's involvement to viral assault, immune response modulation, and adaptive renal epithelial responses following acute infarct or toxic injury, chronic renal failure progression, and kidney cancer development. Clinical trial results correlating KIM-1 with several viral infections and immune system irregularities were also examined. These insights prompt consideration of the potential application for utilizing KIM-1 as a urinary biomarker. Advancements in biomedical technology have led to the extensive deployment of biological markers in clinical practice, preclinical research,

and experimental investigations as measurable indicators of living things, tissue, or organ condition **(Fernandes, Watanabe et al. 2021)**.

The Kim-1 gene, or kidney injury molecule 1, was shown to be significantly displayed in the cell layers of damaged proximal kidney tubes in rats throughout post-ischemic renal membrane healing. This gene appears to be a full homolog of HAVcr-1**(Ortega-Trejo and Bobadilla 2023)**.

1.9.1. KIM-1 Molecule Structure

The human genome contains 11 exons expressed by the HAVcr-1 gene, which is located on the long arm of chromosome 5, at locus 5q33.3 (Gene ID: 26762). The molecular volume of protein can ranges 36 to 44 kDa, and the total amino acid count varies from 334 to 401 depending on the variants produced through alternative mRNA slicing. Following glycosylation, the molecular dimensions of KIM-1 attains 104 kDa. Extracellular, transmembrane, and cytoplasmic domains of KIM-1 are found on the plasma membrane. A short peptide segment, a mucin-like region, and a globular domain resembling the variable portion of immunoglobulins (IgV) make up KIM-1's extracellular domain **(Karmakova, Sergeeva et al. 2021).**

A hydrophobic pocket (metal-ion-dependent binding site, MILIBS) able to interacting a mixture of (PS) is an essential part of the KIM-1 IgV-domain. With death induction, phosphates of translocate from its natural inner region on the membrane of plasma to the outer surface. It instructs lymphocytes and cells of the epithelium to engulf the deceased cells **(Smith et al., 2021).** In cases of tissue damage, KIM-1 in intestinal cells is believed to facilitate the in situ clearance of cellular debris by functioning as a pollutant receptor, due to its capacity to interact with PS. Moreover, the IgV domain can promote the

interaction of ligands with TIM glycoproteins, which altered low-density lipoproteins, P-selectin, and conjugate blood bilirubin.**(Tutunea-Fatan, Arumugarajah et al. 2024).** As shown in figure (1-2).

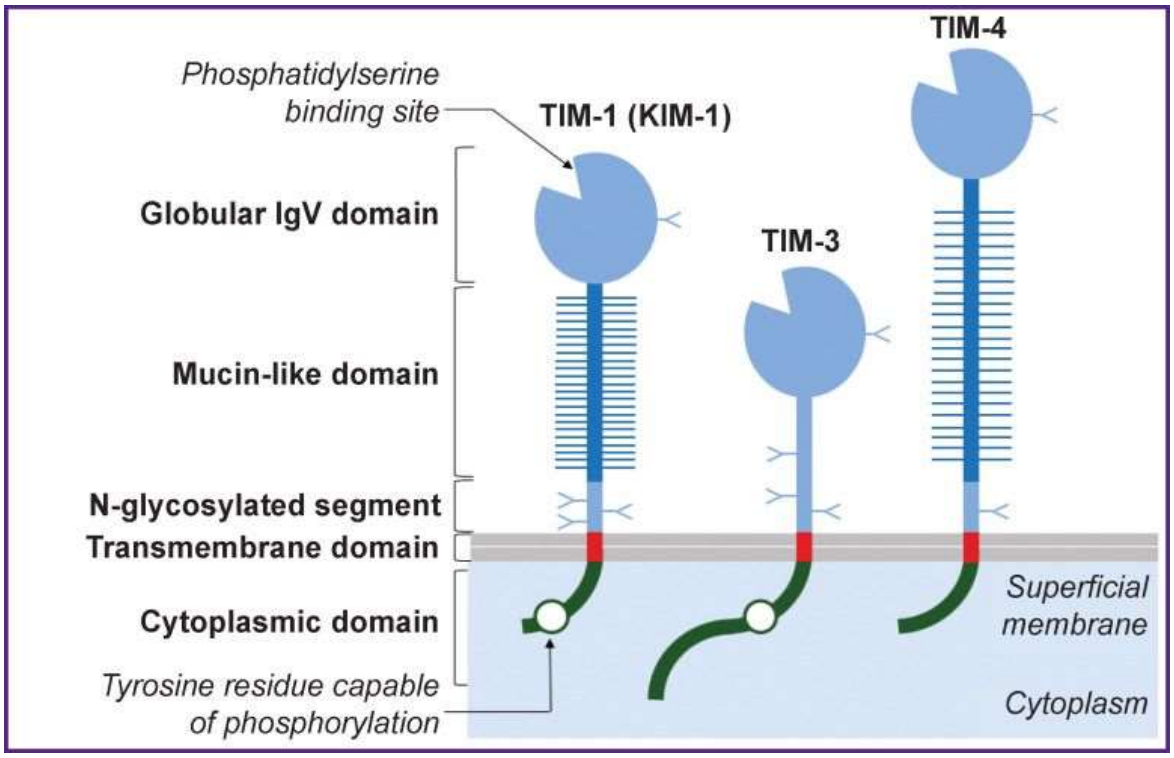


Figure (1-1) Schematic Structure of the TIM Family Glycoproteins

There are tandem repeats of amino acids and places of O-glycosylation in the extremely glycosylated mucin-like area, the biggest part of KIM-1. Though the structure of the domain appears to be critical for KIM-1's ligand conversations its precise functions remain a mystery. Primates present an amazing amount of variation in genetics in these regions **(Ohtani et al., 2012).**

A short peptide tract such as N-glycosylation sites and protease sensitive regions are situated close to the membranes. Proteolytic cleavage of an external segment of KIM-1 at the cell surface results in the creation of free KIM-1, which

has a molecular weight of around 90 kDa. Both blood plasma and urine may show this KIM-1 version.**(Bailly et al., 2002; Zhang et al., 2007).**

The presence or lack of a short polypeptide called a cytoplasmic domain is a defining feature of the various structural variants of KIM-1. The ability of KIM-1 to participate in intracellular signaling is determined by the phosphorylation of this domain in lymphocytes and renal epithelium **(Nemours 2020)** .

1.9.2. KIM-1 Expression in tissues

Appropriate mRNA production of the HAVcr-1 polypeptide in human tissues is distinguished by organ specificity. The number of HAVcr-1 transcripts was largest in the kidneys and lowest in the testicles, but both organs had substantial amounts. Different tissues showed small quantities of specific transcripts. The renal system contains ten times the number of HAVcr-1 transcripts in comparison to other organs and tissues, based on recent findings from deep transcriptome sequencing.

Significant amounts of HAVcr-1 mRNA can be found in the testes, colon and rectum tissues, and peripheral blood leukocytes **(Priego, Parra et al. 2021**).

Studies using polyclonal antibodies against the recombinant HAVcr-1 in the immune system indicate weak to moderate cellular staining in many cell types. This includes cells in the tubules of the kidney epithelium and urethra, the small and large intestine, glands, bronchial epithelium, epidermal layer the endometrium, liver bile duct, gall bladder epithelium, and numerous additional cells. It has been determined that human oligodendrocytes and myocytes from skeletal muscle carry HAVcr-1. Although HAVcr-1 expression is generally low,

it is found in nearly every tissue and organ in the human body **(Zheng, Geng et al. 2021)**.

1.9.3. Functional Role of Kidney Injury Molecule-1

1.9.3.1 KIM-1 in Chronic Kidney Diseases

Chronic kidney disease (CKD) often arises as a consequence of both acute and chronic renal injuries. Persistent hypoxia is considered a major pathogenic factor in CKD. Several mechanisms contribute to this state, including alterations in the post-glomerular microvasculature, activation of the renin-angiotensin system, and increased oxygen utilization by renal cells to counteract oxidative stress and declining glomerular function. Chronic hypoxia ultimately leads to glomerular injury and tubulointerstitial fibrosis. Injured proximal tubular cells, which may adopt a mesenchymal phenotype, also release profibrotic mediators that further drive CKD progression and its complications **(Tanase et al., 2019).**

Hypoxia stimulates upregulation of KIM-1 transcription in proximal tubular cells, contributing to sustained interstitial inflammation. KIM-1 is expressed both on the cell membrane and in soluble form, where it interacts with macrophages and acts as an autocrine–paracrine mediator for tubular and stromal cells. For instance, binding of KIM-1 to LMIR5/CD300b receptors on local myeloid cells induces cytokine and chemokine release, promoting neutrophil recruitment to the injury site. This amplifies local inflammation, hypoxia, and tissue damage, establishing a vicious cycle that accelerates interstitial fibrosis. In this way, increased KIM-1 expression under hypoxic conditions links acute kidney injury (AKI) with chronic renal disease **(Ismail, 2015; Chen et al., 2023).**

Elevated KIM-1 expression and urinary KIM-1 (uKIM-1) levels have been reported in several renal disorders, including focal segmental glomerulosclerosis, proliferative and membranous glomerulonephritis, IgA nephropathy, diabetic and hypertensive nephropathy, chronic allograft nephropathy, and lupus nephritis. However, despite its diagnostic value, uKIM-1 has shown limited utility in predicting CKD prognosis **(Todorov et al., 2023).**

Functionally, KIM-1 can act as a scavenger receptor on renal epithelial cells, converting proximal tubular cells into phagocyte-like cells. This facilitates clearance of apoptotic and necrotic cells from the tubular lumen and may play an important role in innate immune defense and tissue repair after injury. These findings suggest potential therapeutic implications for KIM-1 modulation in protecting the kidney from acute injury and promoting repair mechanisms **(Tajbakhsh et al., 2022).**

Under physiological conditions, KIM-1 is constitutively expressed at very low levels in the kidney and other organs. Its expression markedly increases after ischemia-reperfusion or drug-induced AKI. Features that support its role as a biomarker include its high specificity for proximal tubular epithelial cells compared to other renal or extra-renal tissues, the stability of its soluble ectodomain in urine across a wide pH range, and its sustained expression until complete recovery. Importantly, KIM-1 remains minimally expressed in healthy kidneys, providing a high signal-to-noise ratio. Early studies established a strong association between urinary KIM-1 levels and AKI, confirming its value as a sensitive and reliable biomarker **(Yang et al., 2023).**

- Kim-1 functions as a phosphatidylserine (PS) receptor which recognizes and internalizes apoptotic cells.

- Kim-1 also functions as a scavenger receptor, mediating the uptake of modified low density lipoprotein and necrotic cell debris.
- Kim-1 expression transforms proximal tubular epithelial cells into semiprofessional phagocytes. In the immune system, Kim-1/Tim-1 has been implicated in activation of Th2, Th1 and Th17 differentiation.
- It has also been proposed to be an activating receptor in B cells, dendritic cells and natural killer T cells.
- Many of the experiments leading to these conclusions have relied on antibodies against Kim-1/Tim-1, which have been presumed to be agonists or antagonists, or Kim-1/Tim1-Fc fusion proteins as key reagents **(Schmidt IM, et al.2022).**

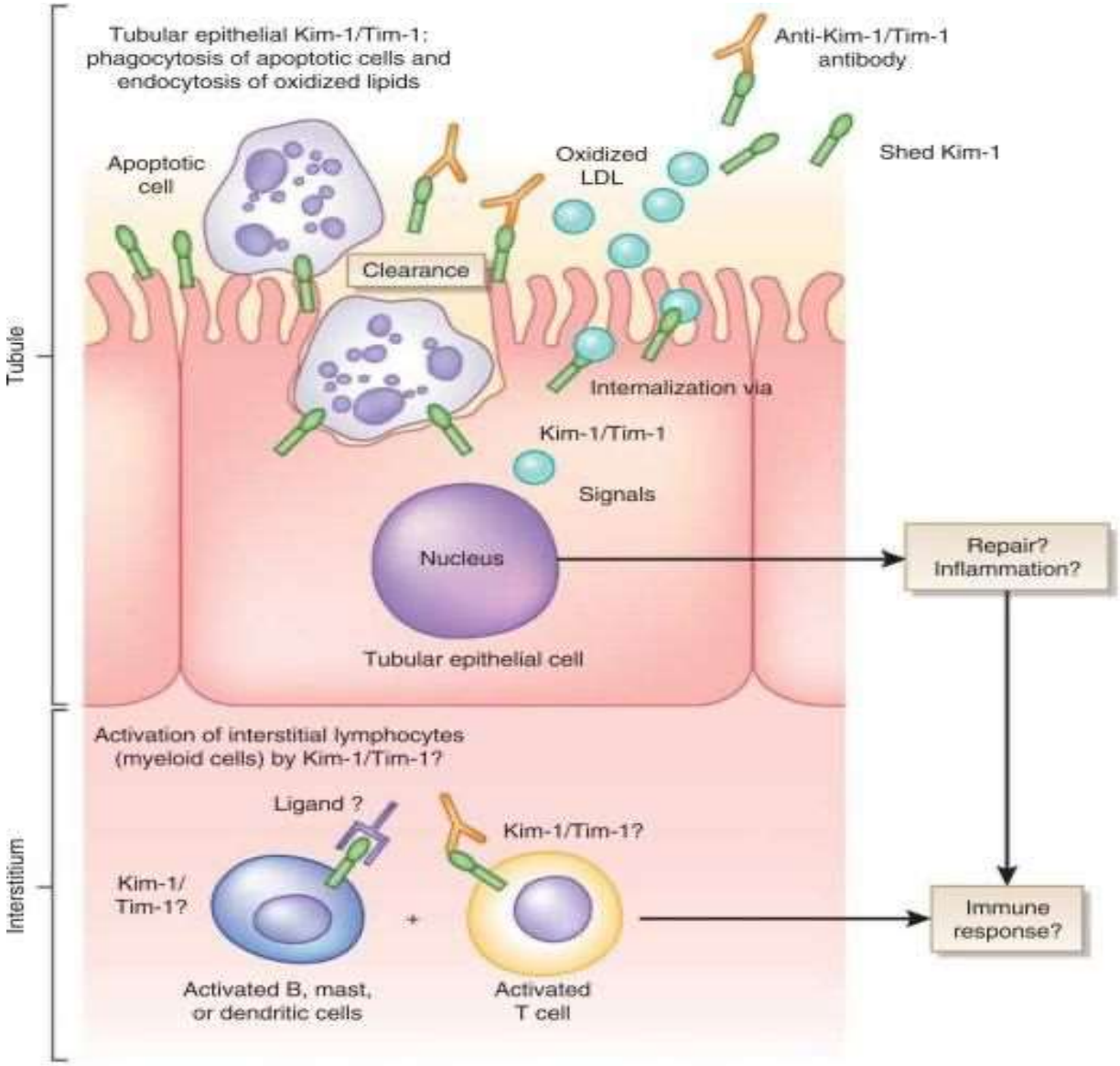


Figure (1-2) Role of Kim-1/Tim-1 in Tubular Epithelial Cell-Mediated Clearance and Immune Activation in the Kidney

1.9.4. Kidney Injury Molecule-1 as a Biomarker for CKD.

A number of characteristics of KIM-1 support its potential as an ideal biomarker of kidney injury: its absence in the normal kidney; its marked upregulation and insertion into the apical membrane of the proximal tubule cell; and persistence

in the epithelial cell until the cell has completely recovered. There is an urgent need for better biomarkers for AKI for to enable timely diagnosis, predict severity and outcome and monitor proximal tubule injury in AKI as well as CKD. Compared to S.Cr, this biomarker reflects kidney tissue injury instead of kidney glomerular filtration rate. Therefore, they offer the potential of early detection of kidney injury. Many studies indicate that KIM-1 is a sensitive and specific marker of kidney injury **(Tabernero, Pescador et al. 2023)**. Typical biomarkers for renal function evaluation in the setting of acute kidney injury (AKI), such as creatinine and blood urea nitrogen, are highly sensitive but imprecise. Moreover, it is important to note that fluctuations in S.Cr and blood urea nitrogen concentrations mostly reflect shifts in filtration capacity and do not always imply injure.

The increase of the ectodomain shed from cells into the urine shortly after proximal tubular kidney injury allows KIM-1 to serve as an early sensitive urinary biomarker in kidney injury detection. The measurement of urinary biomarkers has the potential to determine the risk of renal damage, distinguish between different types of kidney injury, and predict the outcome of several kidney diseases, including AKI, CKD, and transplant rejection, a well as other form of injury**(Simpson 2022).**

1.10. Tissue Inhibitor of Metalloproteinases-2

1.10.1. General Characteristics of Tissue Inhibitor of Metalloproteinases-2

The molecular mass of TIMP-2, a regulatory polypeptide consisting of 194 amino acids, is approximately 21 kDa. The regions at the N-and C-termini are different. The activation of pro-MMP-2 and a decrease of MMP activity are two of the regulatory functions of TIMP-2. The 125 amino acids that form

up the N-terminal domain of the working matrix metalloproteinases (MMPs) primarily inhibit their enzyme activity. This section hinders MMP activity by engaging to its active site in a stoichiometric dimer with a ratio of 1:1**(Delrue and Speeckaert 2024).** The nucleus edge of TIMP-2 may experience conformational changes, as shown by crystallographic X-rays and molecule dynamics simulations. This physical alteration has a possibility to influence TIMP-2's binding affinity and discrimination for various matrix metalloproteinases. Whilst the C-terminal domain has received less attention, it is thought to regulate pro-MMP-2 activity. One way TIMP-2 promotes the metalloproteinase matrix (MMP) activity is by forming a strong, noncovalent bond with MMP-2 (gelatinase A). Membrane-type 1 a type of matrix (MT1-MMP) is activated when pro-MMP-2 bonds with its hemopexin-like realm and non-catalytic parts; this interaction is regulated by TIMP-2. This mechanism is essential for collagenolysis and cellular-mediated tissue remodeling.**(Delrue et al., 2024)**.

The kidney glomeruli and tubular cells are the primary sites of TIMP-2 activity, although it can be detected in many other tissues as well. TIMP-2 plays an important role in kidney development and function, by regulating components of the extracellular matrix (ECM) in kidney tissue. There is rigorous oversight of TIMP-2 expression during and after transcription. The route leading to dialysis and fibrosis is associated with its regulation by cytokines as well as growth factors like making growth factor-beta (TGF-β) **(Kobusiak-Prokopowicz, Kaaz et al. 2021)**. TGF-β activates TIMP-2 expression via many signaling pathways, including small mothers against decapentaplegic and mitogen-activated protein kinase (MAPK). The interplay of these pathways increases TIMP-2 transcriptional activity, which

helps to regulate the ECM turnover. In addition to TGF-β, several other cytokines and growth factors, including fibroblast growth factor (FGF) and epidermal growth factor (EGF), affect TIMP-2 production, but their activities are less well known**(Wolosowicz, Prokopiuk et al. 2024)** .

1.10.2. Mechanism of Action of Tissue Inhibitor of Metalloproteinases-2

In the context of kidney injury, TIMP-2 plays an essential role in irritation, apoptosis, cell growth, cell differentiation, and extracellular matrix turnover, as well as in other biological processes. inhibiting matrix metalloproteinase (MMP) activity, particularly that of MMP-2 (gelatinase A), is the most obvious role of TIMP-2 (Figure 1-3). As a result of TIMP-2's direct inhibition of MMP-2's effective site, the proteolytic enzyme breaks down two vital components of the extracellular matrix: collagen. This inhibition is crucial for preventing fibrosis and controlling ECM restructuring during tissue regeneration **(Peeney, Liu et al. 2022)**. Furthermore, TIMP-2 contributes to the activation of pro-MMP-2. Which accomplished by interacting with MT1-MMP, causing the cellular activation of pro-MMP-2. Which dual involvement shows the complex modulation of TIMP-2 in the ECM dynamics. During AKI, damaged tubular cells express both TIMP-2 and IGFBP-7, which act complementarily. TIMP-2 primarily regulates the ECM turnover and inhibits MMP-2, thereby maintaining tissue homeostasis and preventing kidney fibrosis. To recover from injury and maintain structural and functional integrity, the kidneys require the precise alteration of the ECM. IGFBP-7, on the other hand, promotes the expression of p21 and p27, leading to cell cycle arrest. This combined action helps prevent the proliferation damaged cells and allows for repair, thus offering a powerful predictive tool for AKI. **(Delrue and Speeckaert 2024),** as shown in Figure (1-3).

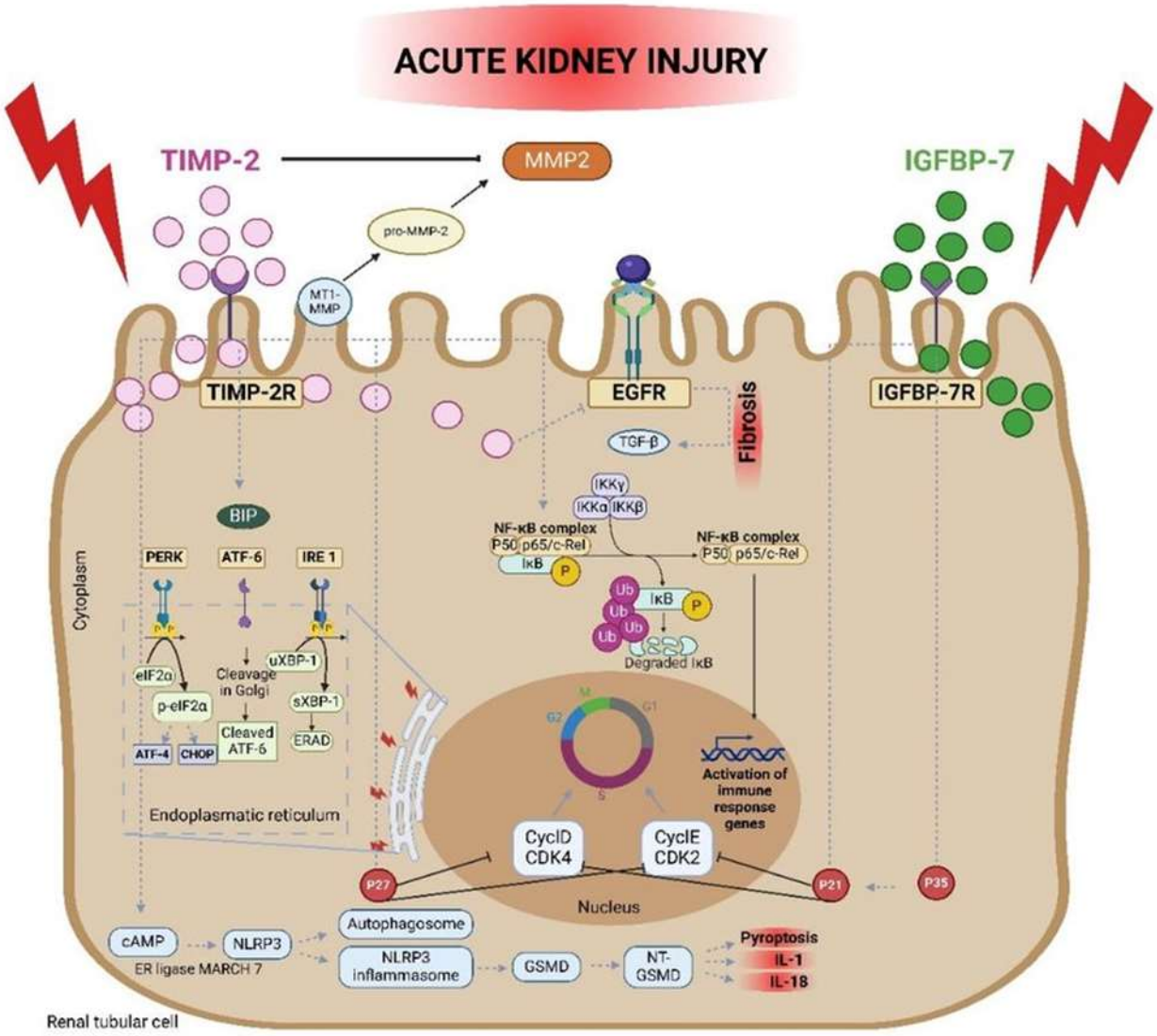


Figure (1-3) (Chematic Overview of the Pathways Affected by TIMP-2 in Kidney Damage. (Minoves, Ouldali et al. 2025).

TIMP-2 plays multiple roles in kidney pathology relevant to CKD. It regulates matrix metalloproteinase-2 (MMP-2) activity by either inhibiting its function or promoting its activation through MT1-MMP. In damaged renal tubular cells, TIMP-2, along with IGFBP-7, induces cell cycle arrest at the G1 phase via inhibition of Cyclin-CDK complexes, thereby preventing the

proliferation of injured or abnormal cells. Additionally, TIMP-2 inhibits the EGFR/TGF-β signaling pathway, which reduces kidney fibrosis—a common complication of CKD. Furthermore, TIMP-2 modulates inflammatory responses by influencing the NF-κB pathway and may contribute to ER stress under pathological conditions. These mechanisms collectively highlight its potential as a biomarker for kidney injury and disease progression.

1.10.3. Clinical Application of Tissue Inhibitor of Metalloproteinases-2

TIMP-2 has emerged as a valuable biomarker for the early prediction of acute kidney injury (AKI), particularly when assessed in combination with IGFBP-7. This combination forms the basis of the NephroCheck assay, which measures their urinary concentrations to generate a risk score for AKI **(Delrue & Speeckaert, 2024).**

The test is especially useful in critical care and perioperative settings, such as intensive care units and post-major surgery, where early identification of high-risk patients enables timely interventions, including hemodynamic optimization, minimization of nephrotoxic exposure, and implementation of renal-protective measures. Multiple clinical trials have demonstrated that the [TIMP-2] × [IGFBP-7] index provides high sensitivity and specificity in predicting AKI, particularly in patients with sepsis, hemodynamic instability, shock, or severe cardiac events. Its application has also been reported in pediatric populations, especially in newborns at risk of AKI following cardiac surgery **(Delrue & Speeckaert, 2024).**

In kidney transplantation, elevated levels of TIMP-2 and IGFBP-7 have been associated with prolonged delayed graft function, adversely affecting graft viability and patient survival **(Cavalcante et al., 2022).**

1.10.4. Functional Role of Tissue Inhibitor of Metalloproteinases-2 in Chronic Kidney Disease.

TIMP-2 (Tissue Inhibitor of Metalloproteinases-2) plays a key role in regulating the extracellular matrix (ECM) turnover and fibrosis, both of which are critical processes in the progression of chronic kidney disease (CKD). The relationship between TIMP-2 and CKD is complex, involving both protective and pathogenic mechanisms. TIMP-2 inhibits matrix metalloproteinases (MMPs), which are responsible for the degradation of ECM components. Controlled inhibition of MMPs helps preserve kidney structure and prevents excessive tissue breakdown **(Kassiri & Khokha, 2005).**

TIMP-2 as a Biomarker for CKD Progression

TIMP-2, often measured alongside IGFBP-7, serves as an early biomarker for kidney stress and acute kidney injury (AKI). In CKD, persistently elevated TIMP-2 levels indicate ongoing renal injury and are associated with an increased risk of progression to end-stage renal disease (ESRD). Clinical studies have demonstrated that higher TIMP-2/MMP-2 ratios are correlated with poorer renal outcomes, highlighting its value as a potential prognostic marker **(Leivonen et al., 2013).**

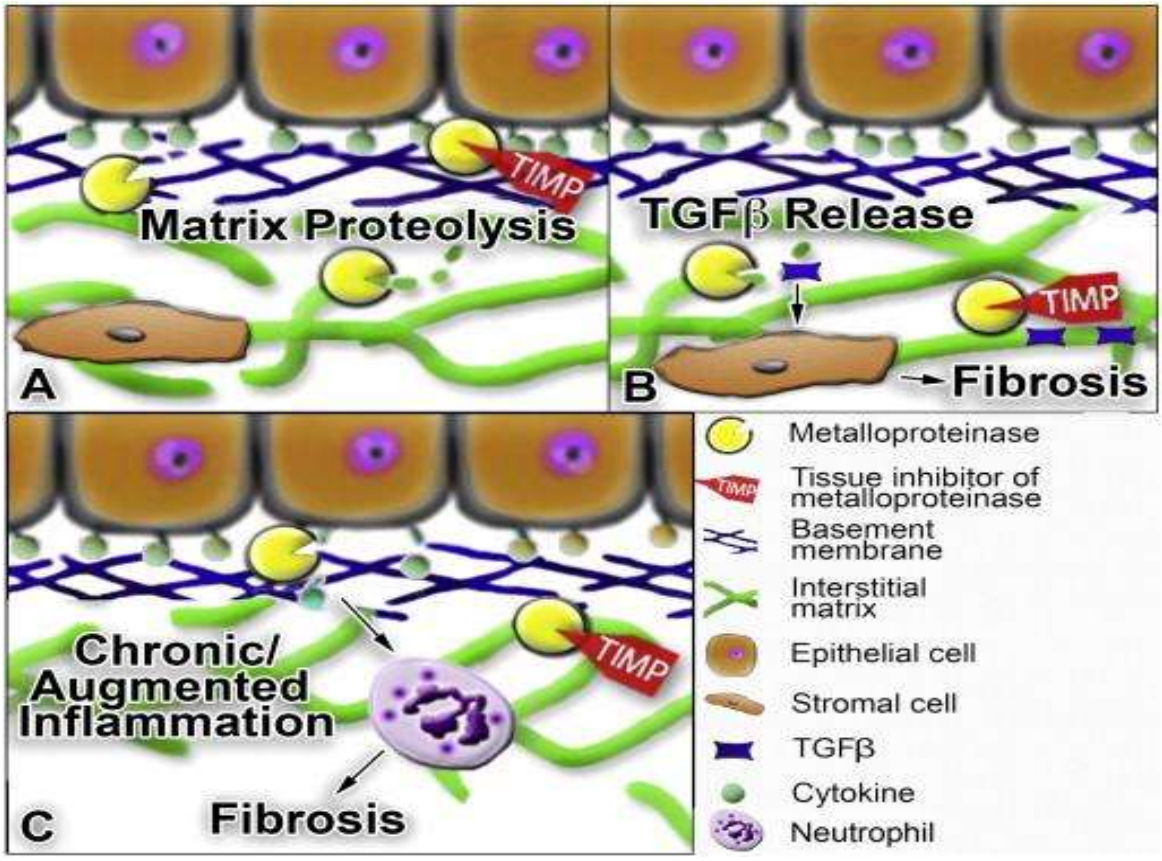


Figure 1-4. Roles of TIMPs in Matrix Remodeling, Inflammation, and Fibrosis in CKD.

The diverse functions of TIMPs in regulating ECM turnover depend on the type of TIMP, the tissue microenvironment, and the specific metalloproteinases they inhibit.

(A) TIMPs directly inhibit ECM proteolysis by suppressing metalloproteinase activity within the ECM.

(B) TGFβ, stored within the ECM, can be released and activated by metalloproteinase activity, leading to increased ECM deposition by stromal cells and promoting fibrosis. By inhibiting metalloproteinases, TIMPs limit TGFβ activation, thereby reducing fibrosis.

(C) Metalloproteinases can modulate cell-surface cytokines and their receptors, contributing to inflammation (e.g., neutrophil chemotaxis). TIMPs inhibit this process, indirectly regulating ECM turnover through the control of inflammation **(Kassiri & Khokha, 2005; Leivonen et al., 2013)**

1.11. Knowledge gap

CKD affects millions globally, often progressing silently until irreversible damage occurs. Traditional biomarkers, such as serum creatinine and estimated glomerular filtration rate (eGFR), lack sensitivity in early disease stages. The combined prognostic and diagnostic value of KIM-1 and TIMP-2 in chronic kidney disease patients remains unclear despite recent studies. This study investigate the value of both biomarkers combined prognostically and diagnostically in correlation with CKD patients.

1.12. Aims of Study:

This study amis to:

1. Assess of serum levels of Kidney injury molecule-1 and Tissue inhibitor of metalloproteinase-2 in Iraqi patients with chronic kidney disease and compare these levels those healthy control group.
2. Study the possibility of utilizing of these two biomarkers as new diagnostic biomarkers in chronic kidney disease.
3. Show the association between serum levels of KIM-1 and TIMP-2 with CKD stages, and demographic features included (age, gender, and body mass index BMI).
4. Assess the relationship between KIM-1 and TIMP-2 levels with classical renal function markers (serum urea, creatinine), bone-mineral metabolism markers (serum calcium, vitamin D, parathyroid hormone), and other biochemical parameters.
5. Explore the association of these biomarkers and biochemical parameters with common comorbidities in CKD patients, such as hypertension, diabetes mellitus, and cardiovascular disease, and smoking.

Chapter Two

Materials and Methods

2.1. Study Design and Ethical Approval.

A case-control study was carried out At AL Imam AL Hussein Medical city in Karbala. The participants were gathered over the duration between November 2024 to Mach 2025. The study protocol was approved by the Ethical Committee of college of medicine university of Kerbala and Kerbala Health Directorate. Verbal informed consent was obtained from all participants or their relatives after clarifying the nature and purpose of the study and objectives to each patient, as show in Figure (2-1).

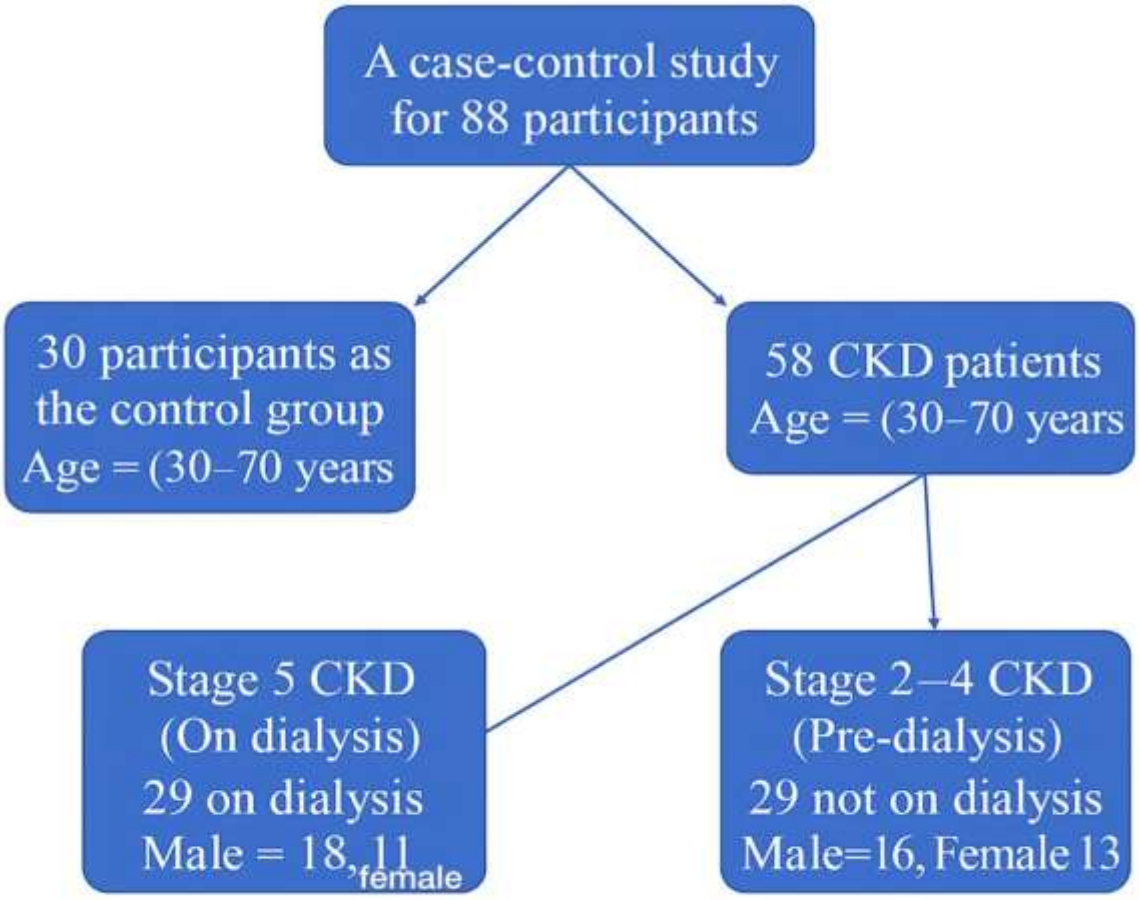


Figure (2.1): Study Design Flowchart

2.2. Patients

2.2.1. Patients Section

The research involved 58 patients. aged between 30 -70 years old. These patients were divided into two subgroups: 29 with Stage 5 Chronic kidney disease (CKD) (on dialysis), and 29 with Stage 2-4 Chronic kidney disease (CKD) (pre-dialysis), as shown in fig (2- 1). They were evaluated and diagnosed by specialist doctor. And recruited from AL Imam AL Hussein Medical city in Karbala. A particular questionnaire form including descriptive information was designed and filled by each patient. The questionnaire included three sections. The first section was comprised of data collected from the patient, including age, gender, smoking status, current treatment, family history of Chronic kidney disease (CKD), physical activity, risk factors for Chronic kidney disease (CKD) including diabetes, hypertension. while second section consisted of a physical examination measuring SBP and DBP, height, and weight. The third section involved blood biochemical tests like Kidney injury molecule-1 (KIM-1), Tissue Inhibitor of Metalloproteinase-2 (TIMP-2), blood urea test, serum creatinine, electrolyte test, serum calcium, vitamin D3 and parathyroid hormone.

Inclusion Criteria

Iraqi Male and female patients with chronic kidney disease where based on diagnosed by a specialized physician according to KDIGO Diagnostic criteria, the clinical medical and history examination.

Exclusion Criteria

Patients with acute kidney injury, acute cardiovascular diseases, recent kidney surgery, recent cardiac events, and renal, lung, or colon cancer were excluded from this study.

2.2.2. Control

A control group of 30 individuals (18 male and 12 female), aged between 30–70 years old as shown in Fig (2-1), were selected from the list of volunteers accompanying the patients and volunteers from outside the hospital. All participants had no history of acute and chronic kidney diseases, and no abnormal renal function tests.

2.2.3. Sample Collection and Processing

Five milliliters of venous blood were collected from each participant. Venous blood was collected from each participant and transferred into serum separator tubes (plain gel tubes, yellow top) to obtain serum and allowed to clot for 30 min – 1 hour*. They were then separated using a centrifuge operating at a speed of 3000 xg for a duration of 20 minutes. The serum samples were divided into three Eppendorf tubes and kept at a temperature of -20 °C in the deep freezer until the time of analysis. To avoid repeated freeze–thaw cycles, one tube was utilized for the measurement of Kidney Injury Molecule-1 (KIM-1) and Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) using the enzyme-linked immunosorbent assay (ELISA) technique. Another Eppendorf tube was utilized to quantify the blood urea, serum creatinine levels, electrolyte levels, and serum calcium , vitamin D3 and parathyroid hormone levels were measured using the Abbott Architect c 4000 immunoassay analyzer.

2.3. Instruments: The instruments that were used in the current study are listed in The Table (2-1).

Table (2-1) The instruments Used in the Study.

NO.	Instruments	Country
1.	Centrifuge	Germany
2.	Deep Freeze	Korea
3.	ELISA System (Bio Tek)	USA
4.	Incubator (Binder)	Germany
5.	Mini VIDAS	French
7.	Abbott Architect c4000	USA

2.4. Tools, Materials and Kits: The Tools, materials and Kits that were used in the current study are listed in the table (2-2).

Table (2-2) Tools and Materials Used in the Study.

No.	Tools and Materials	Country
1.	Pipette (100-1000µl)	DRAGON LAB/USA
2.	Micropipette (10-100µ1)	DRAGON LAB/USA
3.	Eppendorf Tubes 1.5ml	China
4.	Gilson Tips,1000µl (blue)	China
5.	Gilson Micro-tips, 100µl	China
6.	Cotton	China
7.	Eppendorf Tube Racks	China
8.	Syringe	China
9.	Gloves	China
10	Tourniquet	China
11.	Gel tubes	China

Table (2-3): Kits That are Used in The Study.

No.	Name of kit	Company	Country
1	KIM-1 kit	BT LAB	China
2	TIMP-2 kit	BT LAB	China
3	Sodium kit	Linear Chemicals S.L.U.	España
4	Potassium kit	Linear Chemicals S.L.U.	España
6	Urea Nitrogen Reagent kit	Abbott	USA
7	Creatinine kit	Abbott	USA
8	Calcium kit	Abbott	USA
9	25-OH vitamin D kit	Abbott	USA
10	PTH kit	Abbott	USA

2.5. Methods

2.5.1. Calculation of eGFR

Estimated GFR was measured using CKD-EPI Equation (Chronic Kidney Disease Epidemiology Collaboration) (**Inker, L. A., et al. 2021).**

eGFR = 142 x min (Scr/K, 1)^α max(Scr/K, 1)^-1.200 x 0.9938^ age x 1.012 [if female]

Where:

- Scr: Serum creatinine in mg/dL

- Age: Age in years

-k (kappa): 0.7 for females and 0.9 for males

-α (alpha): -0.241 for females and -0.302 for males

- The min (): function takes the smaller value between Scr/k and 1

- The max (): function takes the greater value between Scr/k and 1

- The sex coefficient (1.012) is applied only if the subject is female

2.5.2. Calculation of Body Mass Index

Weight in kilogram (kg) and height in meter (m) were recorded. Body mass index (BMI) was calculated by the following equation:

BMI (kg/m^2) = weight / $(height)^2$

Patients were divided into three categories: Normal weight (BMI 18.5-24.9 kg/m^2), Overweight (25-29.9 kg/m^2), and Obese (≥ 30.0 kg/m^2) **(Donini et al., 2020).**

2.5.3. Determination KIM-1 levels in Human serum by using Sandwich-ELISA Technique:

Test principle

This kit is based on the Enzyme-Linked Immunosorbent Assay (ELISA) principle. The plate was pre-coated with human Kim-1 antibodies. The sample was added, allowing any Kim-1 present to bind to the coated antibodies. Next, a biotinylated human Kim-1 antibody was added, which binds to the captured Kim-1. Streptavidin-HRP is then added and binds to the biotinylated antibody. After incubation, any unbound components where removed by washing. A substrate solution was then added, and color develops proportionally to the amount of Kim-1. The reaction was stopped with an acidic stop solution, and absorbance was measured at 450 nm.

Reagent and materials

Table (2-4): Reagents Provided with the ELISA kit .

Components	Quantity (96T)
Standard solution (12.8ng/ml)	0.5ml xl
Pre-coated ELISA plate	12 * 8 well strips x1
Standard diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop solution	6ml x1
Substrate solution A	6ml x1
Substrate solution B	6ml x1
Wash buffer Concentrate (25x)	20ml x1
Biotinylated Human HAVCR1 antibody	1ml x1
User instruction	1
Plate sealer	2 pics

Preparation of Reagents:

Table (2-5): Dilution of Standards used for sKim-1 assay.

6ng/ml	Standard No.5	120ul Original standard + 120ul Standard diluent
3ng/ml	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
1.5ng/ml	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
0.75ng/ml	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
0.375ng/ml	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

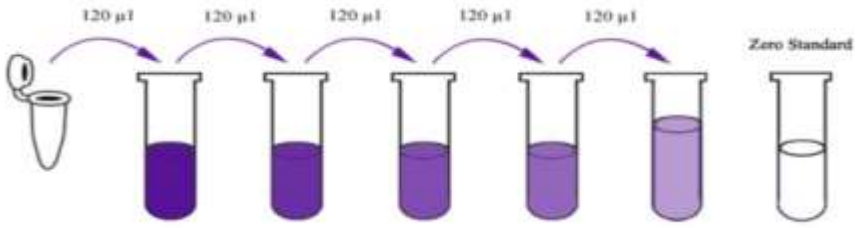


Figure (2-2): Serial Dilution of KIM-1 Standard Solution Used in ELISA"

Table (2-6): Dilution of Standards Used for KIM-1 Assay.

Standard concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
12.8 ng/ml	**6 ng/ml**	**3 ng/ml**	**1.5 ng/ml**	**0.750ng/ml**	**0.375ng/ml**

Assay Procedure

1. All reagents, standard solutions, and samples were prepared as instructed, and all reagents were brought to room temperature before use. The assay was performed at room temperature.

2. The required number of strips for the assay was determined and inserted into the frames for use. The unused strips were stored at 2–8 °C.
3. A volume of 50 µL of standard solution has been added to each standard well. Note: The antibody has not been added to the standard well because the standard solution already contains the biotinylated antibody.
4. A volume of 40 µL of sample has been added to each sample well, followed by 10 µL of Human HAVCR1 antibody. Then, 50 µL of streptavidin-HRP has been added to both sample wells and standard wells (excluding the blank control well). The contents were mixed thoroughly, the plate was covered with a sealer, and incubation has been carried out for 60 minutes at 37 °C.
5. The sealer has been removed, and the plate has been washed five times with wash buffer. During each wash, the wells were soaked with 300 µL of wash buffer for 30 seconds to 1 minute.
6. A volume of 50 µL of substrate solution A has been added to each well, followed by 50 µL of substrate solution B. The plate has been covered with a new sealer and incubated for 10 minutes at 37 °C in the dark.
7. A volume of 50 µL of Stop Solution has been added to each well, and the blue color has changed immediately to yellow.
8. The optical density (OD) of each well has been measured immediately using a microplate reader set to 450 nm, within 10 minutes after the Stop Solution has been added.

Calculation of Results

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. As show in figure (2-2)

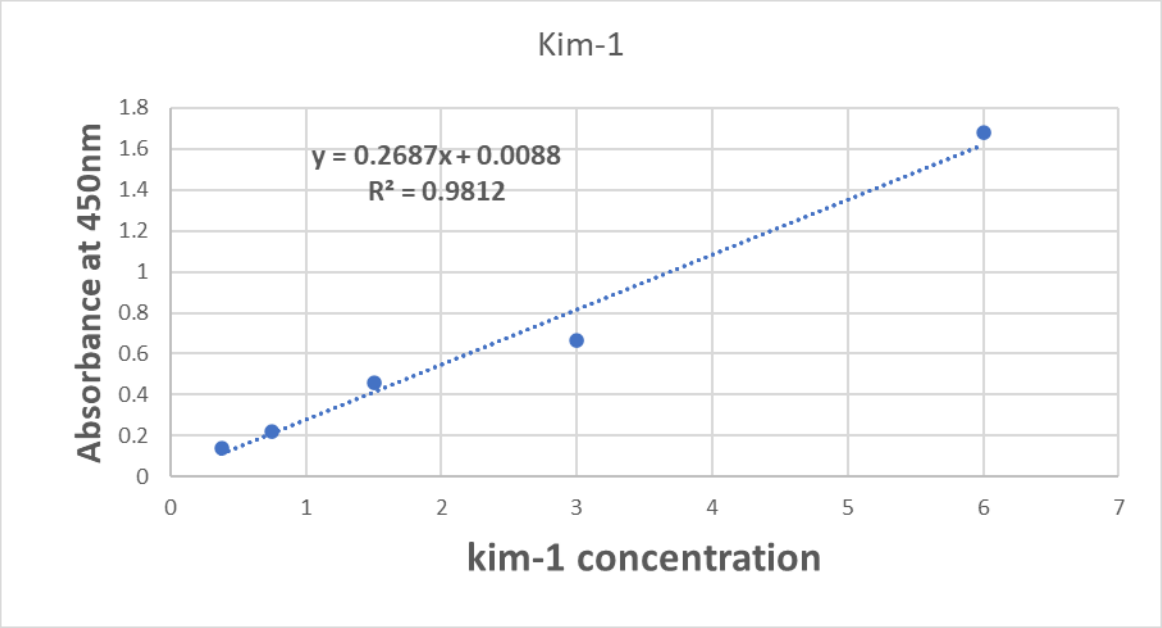


Figure (2-3): Standard Curve for Human Serum Kidney injury molecule-1 (KIM-1) Concentration (ng/mL).

2.5.4. Determination of Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) levels in Human Serum Using The Sandwich-ELISA Technique:

Test Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human TIMP-2 antibody. TIMP-2 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human TIMP-2 Antibody is added and binds to TIMP-2 in the sample. Next ,the streptavidin-HRP is added and binds to the Biotinylated TIMP-2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human TIMP-2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagents and Materials

Table (2-6): Reagents provided with the ELISA Kit.

Components	Quantity (96T)
Standard Solution (240ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human TIMP-2 Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Preparation of reagents:

Table (2-7): Dilution of Standards Used for TIMP-2 Assay.

120ng/ml	**Standard No.5**	**120ul Original standard + 120ul Standard diluent**
60ng/ml	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
30ng/ml	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
15ng/ml	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
7.5ng/ml	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

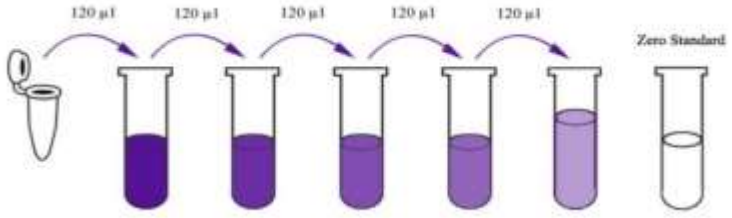


Figure (2-4): Serial Dilution of TIMP-2 Standard Solution Used in ELISA"

Standard concentration	**Standard No.5**	**Standard No.4**	**Standard No.3**	**Standard No.2**	**Standard No.1**
240 ng/ml	120ng/ml	60ng/ml	30ng/ml	15ng/ml	7.5ng/ml

Assay Procedure

1. All reagents, standard solutions, and samples were prepared as instructed, and all reagents were brought to room temperature before use. The assay was performed at room temperature.

2. The required number of strips for the assay was determined and inserted into the frames for use. The unused strips were stored at 2–8 °C.
3. A volume of 50 µL of standard solution has been added to each standard well. Note: The antibody has not been added to the standard well because the standard solution already contains the biotinylated antibody.
4. A volume of 40 µL of sample has been added to each sample well, followed by 10 µL of anti-TIMP-2 antibody. Then, 50 µL of streptavidin-HRP has been added to both sample wells and standard wells (excluding the blank control well). The contents have been mixed thoroughly, the plate has been covered with a sealer, and incubation has been carried out for 60 minutes at 37 °C.
5. The sealer has been removed, and the plate has been washed five times with wash buffer. During each wash, the wells have been soaked with 300 µL of wash buffer for 30 seconds to 1 minute.
6. A volume of 50 µL of substrate solution A has been added to each well, followed by 50 µL of substrate solution B. The plate has been covered with a new sealer and incubated for 10 minutes at 37 °C in the dark.
7. A volume of 50 µL of Stop Solution has been added to each well, and the blue color has changed immediately to yellow.
8. The optical density (OD) of each well has been measured immediately using a microplate reader set to 450 nm, within 10 minutes after the Stop Solution has been added.

Calculation of Results

A standard curve can be drawn by plotting the average optical density (OD) of each standard on the Y-axis and the concentration on the X-axis. Next, make a best-fit curve utilizing the points on the graph.

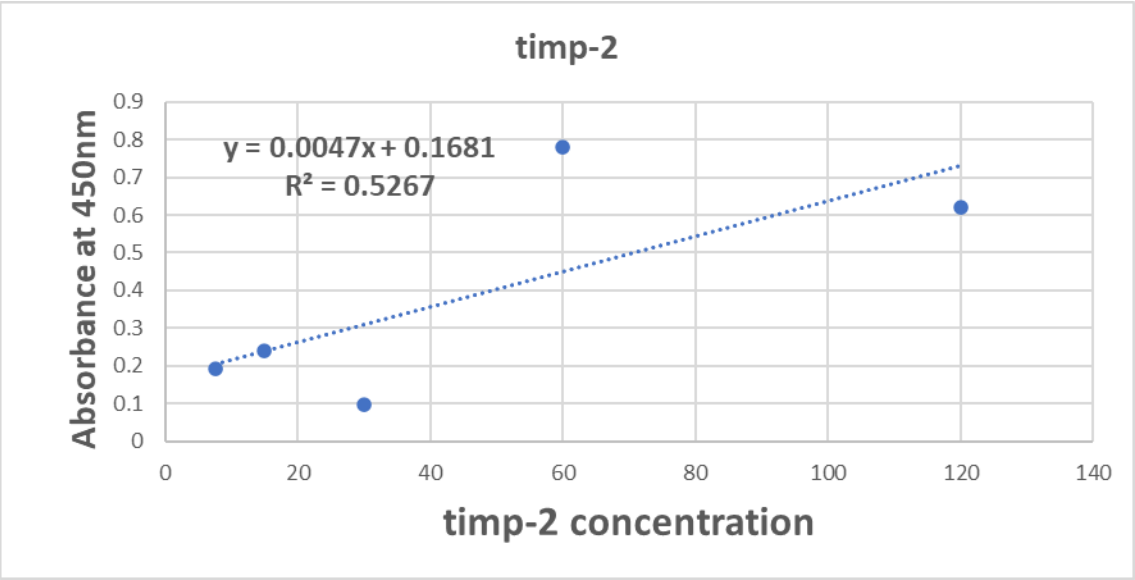
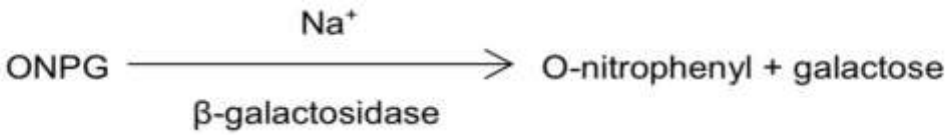


Figure (2-5): Standard Curve for Human Serum of Tissue Inhibitor of Metalloproteinase-2 Concentration (ng/ml).

2.5.5 Determination of Serum Sodium Concentration.

Principle

Sodium is enzymatically determined through the activity of sodium-dependent β-galactosidase using ONPG (o-nitrophenyl-β-D-galactopyranoside) as a substrate. In the presence of sodium ions, ONPG is hydrolyzed by β-galactosidase to produce o-nitrophenyl and galactose. The absorbance of the released o-nitrophenyl is measured at 405 nm and is directly proportional to the sodium concentration.



ONPG = o-nitrophenyl -ß-D-galactopiranos

Table (2.8) Reagents Used for Sodium Assay.

R1	R2	R3
1 x 20 mL	**1 x 10 mL**	**1 x 10 mL**

❖ **Reagent composition**

- R1 Good's buffer (pH 8.5), β-D-galactosidase (<8 U/mL),
- Cryptand (>0.4 mM), Proclin 300 (0.02%).
- R2 Good's buffer (pH 6.5), ONPG (>0.5 mM), Proclin 300 (0.02%).
- CAL Sodium / Potassium standard. Sodium (Na+) 160 mmol/L. / Potassium 6.0 mmol/L.

❖ Procedure

1. Reagents and samples have been brought to room temperature.
2. The required volumes have been pipetted into the labelled cuvettes

Cuvettes	Blank	Sample	Calibrator
R1. Reagent	1.0 mL	1.0 mL	1.0 mL
Sample	-	-	-
Calibrator R2.	-	-	40 µL
Reagent	0.5 mL	0.5 mL	0.5mL

3. Reagents and samples have been brought to room temperature.

   The required volumes have been pipetted into the labelled cuvettes

4. The plate has then been incubated for 2 minutes at 37 °C, and the absorbance (A2) has been read at 405 nm.

❖ **calculation**

$$\textbf{Sodium mmol/L} = \frac{(A2 - A1)\ Sample}{(A2 - A1)\ Calibrator} \textbf{ x C Calibrator}$$

2.5.6 Determination of Serum Potassium Concentration.

Principle

Potassium is determined spectrophotometrically through a kinetic coupling assay system using potassium dependent pyruvate kinase. Pyruvate generated is converted to lactate accompanying conversion of NADH to NAD.

The corresponding decrease of optical density at 380 nm is proportional to the potassium concentration in the serum.

PEP + ADP → Pyruvate + ATP

(via K^+-dependent pyruvate kinase)

Pyruvate + NADH + H^+ → Lactate + NAD^+

(via lactate dehydrogenase)

Table (2.9) Reagents used for potassium assay.

R1	R2	CAL
1 x 20 mL	1 x 5 mL	1 x 3 mL

❖ **Reagent composition**

- R1 LDH <50 KU/L, NADH <10 mmol/L, sodium azide 0,05%

  and stabilizers.

- R2 Pyruvate kinase <50 KU/L, sodium azide 0,05% and stabilizers.

- CAL Sodium / Potassium standard. Sodium (Na+) 160 mmol/L / Potassium (K+) 6.0 mmol/L.

❖ procedure

1. Reagents and samples have been brought to room temperature.
2. The required volumes have been pipetted into the labelled cuvettes.

Cuvettes	**Blank**	**Sample**	**Calibrator**
R1. Reagent	**mL**	**mL**	**mL**
Sample	**-**	**25 µL**	**-**
Calibrator	**-**	**-**	**25µL**

3. The contents have been mixed, then incubated for 5 minutes at 37 °C.
4. add

R2 Reagent	**250 µL**	**250 µL**	**250 µL**

5. The contents have been mixed, then incubated for 1 minute at 37 °C, and the absorbance (A1) has been read at 405 nm.
6. The contents have been mixed, incubated for 3 minutes at 37 °C, and the absorbance (A2) has been read at 405 nm.

❖ Calculation

$$\boldsymbol{Potassium\ mmol/L} = \frac{(\boldsymbol{A2 - A1})\ \boldsymbol{Sample})}{(\boldsymbol{A2 - A1})\ (\boldsymbol{Calibrator})} \textbf{x C Calibrator}$$

2.5.7 Determination of Serum Creatinine Concentration.

Principle

At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. the rate of increase in absorbance at 500 nm due to formation of this complex is directly proportional to the concentration of creatinine in the sample (Wong, (1999).

Methodology: Kinetic Alkaline Picrate

Normal range: male (0.72-1.25mg/dl), female (0.57-1.11 mg/dl).

Table (2.10) Reactive Ingredients of Serum Creatinine. (Abbott, USA).

Reactive ingredients	Concentration
R1 sodium hydroxide	0.8 mol/L
R2 picric acid	24mol/L

❖ Measurement of Serum Creatinine Level

$$\text{Creatinine + Picric acid} \xrightarrow[\text{Alkaline pH}]{} \text{Yellow–orange complex}$$

Procedure: 100 microliter of separated serum was placed in cuvate to be analyzed.

Calculations:

The concentration of the serum creatinine is measured automatically by using architect Abbott c4000 chemistry analyzer.

2.5.8 Determination of Serum Urea Level

Principle

The urea nitrogen assay is a modification of a totally enzymatic procedure first described by Talke and Schubert (Talke 1965). The test is performed as a kinetic assay in which the initial rate of the reaction is linear for a limited period of time. Urea in the sample is hydrolyzed by urease to ammonia and carbon dioxide. The second reaction, catalyzed by glutamate dehydrogenase

(GLD) converts ammonia and α-ketoglutarate to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide (NADH)to nicotinamide adenine dinucleotide (NAD). Two moles of NADH are oxidized for each mole of urea present. The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample (Abbott, USA).

Table (2.11) Reactive Ingredients of Serum Urea Level (Abbott, USA).

Reactive ingredients	Concentration
R1 NADH	2.95mmol/L
R2 α-ketoglutaric acid	99.8 mmol/L
urease (jack bean)	23.5KU/L
GLD (beef liver)	63.5 KU/L
Adenosine diphosphate	7.6 mmol/L

❖ **Measurement of Serum Urea Level**

Urease

$$\text{Urea} + 2H_2O \longrightarrow\longrightarrow 2NH_4^+ + CO_3^{2-}$$

GLDH

$$NH_4^+ + \text{2-oxoglutarate} + NADH \longrightarrow\longrightarrow \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentrations is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm.

Procedure:

100 µL of separated serum was putted in cuvate to be analyzed.

Calculations

The concentration of the serum urea measured automatically by using architect Abbott c4000 chemistry analyzer.

2.5.9 Determination of Serum Calcium Level

Principle:

Arsenazo-lll dye reacts with calcium in an acid solution to form a blue-purple complex. The color developed is measured at 660 nm and is proportional to the calcium concentration in the sample. (Abbott, USA).

Table (2.12) Reactive ingredients of serum calcium level (Abbott, USA).

Reactive Ingredients	Concentration
R1 **Arsenazo-III dye** **Sodium acetate**	0.94 mmol/L 271 mmol/L

❖ **Calculations:**

The concentration of the serum calcium measured automatically by using architect Abbott c4000 chemistry analyzer.

2.5.10 Determination of Serum Vitamin D Concentration Level

Principle:

The archi25-OH Vitamin D assay is a quantitative delayed one step competitive immunoassay to determine the presence of vitamin D in human serum and plasma. It employs CMIA technology with flexible assay protocols, referred to as Chemillex.

1. The sample, assay diluent, and paramagnetic anti-vitamin D coated microparticles are combined. The 25-OH vitamin D present in the sample is displaced from the vitamin D binding protein and binds to the coated microparticles, forming an antigen antibody complex.
2. After incubation, a conjugate containing acridinium-labeled vitamin D was added to the reaction mixture and binds the unoccupied binding sites of the anti-vitamin D coated microparticles
3. After further incubation and washing, Pre-Trigger and Trigger Solutions were added to the reaction mixture.

4. The resulting chemiluminescent reaction is measured in relative light units (RLUs). There is a relationship between the amount of 25-OH vitamin D in the sample and the RLUs detected by the ARCHITECT System optics.

Table (2.13) Reagents used for 25-OH vitamin D assay (Abbott, USA).

Microparticles	**27ml**
Conjugate	**26mL**
Assay diluent	**50.9mL**

1. **Conjugate:** Acridinium-labeled vitamin D in MES Buffer and surfactant. Minimum concentration 12 ng/mL labeled vitamin D.
   Preservative: Sodium Azide.
2. **Assy Diluent:** Citrate buffer with EDTA, Methanol, 8-anilino-1-naphthalene sulfonic acid (ANSA), and surfactant. Preservative: ProClin 300.

❖ Assy procedure

1. Before loading the reagent kit on the system for the first time, the microparticle bottle has been mixed to resuspend the microparticles that may have settled during shipment. After the first time, the microparticles have been loaded, no further mixing is required. Invert the microparticle bottle 30 times.
2. Once the microparticles have been resuspended, place a septum on the bottle. Load the reagent kit on the architect system, Verify that all necessary reagents are present, Ensure that separe present on all reagent bottles.

3. verify adequate sample cup volume is present prior to running the test.

4. ARCHITECT 25-OH Vitamin D calibrators and controls have been prepared.

5. Calibrators and controls have been mixed by gentle inversion before use.

6. Bottles have been held vertically, and the recommended volumes have been dispensed into each respective sample cup.

- **Calculation**

The architect 25-OH Vitamin D assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

2.6 Determination of Serum Parathyroid Hormone (PTH) Concentration Level.

Principle

The architect Intact PTH assay is a two-step sandwich immunoassay for the quantitative determination of intact PTH in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. The sample, assay diluent, and anti-PTH coated paramagnetic, microparticles are combined. Intact PTH present in the sample binds to the anti-PTH coated microparticles. After washing, anti-PTH acridinium-labeled conjugate is added to create a reaction mixture. Following another wash cycle, Pre-Trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of intact PTH in the sample and the RLUs detected by the ARCHITECT iSystem optics.

Table (2.14) Reagents used for Parathyroid Hormone (PTH) Assay (Abbott, USA).

Microparticles	**1 * 6.6mL**
Conjugate	1 * 5.9mL
Assay diluent	1 * 10.0mL

1. **Microparticles:** Anti-PTH (goat, polyclonal) acridinium-labeled coated microparticles in TRIS buffer. Minimum concentration: 0.05% solids. Preservative: sodium azide.
2. **Conjugate:** conjugate in MES buffer with protein (bovine, goat) stabilizer. Preservative: sodium azide.
3. **Assay diluent:** Intact PTH Assay Diluent containing phosphate buffer with protein (bovine, goat) stabilizer. Preservative: sodium azide.

❖ **Assay Procedure**

1. Before loading the reagent kit on the system for the first

time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. Invert the microparticle bottle 30 times.

place a septum on the bottle, Load the reagent kit on the architect System. Verify that all necessary reagents are present, Ensure that spectrum are present on all reagent bottles.

2.Prepare architect intact PTH Calibrators and Controls.

3.Mix calibrator(s) and controls by gentle inversion before use.

4.Hold bottles vertically and dispense recommended volumes

into each respective sample cup. Recommended volumes, for each calibrator: 15 drops, for each control: 10 drops.

❖ **Calculation**

The architect intact PTH assay uses a point to point data reduction method to generate a calibration curve.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows 10 (IBM SPSS 26.0, Chicago, IL, USA) program was used for the statistical assessments. Descriptive statistical approaches were used to analyze the data from each group. Scale variables were represented by their Median (Min-Max) and interquartile range (IQR), whereas values for categorical variables were reported as the number of occurrences (n) and \the corresponding percentage (%). The Shapiro-Wilk test was utilized to confirm the normality of the data distribution.

Additionally, an inferential statistical approaches are used to analyze the abnormal distribution, The Mann-Whitney U test was used to compare continuous variables between two groups. The Kruskal-Wallis test was employed to compare continuous variables among multiple groups. The correlation within the case study was evaluated by comparing biomarkers using the Spearman rank test.

The association between the analyzed factor were estimated using odds ratios (ORs) and 95% Confidence Interval Range which calculated by a non-conditional logistic regression.

Analytical statistical studies indicated significant differences in categorical variables among the parameters. All hypothesis tests with p-values less than 0.05 (two-sided) were deemed to have statistical significance.

The optimal threshold with high specificity and sensitivity for critical cases was detected using receiver operating characteristic (ROC) analysis

Chapter Three

Results

3. Results

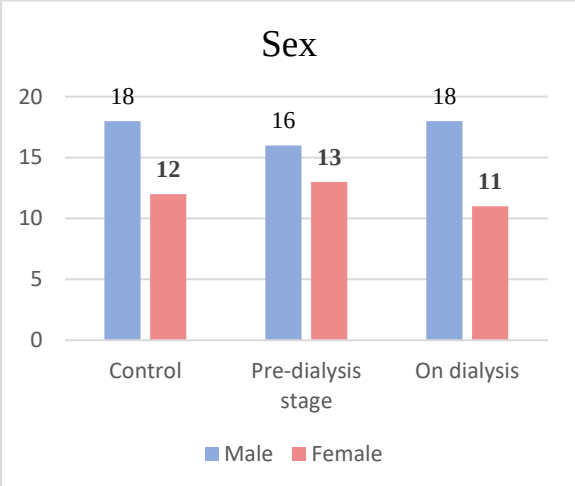
3.1. Demographic and Clinical Characteristics

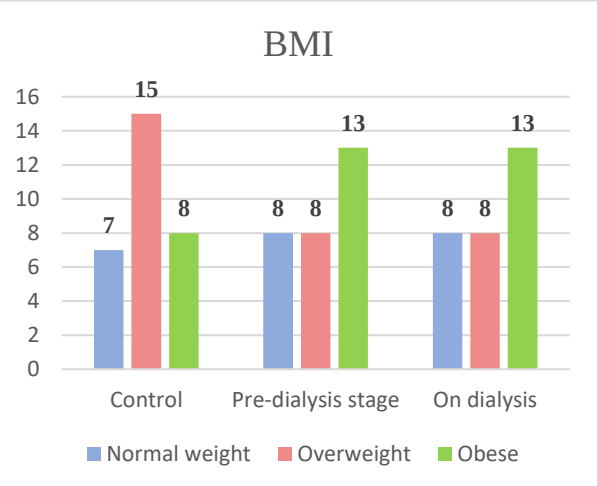
The demographic and clinical characteristics of patients with chronic kidney disease and control groups were summarized in Table (3-1) and Figures (3-1), (3-2). The proportion of patients aged (30-40 years) was 12.1%, while the proportion of patients aged (41-51 years) was 13.8%, while the proportion of patients aged (52-62 years) was 31.0%. Patients aged 62 years and above account for 43.1%.

In the control group, the proportion of participants aged 30-40 year was 50%, the proportion of people aged 41-51 year was 20%, the percentage of people aged (42-62 years) was 20٪, and the percentage of people aged 62 years and above was 10%. The sex distribution among the studied groups was,58% male and 41% female in the patient group. In control group 60% were male and 40% were female. The patients' BMI categories were distributed as follows: 50.0%were classed as having a normal BMI, 50.0% were classified as overweight, and 39.4%were classified as obese. The control group consists of individuals with the following proportions: 27.6% have a normal BMI, 50.0% were overweight, and 44.8% were obese. The medical history of patients CKD was obtained by a questionnaire, revealing that around 44.8% of them were smokers, 53.6% had diabetes mellitus (DM), 72.0% had hypertension, 81.0% had a family history of chronic kidney disease (CKD), 60.6 % had heart disease and 60% engaged in regular physical activity.

Table (3-1). Demographic and Medical History Characteristics of Study Participants

Characteristics			Group	
			Control n=30	Patient n=58
Demographic characteristics	Sex	Male	18 (60.0%)	34 (58.6%)
		Female	12 (40.0%)	24 (41.4%)
	Age	30-40 years	15 (50.0%)	7 (12.1%)
		41-51 years	6 (20.0%)	8 (13.8%)
		52-62 years	6 (20.0%)	18 (31.0%)
		>62 years	3 (10.0%)	25 (43.1%)
	BMI	Normal weight	7 (23.3%)	16 (27.6%)
		Overweight	15 (50.0%)	16 (27.6%)
		Obese	8 (26.7%)	26 (44.8%)
Past Medical History	Ischemic Heart Disease	No	28 (93.3%)	40 (69.0%)
		Yes	2 (6.7%)	18 (31.0%)
	Family history of CKD	No	29 (96.7%)	41 (70.7%)
		Yes	1 (3.3%)	17 (30.5%)
	Hypertension	No	23 (76.7%)	4 (7.0%)
		Yes	7 (23.3%)	54 (93.0%)
	D.M	No	27 (90.0%)	21 (36.2%)
		Yes	3 (10.0%)	37 (63.8%)
	Smoking	No	20 (66.7%)	32 (55.2%)
		Yes	10 (33.3%)	26 (44.8%)
	Physical activity(Yes /No)	No	(30/0)	(36 /24)
		Yes	100%	(60% /40%)





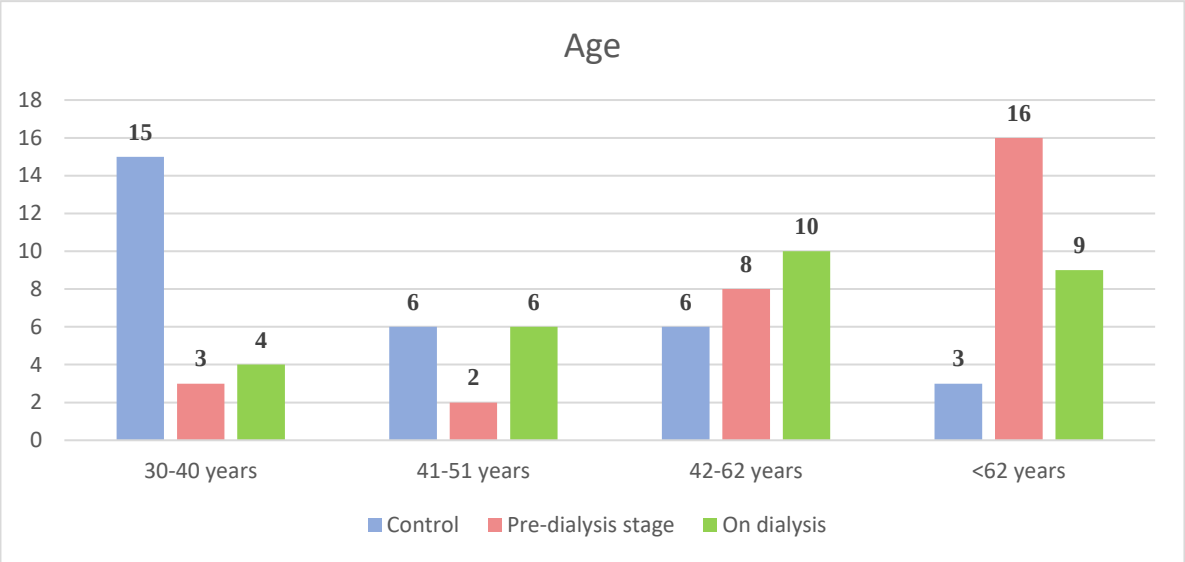


Figure (3-1): Distribution of patients and Control group according to sex, age group, BMI.

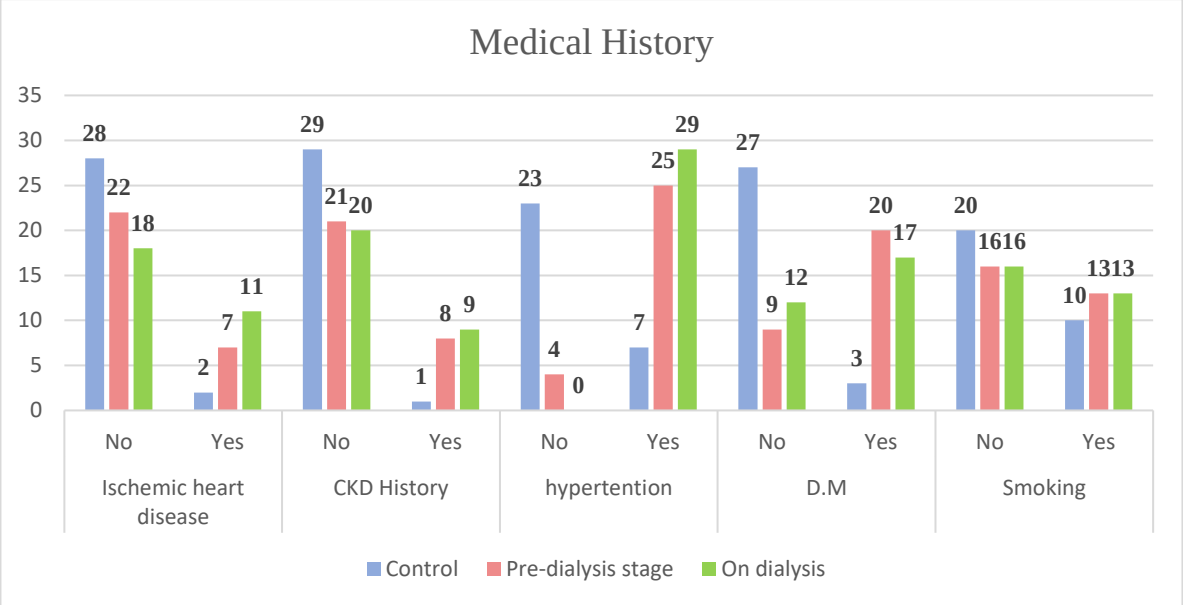


Figure (3-2): Distribution of Patients and Control Group According to Medical History of Participants.

3.2. Determination of the Serum Levels of KIM-1 and TIMP-2 in The CKD Group Compared to The Control Group

The difference in the serum level of KIM-1 between the patient CKD group and the control group was not statistically significant (p=0.066). The median (Min-Max) and IQR were 0.51 (0.17 – 1.91), 0.33for CKD group and 0.46 (0.29 – 1.58),0.13 for control group respectively.

On the other hand, patients with CKD showed a significantly elevated serum level of TIMP-2 ($p < 0.004$) compared to the control group, the median (Min-Max), IQR were 0.233(0.14 – 1.45), 0.23and 0.199 (0.14 – 1.45),0.050 respectively. as shown in table (3-2).

Table (3-2). Comparison of Study Marker levels Between Patient and control Groups.

Biomarker	Control n=30		Patients n=58		P-value
	Median (min-max)	IQR	Median (min-max)	IQR	
KIM-1ng/mL	0.46 (0.29-1.58)	0.13	0.51 (0.17-1.91)	0.33	0.066
TIMP-2ng/mL	0.199 (0.14-1.45)	0.273	0.233 (0.14 – 1.45)	0.23	0.004

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.3. Comparison of Serum Levels of KIM-1 and TIMP-2 Among Pre-Dialysis, On-Dialysis Patients, and the Control Group

When serum levels of KIM-1were compared among CKD patient subgroups (on-dialysis and pre-dialysis) with the control group, higher levels were

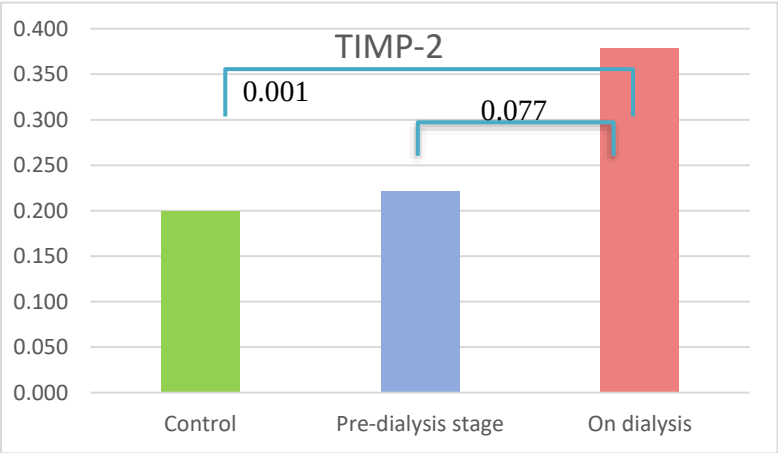
observed significantly in the on-dialysis group compared to both the control and pre-dialysis stages groups. (p-value was 0.001). This suggests that KIM-1 may be a marker elevated in patients undergoing dialysis. The median (Min-Max) and IQR were 0.689 (0.22-1.91) 0.900, 0.446 (0.17-1.45) 0.190, 0.462(0.29-1.58) 0.31 respectively.

On the other hand, a statistically significant difference in serum levels of TIMP-2 was observed when comparing CKD subgroups with the control group (p=0.001). The median (Min-Max) and IQR were 0.379 (0.14-1.57) 0.610, 0.222 (0.12-0.77) 0.260, 0.199 (0.14-1.45) 0.273 respectively, as shown in table (3-3) and figure (3-3).

Table (3-3). Comparison of Serum Levels of KIM-1 and TIMP-2 Among Pre-Dialysis, On-Dialysis Patients, and the Control Group

Marker	Control n=30 (a)		Pre-dialysis n=29 (b)		On dialysis n=29 (c)		P-value (ac)
	Median (min-max)	IQR	Median (min-max)	IQR	Median (min-max)	IQR	
KIM-1ng/mL	0.462 (0.29-1.58)	0.31	0.446 (0.17-1.45)	0.190	0.689 (0.22-1.91)	0.900	0.001
TIMP-2ng/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

Kruskal-Wallis Test was, significant at p ≤ 0.05; N: number , IQR: interquartile ranges, Min: Minimum, Max: Maximum



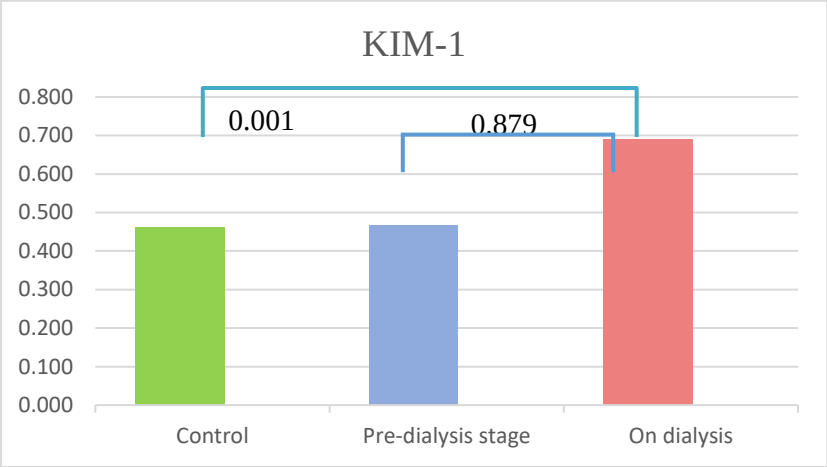


Figure (3-3): The differences in serum levels of KIM-1 and TIMP-2 among on-dialysis, pre-dialysis patient groups, and the control group. The Mann–Whitney test was considered significant at $p \leq 0.05$.

3.4. Differences in Serum KIM-1 and TIMP-2 Levels Across Stages of CKD

The study findings indicated that there was no statistically significant difference of serum levels KIM-1 (p=0.089). Suggests a trend toward statistical significance among different stages of CKD Patients, but not quite reaching it possibly due to small sample size of 2 stage and 3 stage. The median (Min-Max), IQR of KIM-1 were 0.384 (0.17-0.51) 0.26, 0.46 (0.43-0.93) 0.16, 0.414 (0.19-0.90) 0.12, 0.689 (0.22-1.91) 0.90 respectively. The Median values increase from Stage 3 to Stage5 suggesting a trend of rising KIM-1levels with disease progression. The IQR also increases, indicating more variability in severe stages. Also, there was no statistically significant difference in serum levels TIMP-2 (p=0.450) across stages of CKD Patients. The median (Min-Max), IQR of TIMP-2 were 0.173 (0.16-0.25) 0.07,0.207 (0.12-0.27) 0.07,0.225 (0.16-0.41) 0.07, 0.335 (0.14-1.57) 0.50 respectively, as shown in table (3-4**)**

Table (3-4). Differences in Serum KIM-1 and TIMP-2 Levels Across Stages of CKD

Stages									
Marker	Stage 2 n=8		Stage 3 n=7		Stage 4 n=14		Stage 5 n=29		
	Median (min-max)	IQR	Median (min-max)	IQR	Median (min-max)	IQR	Median (min-max)	IQR	P-value
KIM-1 ng/mL	0.384 (0.17-0.51)	0.26	0.461 (0.43-0.93)	0.56	0.414 (0.19-0.90)	0.32	0.689 (0.22-1.91)	0.90	0.089
TIMP-2 ng/mL	0.173 (0.16-0.25)	0.22	0.207(0.12-0.27)	0.17	0.225 (0.16-0.41)	0.27	0.335 (0.14-1.57)	0.50	0.450

Kruskal-Wallis Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

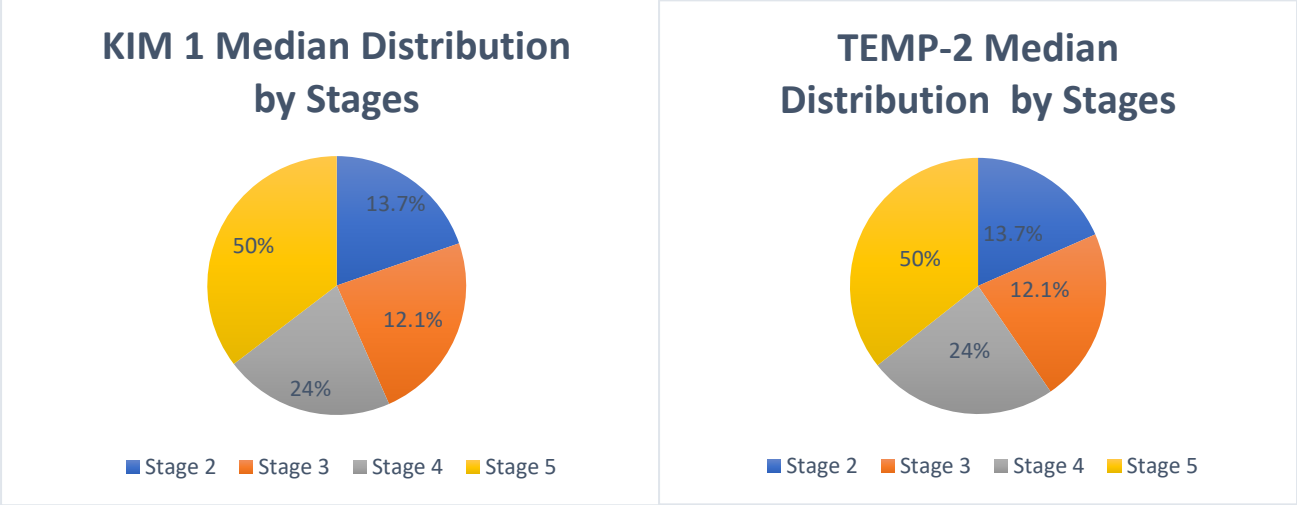


Figure (3-4): Pie Chart Showing the Stage-wise Distribution of KIM-1 and TIMP-2 in Patients with Chronic Kidney Disease According to Stage of Disease.

3.5. Differences of the Serum Levels of KIM-1 and TIMP-2 Between Male and Female in Patient with CKD

The study results showed that there was no statistically significant difference in serum levels of KIM-1and TIMP-2 between male and female patients with CKD. The median (Min-Max) of KIM-1 was 0.495(0.22-1.87) and interquartile range (IQR) (0.33) in male, while 0.533(0.17-1.91), IQR= 0.59 in female, (p=0.856)

The median (Min-Max) of TIMP-2 was 0.229 (0.12-1.57) and interquartile range (IQR) (0.28) in male while 0.245 (0.16-1.10), IQR= 0.20 in female, (p=0.449) respectively, as shown in table (3-5).

Table (3-5). Comparison of the Serum Levels of KIM-1 and TIMP-2 Between Male and Female in Patient with CKD

Sex					
Biomarker	**Male n=34**		**Female n=24**		P-value
	Median (min-max)	**IQR**	**Median (min-max)**	**IQR**	
KIM-1 ng/mL	0.495 (0.22-1.87)	0.33	0.533 (0.17-1.91)	0.59	0.856
TIMP-2 ng/mL	0.229 (0.12-1.57)	0.28	0.245 (0.16-1.10)	0.20	0.449

Mann-Whitney Test was, significant at p ≤ 0.05; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.6. Differences of the Serum levels of KIM-1 and TIMP-2 Among Age Categories in Patients with CKD.

The study results showed that there were no statistically significant differences in KIM-1 and TIMP-2 serum levels between individuals with CKD aged 30-40, 41-51 ,52-62 and those aged 62 or above (P=0.580) and (P=0.609) respectively. The median (Min-Max), (IQR) of KIM-1were 0.446 (0.22-0.72) 0.32, 0.650 (0.27-1.45) 0.42, 0.484 (0.38-1.87) 0.55, 0.553 (0.17-1.91) 0.39 respectively.

The median (Min-Max) (IQR), of TIMP-2 were 0.267 (0.17-0.77) 0.31, 0.208 (0.14-1.38) 0.22, 0.249 (0.12-1.57) 0.32, 0.235 (0.16-1.10) 0.23 respectively, as shown in table (3-6).

Table (3-6). Differences of the Serum levels of KIM-1 and TIMP-2 Among Age Categories in Patient with CKD.

Age									
Biomarker	**30-40 years n=7**		**41-51 years n=8**		**52-62 years n=18**		**>62 years n=25**		**P-value**
	Median (min-max)	**IQR**	**Median (min-max)**	**IQR**	**Median (min-max)**	**IQR**	**Median (min-max)**	**IQR**	
KIM-1 ng/mL	0.446 (0.22-0.72)	0.32	0.650 (0.27-1.45)	0.42	0.484 (0.38-1.87)	0.55	0.553 (0.17-1.91)	0.39	0.580
TIMP-2 ng/mL	0.267 (0.17-0.77)	0.31	0.208 (0.14-1.38)	0.22	0.249 (0.12-1.57)	0.32	0.235 (0.16-1.10)	0.23	0.609

Kruskal-Wallis Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.7. Differences of the serum levels of KIM-1and TIMP-2 Between BMI Categories in Patient with CKD.

The study findings indicated that there were no statistically significant difference in serum levels of KIM-1 and TIMP-2 (p=0.761) and (p=0.583) among different BMI categories (normal, overweight and obese) in patient with CKD. The median (Min-Max), IQR of KIM-1 were 0.5528 (0.22-1.45) 0.40, 0.494 (0.34-1.87)0.39,0.494 (0.17-1.91) 0.39 respectively.

The median (Min-Max), IQR of TIMP-2 were 0.207(0.14-1.38) 0.27,0.234 (0.12-1.45) 0.25, 0.243 (0.14-1.44) 0.24 respectively, As shown in table (3-7).

Table (3-7). Differences in Serum levels of KIM-1and TIMP-2 Among BMI Categories in Patient with CKD.

Biomarker	Normal weight n=16		Overweight n=16		Obese n=26		P-value
	Median (min-max)	IQR	Median (min-max)	IQR	Median (min-max)	IQR	
KIM-1 ng/mL	0.552 (0.22-1.45)	0.40	0.494 (0.34-1.87)	0.39	0.494 (0.17-1.91)	0.39	0.761
TIMP -2 ng/mL	0.207 (0.14-1.38)	0.27	0.234 (0.12-1.45)	0.25	0.243 (0.14-1.44)	0.24	0.583

Kruskal-Wallis Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.8. Differences in KIM-1and TIMP-2 Serum levels Between Smokers and Non-Smokers Patient with CKD.

The study results showed a statistically significant difference in KIM-1serum levels (p=0.011) between patients with CKD who were smoker and those who did not have smoking the median (Min-Max), IQR of Kim-1 were 0.633(0.39-1.59)0.43, 0.444(0.26-1.91)0.26 respectively.

In contrast there were no statistically significant differences in TIMP-2 serum levels (p=0.662) between CKD patients with a smoking and those without" the median (Min-Max), IQR of TIMP-2 were 0.252(0.12-1.57)0.39, 0.233(0.14-1.10) 0.18 respectively, as shown in table (3-8).

Table (3-8). Differences in Serum levels of KIM-1and TIMP-2 Between Smoker and Non-smoker Patients with CKD.

Smoking Status					
Biomarker	**Smoker n=26**		**Non-Smoker n=32**		**P-value**
	Median (min-max)	**IQR**	**Median (min-max)**	**IQR**	
KIM-1 ng/mL	0.633 (0.39-1.59)	0.43	0.444 (0.26-1.91)	0.36	0.011
TIMP-2 ng/mL	0.252 (0.12-1.57)	0.39	0.233 (0.14-1.10)	0.18	0.662

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.9. Differences in Serum KIM-1 and TIMP-2 Levels in CKD Patients With and Without Ischemic Heart Disease.

The study results showed the there was no statistically significant difference in KIM-1serum levels (p=0.449) between CKD Patients with and without ischemic heart disease. The median (Min-Max), IQR of KIM-1 were 0.519 (0.34-1.91) 1.04, 0.493 (0.17-1.59) 0.29 respectively.

In contrast there was slightly elevated in TIMP-2 serum levels (p=0.079) between CKD patients with ischemic heart disease. It Possible trend toward higher levels in ischemic heart disease, but not statistically significant. The median (Min-Max), IQR of TIMP-2 were 0.334 (0.16-1.57) 0.51, 0.229 (0.12-1.45) 0.15 respectively.

Table (3.9). Comparison of Serum Levels KIM-1 and TIMP-2 in CKD Patients With and Without Ischemic Heart Disease

Ischemic Heart disease					
Biomarker	**Ischemic Heart Disease n=18**		**Non- Ischemic Heart Disease n=40**		**P-value**
	Median (min-max)	**IQR**	**Median (min-max)**	**IQR**	
KIM-1 ng/mL	0.519 (0.34-1.91)	1.04	0.493 (0.17-1.59)	0.29	0.449
TIMP-2 ng/mL	0.334 (0.16-1.57)	0.51	0.229 (0.12-1.45)	0.15	0.079

Mann-Whitney Test was, significant at p ≤ 0.05; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.10. Differences of Serum Levels KIM-1 and TIMP-2 in Diabetic and Non-Diabetic Patients with CKD.

The study findings indicated that there was no statistically significant difference in KIM-1 serum levels (p=0.419) among Diabetic and non-Diabetic in patient with CKD. the median (Min-Max), IQR of KIM-1 were 0.518 (0.19-1.87) 0.40, 0.496 (0.17-1.91) 0.38 respectively. Conversely, there was a statistically significant difference in TIMP-2 levels (p=0.027) among Diabetic and non-Diabetic in patient with CKD, the median (Min-Max), IQR of TIMP-2 were 0.267 (0.12-1.57) 0.26, 0.205 (0.14-1.10) 0.11 respectively, as shown in table (3-10).

Table (3-10). Differences of Serum Levels KIM-1 and TIMP-2 in Diabetic and Non-Diabetic patients with CKD.

Diabetic Status					
Biomarker	**Diabetic n=37**		**Non-Diabetic n=21**		**P-value**
	Median (min-max)	**IQR**	**Median (min-max)**	**IQR**	
KIM-1 ng/mL	0.518 (0.19-1.87)	0.40	0.496 (0.17-1.91)	0.38	0.419
TIMP-2 ng/mL	0.267 (0.12-1.57)	0.26	0.205 (0.14-1.10)	0.28	0.027

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.11. Differences of Serum Levels KIM-1 and TIMP-2 between Hypertension and Non-Hypertension in Patient with CKD.

The study results showed a statistically no significant difference in KIM-1 and TIMP-2 serum levels between hypertension and non-hypertension patient with CKD (p=0.625) and (p=0.604) respectively. the median (Min-Max), IQR of KIM-1 were 0.507 (0.17-1.91) 0.43, 0.521 (0.39-0.66) 0.24 respectively. The median (Min-Max), IQR of TIMP-2 were 0.235 (0.12-1.57)0.27, 0.239 (0.17-0.27) 0.08 respectively, as shown in table (3-11)

Table (3-11). Differences in Serum Levels KIM-1 and TIMP-2 Between Hypertensive and Non-Hypertensive Patients with CKD.

Blood pressure status					
Biomarker	**Hypertensive n=54**		**Non- Hypertensive n=4**		**P-value**
	Median (min-max)	**IQR**	**Median (min-max)**	**IQR**	
KIM-1 ng/mL	0.507 (0.17-1.91)	0.43	0.521 (0.39-0.66)	0.24	0.625
TIMP-2 ng/mL	0.235 (0.12-1.57)	0.27	0.239 (0.17-0.27)	0.28	0.604

Mann-Whitney Test was, significant at $p \leq 0.05$; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.12. Comparison of Urea and Creatinine Levels Among In-Dialysis, Pre-Dialysis Groups and Control Groups in Patients with CKD.

The study showed statistically significant difference in urea and creatinine levels (p= <0.001) and (p= <0.001) in sub-groups of CKD Patients. The median (Min-Max), IQR of urea and creatinine in-dialysis, pre-dialysis and control groups were 138 (90-189) 22.5, 114 (34-389) 72.5, 35 (20-44) 12.7 and 8.3 (5-14) 2.55, 2.7 (0.70-12.0) 1.55, 0.75 (0.40-1.10) 0.37 respectively. Urea levels are significantly higher in both pre-dialysis and dialysis groups compared to the control group .Creatinine levels increase progressively from control to pre-dialysis to dialysis.

Table (3-12). Comparison of Renal Functions Tests According to Study Groups

Tests	Control n=30		Pre-dialysis n=29		On dialysis n=29		P-value
	Median (min-max)	IQR	Median (min-max)	IQR	Median (min-max)	IQR	
eGFR (mL/min/1.73 m²)	-	-	26 (38-83.3)	29.95	6 (4-11)	2.55	<0.001
Urea (mg/dL)	35 (20-44)	12.75	114 (34-389)	72.5	138 (90-189)	22.5	<0.001
Creatinine (mg/dL)	0.75 (0.40-1.10)	0.37	2.7 (0.70-12.0)	1.55	8.3 (5-14)	2.55	<0.001

Kruskal Test, Mann-Whitney Test was, significant at p ≤ 0.05; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

3.13. Comparison of Electrolyte and Vitamin D3 Parathyroid Hormone levels Among Study Groups.

The study showed there are significant differences among study sub-groups according to all tests (Na, K, Ca, vit D3 and PTH) all (p=<0.001). the median (Min-Max), IQR of all tests (Na, K, Ca, vit D3 and PTH) in-dialysis, pre-dialysis and control groups. For Na 140 (130-146) 6.5, 135 (123-149) 7, 147.5(135-155) 6.25 for K 5.2 (3.39-7.3) 1.3,4.11 (2.7-5.9) 1.2, 4.55 (3.8-5.2) 0.70 for Ca 7.9 (5.1-9.7) 1.9, 7 (3.7-8.3) 1.8, 9.45 (7.7-10.5) 1.05 for Vit D3 17 (6.0-33.0) 7.7, 7.9 (1.5-22.0) 5.8, 48.5 (4.9-79.0), 1.05 for PTH 355 (104-949) 293.5, 95 (23.5-285.2) 80.2. respectively, as shown in table (3-13).

Table (3-13). Comparison of Electrolytes and Vitamin D3 levels tests Among Across Study Groups

Tests	Control n=30		Pre-dialysis n=29		On dialysis n=29		P-value
	Median (min-max)	IQR	Median (min-max)	IQR	Median (min-max)	IQR	
Na mmol/L	147.5 (135-155)	6.25	135 (123-149)	7	140 (130-146)	6.5	<0.001
K mmol/L	4.55 (3.8-5.2)	0.70	4.11 (2.7-5.9)	1.2	5.2 (3.39-7.3)	1.3	<0.001
Ca mg/dL	9.45 (7.7-10.5)	1.05	7 (3.7-8.3)	1.8	7.9 (5.1-9.7)	1.9	<0.001
Vit D3 (ng/mL)	48.5 (4.9-79.0)	32.6	7.9 (1.5-22.0)	5.8	17 (6.0-33.0)	7.7	<0.001
PTH (pg/mL)	46 (38-99)	14.3	95 (23.5-285.2)	80.2	355 (104-949)	293.5	<0.001

Kruskal-Wallis Test was, significant at p ≤ 0.05; N: number; IQR: interquartile range, Min: Minimum, Max: Maximum

Note: Normal reference ranges: Na: 136–146 mmol/L, K: 3.5–5 mmol/L, Ca: 8.6–10.3 mg/dL, Vit D3: ≥30 ng/ml, PTH: 10–65 pg/ml.

3.14. Correlation Between KIM-1 and TIMP-2 Levels in Patients with Chronic Kidney Disease (CKD)

The study showed significant, and moderate positive correlation between KIM-1 and TIMP-2 (p=<0.001) in patients with CKD. Correlation Coefficient was (0.411).

Table (3-14). Correlation Coefficients of KIM-1 with TIMP-2 in Patients with CKD

Biomarker	r	P-value
(KIM-1) - (TIMP-2) ng/mL	0.411	<0.001

Spearman Rank Test; *Correlation is significant at the 0.05 level; + = positive ; r: pearson correlation coefficients

3.15. Correlation of KIM-1 and TIMP-2 with Renal Function Parameters and Electrolyte, Vitamin D3 and Parathyroid Hormone in Patients with Chronic Kidney Disease (CKD)

In the current study, both biomarkers KIM-1 and TIMP-2 are significantly and negative correlation with estimated glomerular filtration rate (eGFR) ($r = -0.439$, $p = 0.001$), ($r = -0.418$, $p = 0.001$).

On the other hand, both biomarkers KIM-1 and TIMP-2 are significantly and positive correlation with creatinine levels ($p=<0.001$). KIM-1 and TIMP-2 showed non-significant Positive correlation with Urea ($p=0.355$), ($p=0.287$).

KIM-1 demonstrated non-significant negative correlation with Na ($p=0.307$) and non-significant positive correlation with K ($p=0.510$), and non-significant negative correlation with Ca and vit D3. ($p=0.309$) and ($p=0.118$) respectively.

TIMP-2 demonstrated non-significant negative correlation with Na ($p=0.313$) and non-significant positively correlation with K, Ca and vit D3. ($p=0.383$), ($p=0.295$) and ($p=0.623$) respectively.

KIM-1 demonstrated non-significant positively correlation with PTH ($p=0.227$), and showed statistically significant negative correlations with eGFR ($p=0.001$).

TIMP-2 demonstrated statistically significant positive correlations with PTH ($p=0.020$), also showed significant negative correlations with eGFR ($p=0.001$). as show in table (3-15).

Table (3-15). Correlation of KIM-1 and TIMP-2 with Renal Parameters and Electrolyte, Vitamin D3 and Parathyroid Hormone in Patients (CKD)

Tests	KIM-1	P-value	TIMP-2	P-value
eGFR (mL/min/1.73 m²)	-0.439	0.001	-0.418	0.001
Urea (mg/dL)	0.100	0.355	0.115	0.287
Creatinine (mg/dL)	0.375	<0.001	0.400	<0.001
Na mmol/L	-0.110	0.307	-0.109	0.313
K mmol/L	0.071	0.510	0.094	0.383
Ca mg/dL	-0.110	0.309	-0.113	0.295
D3 (ng/mL)	-0.168	0.118	-0.053	0.623
PTH (pg/mL)	0.161	0.227	0.305	0.020

Spearman Rank Test; *Correlation is significant at the 0.05 level; - = negative; r: pearson correlation coefficients

3.17. The Odd Ratio of KIM-1 and TIMP-2 in Patients with CKD.

The odd ratio for KIM-1 in relation to CKD presence was not statistically significant (OR= 1.625, 95% CI: 0.948- 27.207, P=0.058), indicating association between KIM-1 and CKD presence in the studied population.

The odd ratio for TIMP-2 in relation to CKD presence was not statistically significant (OR= 1.907, 95% CI: 0.741- 61.233, P=0.090), indicating a positive association between the exposure and elevated TIMP-2 levels. Patients appear more likely to have elevated TIMP-2, but the association is modest.as shown in table (3-17).

Table (3 -17). The Odd Ratio of KIM-1 and TIMP-2 in Patients With CKD.

Biomarker	Odds Ratio	CI 95%		P-value
		Lower	Upper	
KIM-1 ng/mL	1.625	0.948	27.207	0.058
TIMP-2 ng/mL	1.907	0.741	61.233	0.090

OR: Odds Ratio, CI; Confidence Interval,

3.18. Receiver Operating Characteristic (ROC) .

3.18.1. ROC Curve and AUC Analysis of the KIM-1 in Pre - dialysis CKD Patients Compared to the Control Group.

ROC curve and AUC analyses were conducted to evaluate the diagnostic performance of KIM-1 in distinguishing pre-dialysis CKD patients from healthy controls, data are presented in Figure (3-5) and table (3-18). The results demonstrated that KIM-1 had no significant discriminative ability in this context. AUC =0.489 , Sensitivity% =52%, Specificity% = 44%, Cut-off points = 0.45 (ng/L).

Table (3-18). AUC, Optimal Cut-off, Sensitivity, and Specificity of KIM-1 Based on ROC Analysis in Pre-dialysis CKD Patients Compared to Controls Group

Groups	AUC	P-value	Cut off	Sensitivity	Specificity
Control – Pre dialysis	0.489	0.897	≥ 0.45 ng/L	0.52	0.44

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

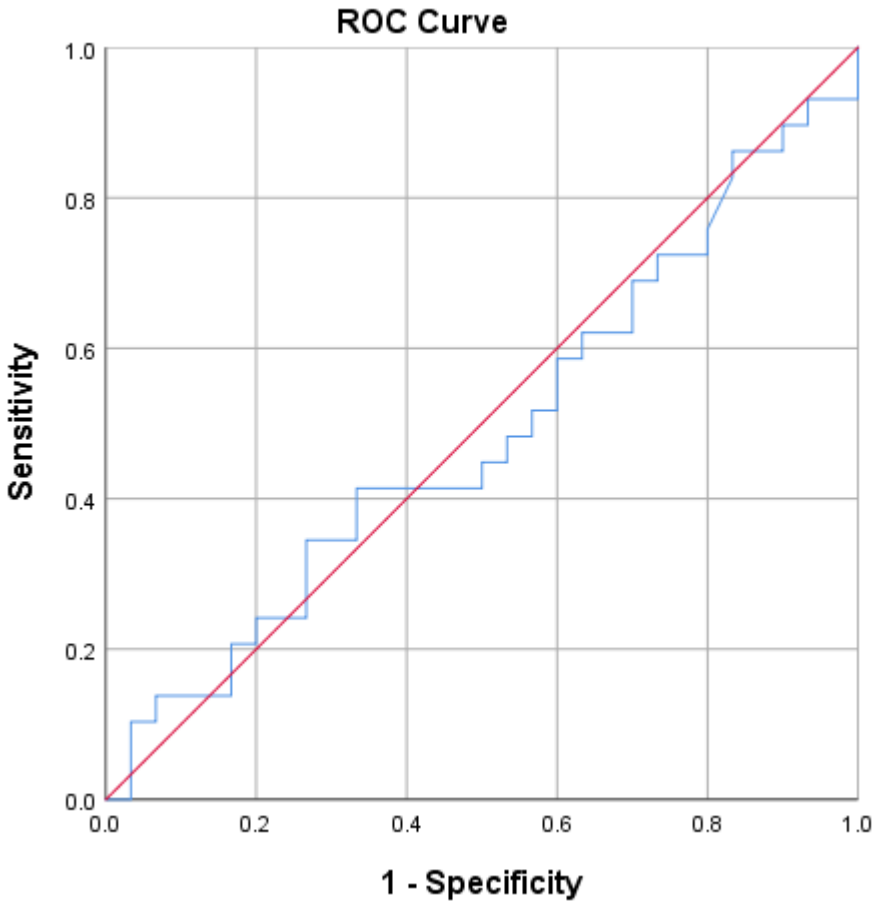


Figure (3-5): Receiver Operating Characteristic(ROC) of KIM-1 in Pre-dialysis CKD Patients Compared to Control Group .

3.18.2. ROC Curve and AUC Analysis for the KIM-1 for CKD patients On-dialysis Group Compared to the Control Group.

ROC curve and AUC analysis for the KIM-1 for On-dialysis CKD patients group compared to control group were performed. Results of the receiver operating curve (ROC) and AUC analysis for the KIM-1 diagnostic parameter showed that KIM-1 have a fair performance On-dialysis CKD patients group, data are presented in Figure (3-6) and table (3-19), KIM-1 had AUC =0.751, Sensitivity% = 79%, Specificity% = 67%, Cut-off points = 0.48 (ng/L).

Table (3-19). AUC, Optimal Cut-off, Sensitivity, and Specificity of KIM-1 Based on ROC Analysis In-dialysis CKD Patients compared to The Controls group

Groups	AUC	P-value	Cut off	Sensitivity	Specificity
Control vs. on-dialysis	0.751	0.001	≥ 0.48 ng/L	0.79	0.67

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

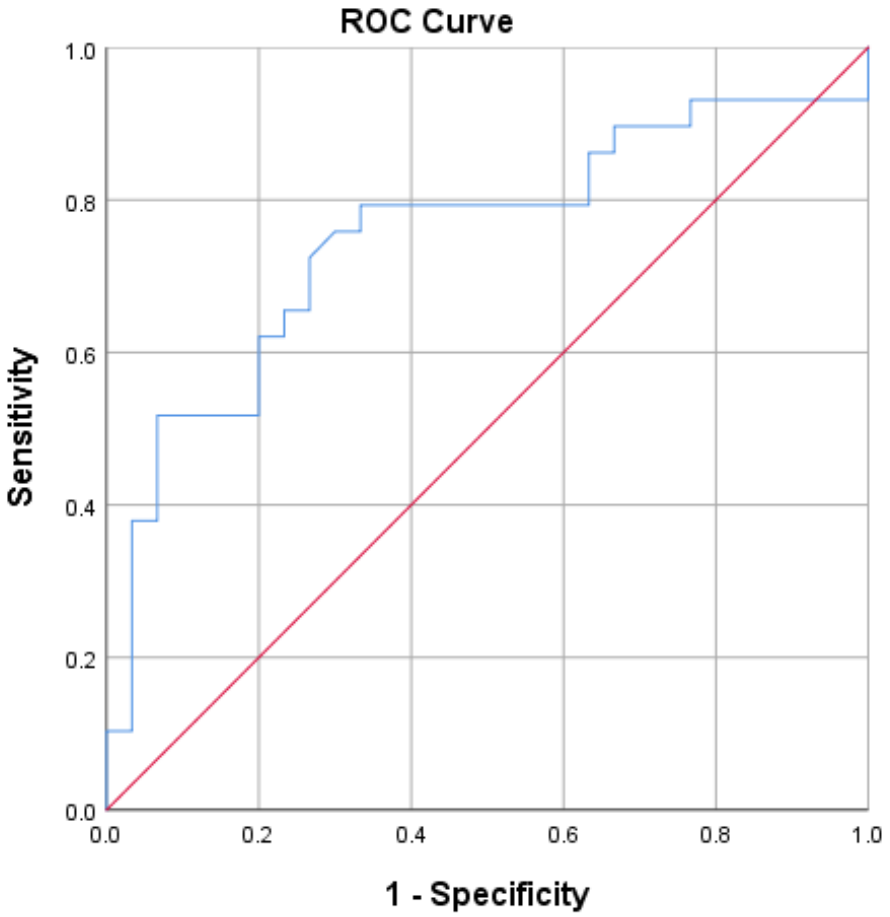


Figure (3-6): Receiver Operating Characteristic (ROC) of KIM-1 for on-dialysis CKD patients Compared to Control Group.

3.18.3. AUC, Optimal Cut-off, Sensitivity, and Specificity of KIM-1 for Pre-dialysis Compared to On-dialysis CKD Patients Based on ROC Analysis

KIM-1 demonstrates fair diagnostic performance in distinguishing pre-dialysis from on-dialysis CKD patients, with moderate sensitivity and weak specificity, data are presented in Figure (3-7) and table (3-20), KIM-1 had AUC =0.743, Sensitivity% =79%, Specificity% = 57%, Cut-off points = 0.48 (ng/L).

Table (3-20). AUC, Optimal Cut-off, Sensitivity, and Specificity of KIM-1 for Pre-dialysis Compared to On-dialysis CKD Patients Based on ROC Analysis

Groups	AUC	P-value	Cut off	Sensitivity	Specificity
Pre -In-dialysis	0.743	0.001	≥ 0.48 ng/L	0.79	0.57

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

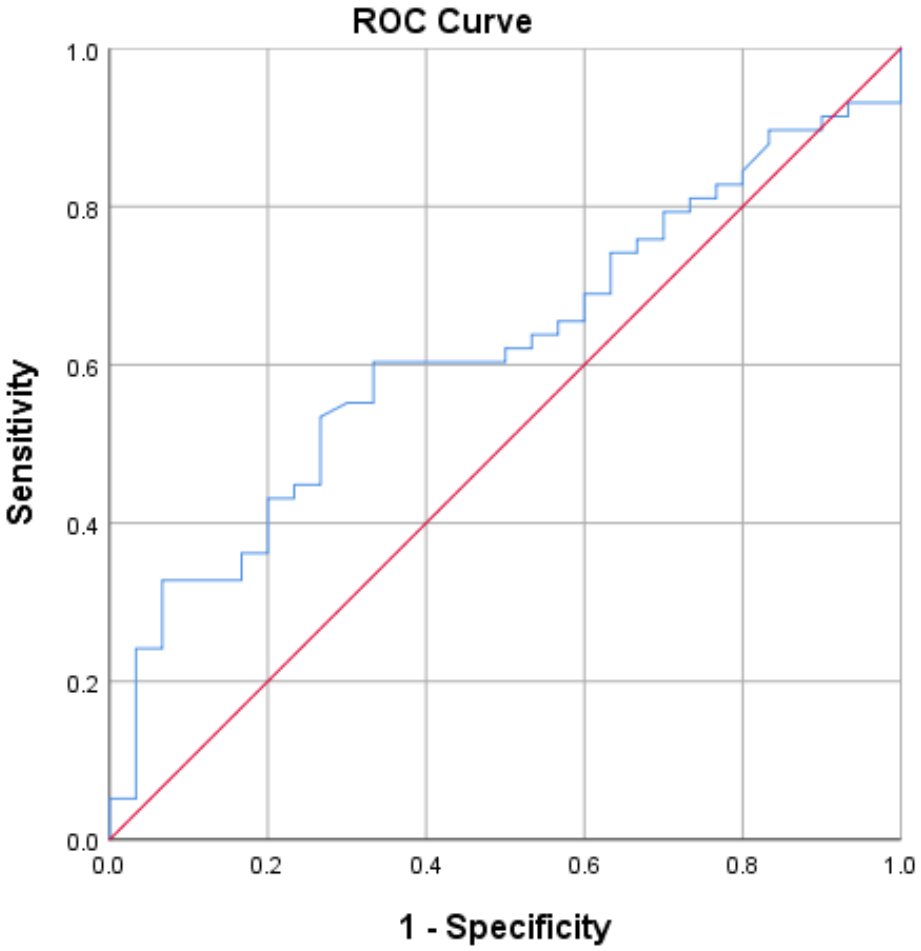


Figure (3-7): Receiver Operating characteristic (ROC) Analysis of KIM-1 for Pre-dialysis and On-dialysis CKD Patients.

3.20.1 ROC Curve and AUC Analysis of TIMP-2 in Pre -dialysis CKD Patients Compared to the Control Group.

ROC Curve and AUC Analysis for TIMP-2 showed a ability to differentiate between Pre -dialysis CKD patients and control Group (AUC = 0.634) However, the discrimination is not statistically significant (p = 0.077). Data are presented in Figure (3-8) and table (3-22). TIMP-2 had AUC =0.634, Sensitivity% =65%, Specificity% = 60%, Cut-off points = 0.207 (ng/L).

Table (3-22). AUC, Optimal Cut-off, Sensitivity, and Specificity of TIMP-2 Based on ROC Analysis in Pre-dialysis CKD Patients Compared to Controls Group.

Groups	AUC	P-value	Cut off	Sensitivity	Specificity
Control – Pre dialysis	0.634	0.077	≥ 0.207 ng/L	0.65	0.60

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

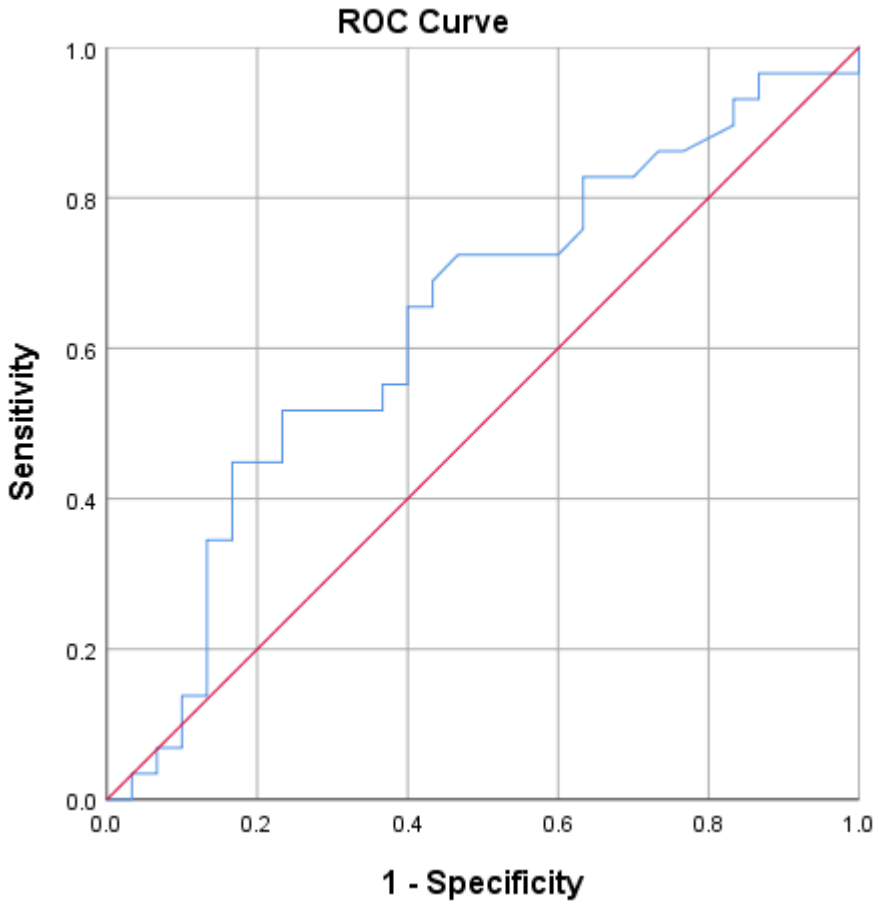


Figure (3-8): Receiver Operating Characteristic (ROC) of TIMP-2 in Pre-dialysis CKD Patients Compared to Control Group.

3.20.2. ROC Curve and AUC Analysis of TIMP-2 On-dialysis CKD patients Group Compared to the Control group.

ROC curve and AUC analysis of the TIMP-2 for on-dialysis CKD patients group compared to control group were performed. The results of the receiver operating curve (ROC) and AUC analysis of the TIMP-2 demonstrated fair discriminatory performance in distinguishing of on-dialysis CKD patients group from Control, The result was statistically significant (p=0.002), the data

are presented in Figure (3-9) and table (3-23), TIMP-2 had AUC =0.715, Sensitivity% =76%, Specificity% = 60%, Cut-off points = 0.208 (ng/L).

Table (3-23). AUC, Optimal Cut-off, Sensitivity, and Specificity of TIMP-2 Based on ROC Analysis On-dialysis CKD Patients Compared to Controls Group.

Groups	AUC	P-value	Cut off	Sensitivity	Specificity
Control–In-dialysis	0.715	0.002	≥ 0.208 ng/L	0.76	0.60

ROC: Receiver operating characteristic; significant at p ≤ 0.05; AUC; Area under curve, P-value.

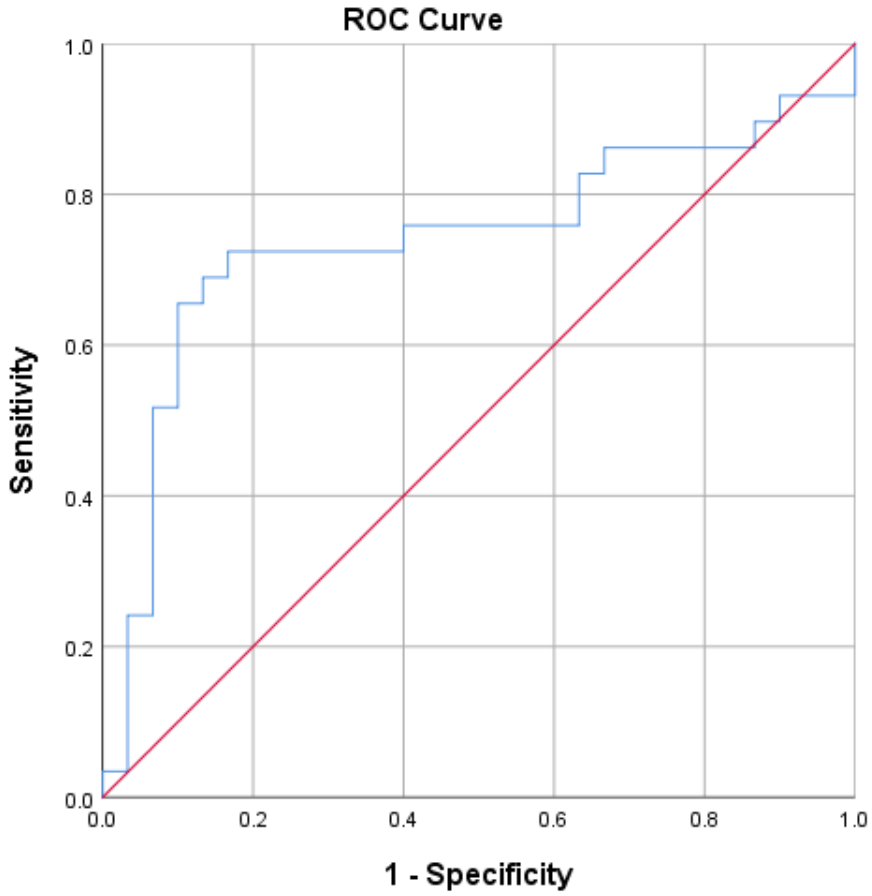


Figure (3-9): Receiver Operating Characteristic (ROC) of TIMP-2 On-dialysis Patients compared to Control Group.

3.20.3. AUC, Optimal Cut-off, Sensitivity, and Specificity of TIMP-2 in Pre-dialysis Compare to On-dialysis CKD Patients Based on ROC Analysis

ROC curve and AUC analysis for the TIMP-2 showed the diagnostic performance of TIMP-2 in distinguishing pre-dialysis CKD patients from on-dialysis CKD patients. TIMP-2 shows fair accuracy (AUC = 0.715) and statistically significant discrimination (p = 0.005) between pre-dialysis and on-dialysis CKD patients. with high sensitivity and moderate specificity. Data are presented in Figure (3-10) and table (3-24), TIMP-2 had AUC =0.715, Sensitivity% =71%, Specificity% = 71%, Cut-off points = 0.230 (ng/L).

Table (3-24). AUC, Optimal Cut-off, Sensitivity, and Specificity of TIMP-2 Based on ROC Analysis.

Groups	AUC	P-value	Cut off	Sensitivity	Specificity
Pre -In-dialysis	0.715	0.005	0.230 ng/L	0.71	0.63

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

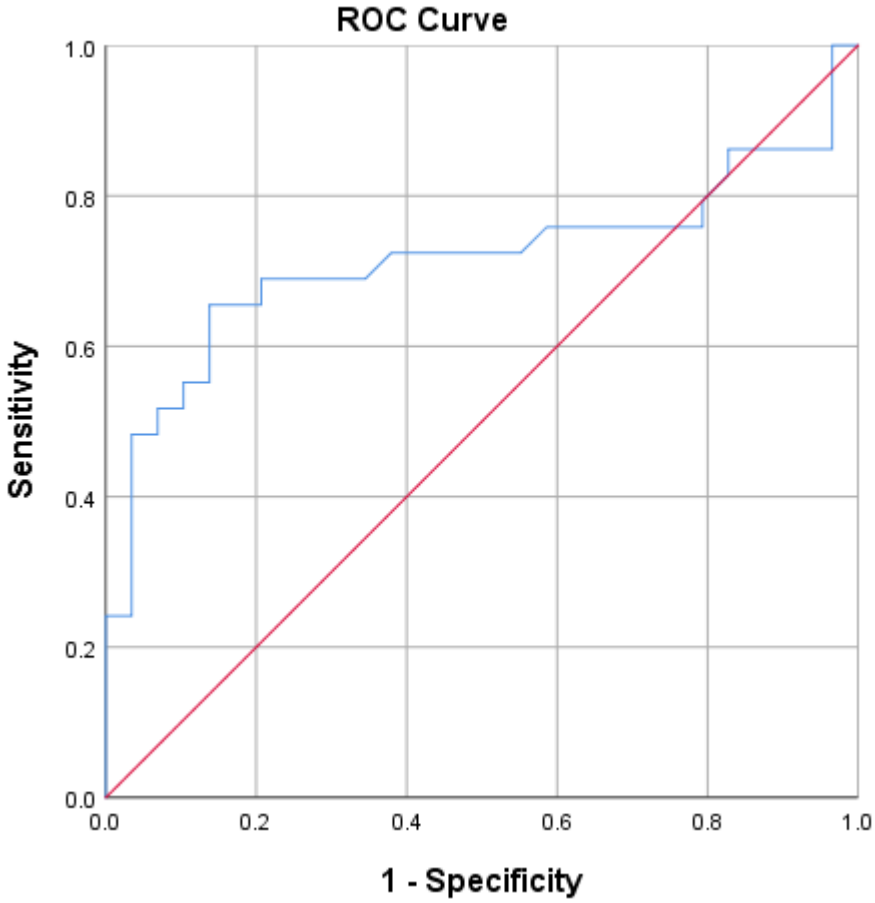


Figure (3-10): Receiver Operating Characteristic (ROC) of TIMP-2 On-dialysis Patients Compared to Pre-dialysis Patients Group.

Chapter four
Discussion

4.1. Demographics and Clinical Characteristics

In the current study, the percentage of male participants was slightly higher than that of females, as shown in Tables 3–1. While sex-based differences were not statistically significant for the biomarkers assessed (KIM-1 and TIMP-2), the role of sex in chronic kidney disease (CKD) remains a significant consideration in the broader literature.

Sex differences affect CKD prevalence, progression, and outcomes. Although CKD is more common in women, men typically show faster progression and worse prognosis **(Carrero et al., 2018).** This may relate to sex hormones—estrogens have protective anti-inflammatory effects, while androgens may promote renal injury **(Neugarten et al., 2019).** Moreover, recent findings identified 23 sex-specific genetic variants associated with kidney function, highlighting hormonal influences on gene expression in CKD **(Scholz et al., 2024).**

The age distribution of CKD patients in this study showed that 7 (12.1%) were aged 30–40 years, 8 (13.8%) were 41–51 years, 18 (31.0%) were 52–62 years, and 25 (43.1%) were older than 62 years. Thus, the majority of patients were above 51 years of age. This pattern highlights the strong association between CKD and advancing age, consistent with the Global Burden of Disease Study. This result is consistent with a large body of research, such as the Global Burden of Disease Study, which showed that aging is a substantial and independent risk factor for the onset and advancement of CKD, Reduced GFR, nephron loss, and decreased perfusion are examples of age-related declines in kidney function that can lead to chronic kidney disease **(Lu et al., 2024).**

In the current study, only 27.6% of CKD patients had a normal BMI, while 27.6% were overweight and 44.8% were obese. Thus, the majority of patients had excess body weight, supporting the established link between adiposity and obesity-related glomerulopathy through mechanisms such as hyperfiltration and glomerulosclerosis." even in the absence of other comorbidities. Across all age groups, obesity is independently linked to higher albuminuria and lower eGFR, according to a large cohort study **(Garofalo et al., 2023).** Obesity also raises the risk of CKD and End-Stage Renal Disease **(**ESRD**)** progression significantly, according to a meta-analysis of more than 5 million people **(Wang et al., 2022).**

Additionally, in the present study, 63.8% of the patients were diagnosed with type 2 diabetes, while 93% had hypertension. These findings are consistent with existing literature identifying both conditions as the leading modifiable risk factors for chronic kidney disease (CKD).

Type 2 diabetes is the most common cause of CKD globally. Persistent hyperglycemia leads to glomerular hyperfiltration, thickening of the glomerular basement membrane, and mesangial expansion, culminating in diabetic kidney disease (DKD). A 2022 global analysis estimated that over 40% of individuals with type 2 diabetes will develop some form of CKD during their lifetime **(Thomas et al., 2022).** Moreover, patients with both diabetes and CKD are at significantly greater risk of cardiovascular complications and mortality, underscoring the importance of early detection and glycemic control.

Hypertension is a major contributor to the development and progression of CKD. It causes vascular damage, leading to nephron loss and reduced renal function. Findings from the Atherosclerosis Risk in Communities (ARIC)

study showed that individuals with baseline hypertension had a significantly higher risk of incident CKD and experienced a more rapid decline in eGFR over a 30-year follow-up period **(Ballew et al., 2021).**

The coexistence of obesity, diabetes, and hypertension in a large proportion of patients in this study illustrates the complex, interconnected nature of CKD risk factors. These findings support the need for integrated management approaches that target multiple cardiometabolic pathways to prevent or slow CKD progression.

Over one-third of the patients in the current study reported not working out regularly. As physical inactivity is a known modifiable risk factor for the development of chronic kidney disease (CKD), as well as for the disease's progression and related cardiovascular morbidity, this conclusion is particularly important. Kidney failure is a result of poor metabolic profiles, including insulin resistance, obesity, hypertension, and systemic inflammation, which have all been linked to sedentary behavior. Several recent studies have emphasized the protective role of physical activity in kidney health. A 2022 meta-analysis by Zhang et al. found that individuals with higher levels of physical activity had a significantly lower risk of incident CKD and slower eGFR decline. Moreover, regular physical exercise has been associated with improved blood pressure control, enhanced glycemic regulation, and reduced albuminuria in patients with early-stage CKD **(Sharma et al., 2023).**

4.2. Kidney Injury Molecule-1 (KIM-1)

In current study, KIM-1 demonstrated limited utility in distinguishing between healthy individuals and pre-dialysis CKD patients, with poor sensitivity and specificity. These results suggest that while KIM-1 may be elevated in the presence of kidney injury, its discriminatory power in the early stages of CKD is inadequate. These findings are consistent with prior evidence suggesting that KIM-1 performs poorly as an early marker of CKD despite its elevation in kidney injury **(Almulhim, 2025).**

Even though tubular injury raises KIM-1 levels, new research indicates that KIM-1 might not be sensitive enough to detect CKD in its early stages. Combining KIM-1 with additional tubular stress biomarkers may increase diagnostic accuracy **(Kaddah et al., 2024; Clinica Chimica Acta, 2024).**

The inability of KIM-1 to reliably differentiate early CKD stages from healthy states may stem from its expression being more reflective of tubular injury rather than overall kidney function decline. Since early CKD may involve minimal or heterogeneous tubular damage, KIM-1 levels may not consistently rise until later stages or during acute episodes.

The results of the current study showed statistically significant difference in the concentration of KIM-1 on dialysis patients compared to pre-dialysis stages and control group, as shown in Table (3-3). although the overall difference in KIM-1 serum levels between CKD patients and controls did not reach statistical significance. A clear elevation trend was observed. Importantly, KIM-1 levels were significantly higher on dialysis patients compared to pre-dialysis stages and control groups, suggesting that KIM-1

may be a marker of advanced tubular injury in end-stage renal disease (ESRD).

KIM-1 is a sensitive indicator of tubular cell injury in the kidneys. It is not specific to one single cause, but rather it increases in many types of kidney injury—whether caused by reduced blood flow (ischemia), toxic substances (such as certain drugs), or inflammation. This makes KIM-1 a reliable biomarker for detecting ongoing damage in the kidney tubules, regardless of the underlying cause. KIM-1 is upregulated in response to various types of tubular injury—including ischemic, toxic, and inflammatory damage—and is now recognized as a reliable biomarker for both acute and chronic tubular pathologies **(Tang et al., 2025).**

The current study found that in patients with chronic kidney disease, particularly those with diabetic nephropathy, KIM-1 levels are associated with an increased risk of developing end-stage kidney disease (ESKD). KIM-1 may be a useful biomarker for evaluating tubular damage and forecasting unfavorable renal outcomes, according to this **(Sarnak et al., 2022).**

Recent research has shown that serum KIM-1 (sKIM-1) may be useful as a biomarker for prognosis in chronic kidney disease. Increased plasma KIM-1 levels were found to be independently linked to tubulointerstitial inflammation, fibrosis, and mesangial expansion in both the Boston Kidney Biopsy Cohort and the CRIC Study. Furthermore, even after controlling for baseline kidney function, higher sKIM-1 concentrations were associated with a significantly higher risk of developing end-stage kidney disease (ESKD) **(Waikar et al., 2022).**

The current study observed no statistically significant differences in KIM-1 between age groups, genders and BMI in patients. As indicated in the tables (3-5) (3-6). (3-7). KIM-1 is a well-known indicator of proximal tubular injury, but according to a number of studies, its level is not significantly impacted by demographic factors like age, gender, or body mass index (BMI). Sex had no effect on KIM-1 baseline levels or diagnostic performance in patients with renal injury, according to **Han et al. (2020).** Similarly, age has no effect on the expression of the biomarker, as demonstrated by **Ratliff et al. (2021),** who found no significant correlation between age and serum KIM-1 levels at different stages of CKD. In addition, **Wang et al. (2021)** discovered that, after controlling for renal function, KIM-1 levels did not independently correlate with BMI, despite the fact that obesity is associated with glomerular hyperfiltration and albuminuria. These findings collectively indicate that KIM-1 levels reflect kidney injury rather than patient demographic characteristics, and that its diagnostic utility may not be influenced by age, gender, or obesity. The lack of significant association between KIM-1 levels and demographic variables such as age, sex, and BMI can be attributed to its biological origin and regulation. KIM-1 is not a product of systemic metabolism, but a stress-induced protein specifically upregulated in injured proximal tubular epithelial cells. Unlike serum creatinine, which is influenced by muscle mass and thus varies with age, sex, and body size, KIM-1 reflects localized epithelial injury regardless of external physiological characteristics. This mechanistic specificity allows it to serve as a demographically neutral biomarker, with its levels determined primarily by the extent of renal tubular damage rather than patient profile.

According to the current study, smokers with CKD had significantly higher serum KIM-1 levels than non-smokers according to the table **(3–8),** These results are consistent with earlier research showing elevated KIM-1 levels in smokers' serum and urine, particularly in those with underlying kidney dysfunction **(Yacoub et al., 2010; He et al., 2021; Oliveira et al., 2022).** It has been demonstrated that the harmful substances found in cigarette smoke, such as nicotine, cadmium, and reactive oxygen species, directly damage tubular epithelial cells, which raises KIM-1 expression as a result of the injury response.

KIM-1 levels among patients with ischemic heart disease did not differ statistically significantly, according to the current study. **as shown in the tables (3–9).** This conclusion is corroborated by a recent study by **Sakaguchi et al. (2024),** which found that neither cardiovascular events nor left ventricular dysfunction were significantly correlated with circulating KIM-1 levels in hemodialysis patients. This suggests that KIM-1 has limited utility as a cardiac-specific biomarker in this population.

The results of the study showed that there was no statistically significant difference in serum KIM-1 levels between CKD patients with diabetes and those without diabetes **as shown in Table (3-10).** This implies that even though diabetes is a known risk factor for the development of CKD, once CKD is established, elevated levels of KIM-1 in the bloodstream are not always the consequence of diabetes. Because of a number of common pathological processes, including ischemia, oxidative stress, and inflammation, both diabetic and non-diabetic patients may exhibit active tubular epithelial injury, which is specifically reflected by the biomarker KIM-1.

The current study's lack of a statistically significant difference in serum KIM-1 levels between CKD patients with diabetes and those without is in line with earlier findings. In their study of KIM-1 and TNF receptor levels in patients with type 2 diabetes and normal albuminuria, for example, **Heerspink et al. (2025)** discovered that elevated KIM-1 levels were not substantially linked to the advancement of CKD. This supports the idea that, even though KIM-1 might indicate ongoing tubular damage, it might not be enough to distinguish between the progression of CKD based only on diabetic status. According to these results, the degree of renal tubular damage was probably similar in the diabetic and non-diabetic CKD patients in this study, and KIM-1 expression in the blood indicates the severity of tubular injury rather than its cause. Consequently, the lack of a significant difference highlights KIM-1's function as a general indicator of tubular pathology rather than one unique to diabetes, without diminishing its clinical significance.

As shown in Table (3-11), the current study did not find any statistically significant differences in KIM-1 levels between CKD patients with hypertensive and without hypertensive. These results are in line with those of **Sîrcuța et al. (2024),** who looked at plasma KIM-1 levels in hemodialysis patients. Their research revealed a significant correlation between KIM-1 levels and anemia and inflammation markers, but not with the presence of hypertension. This finding can be explained by the distinct pathophysiological mechanisms underlying tubular injury versus glomerular damage. KIM-1 is a transmembrane protein specifically upregulated in proximal tubular epithelial cells in response to direct tubular injury, such as ischemia, toxin exposure, or inflammation. In contrast, hypertension primarily affects glomerular capillaries and vascular structures in the early

stages, rather than inducing immediate tubular epithelial damage. Therefore, unless hypertension leads to chronic, severe ischemic injury extending to the tubular compartment, it may not elicit a marked increase in KIM-1 expression. Previous studies have similarly reported that KIM-1 elevation is more closely associated with tubulointerstitial pathology rather than systemic hemodynamic disturbances alone (**Sabbisetti et al., 2014; Vaidya & Aeddula, 2023).** This supports the interpretation that KIM-1 reflects a specific pattern of localized renal injury, which may not be uniformly present in all hypertensive CKD patients.

In the present study, serum KIM-1 demonstrated a moderate positive correlation with serum creatinine and a moderate negative correlation with estimated glomerular filtration rate (eGFR), while its correlation with serum urea was weak and not statistically significant. These relationships are consistent with the biological role of KIM-1 as a sensitive indicator of proximal tubular injury. As kidney function deteriorates, the decline in filtration capacity leads to accumulation of metabolic waste, increased oxidative stress, and activation of inflammatory pathways, all of which contribute to tubular cell damage and subsequent upregulation of KIM-1 expression. This aligns with findings from **Parikh et al. (2023)**, who reported that elevated KIM-1 levels were predictive of eGFR decline and offered superior sensitivity to some traditional renal markers, particularly in identifying early tubular damage prior to overt changes in creatinine.

Serum KIM-1 levels in this study did not correlate statistically significantly with a number of biochemical markers that are frequently changed in chronic kidney disease. More specifically: sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), Parathyroid hormone (PTH): vitamin D3. in accordance with the

tables (3-15). According to these findings, KIM-1 does not correlate with serum electrolyte or vitamin D levels, nor does it represent secondary hyperparathyroidism or dysregulation of mineral metabolism in CKD patients.

The results of the current study on KIM-1 are corroborated by Suresh et al. (2025), who found that KIM-1 levels did not correlate with vitamin D status or serum electrolyte levels, nor did they indicate secondary hyperparathyroidism or abnormalities in mineral metabolism in CKD patients. This supports the idea that tubular injury, not systemic metabolic dysregulation, is the primary cause of KIM-1. Thus, although KIM-1 is a valuable biomarker for kidney damage detection, it might not be appropriate for evaluating CKD-related mineral bone disease (CKD-MBD**) (Suresh et al., 2025).**

According to **Sabbisetti et al. (2014) and Han et al. (2022),** KIM-1 levels have a strong correlation with glomerular filtration rate and tubular injury markers, but not with systemic metabolic disorders. These findings lend credence to this interpretation. According to their research, KIM-1 is a localized biomarker that is driven by injury and is not influenced by systemic biochemical changes that are frequently seen in CKD, especially those associated with endocrine adaptations and calcium-phosphate imbalance. Furthermore, **Cheng et al. (2020)** stressed that although PTH and vitamin D3 are closely associated with CKD-MBD (Mineral and Bone Disorder), they do not directly trigger the expression of KIM-1 unless there is obvious tubular cell damage.

These findings together reinforce the specificity of KIM-1 as a tubular injury biomarker rather than a reflection of systemic biochemical derangement. Its

lack of correlation with sodium, potassium, calcium, PTH, and vitamin D3 strengthens its role as a structural, not metabolic, indicator of nephron integrity.

KIM-1 is a marker of kidney tubular injury, but not a marker of the underlying cause of the injury. Therefore, the etiological factors leading to CKD such as diabetes, hypertension, or nephrotoxicity do not directly affect KIM-1 levels. Rather, its elevation reflects the presence and extent of tubular damage regardless of the cause.

4.3. Tissue Inhibitor of Metalloproteinase-2

In the current study, serum TIMP-2 levels were significantly higher in CKD patients compared to healthy controls, As indicated in the tables (3-2). This result underscores the utility of TIMP-2 as a sensitive marker of tubular epithelial stress and subclinical injury, particularly in chronic kidney disease. This finding aligns with those of **Yang et al. (2021) and Zhou et al. (2020),** who reported that TIMP-2 levels were significantly increased in both serum and urine of CKD patients compared to controls, particularly in those with stage 3 or higher. Yang et al. emphasized that TIMP-2 elevations are strongly associated with renal tubular cell damage and interstitial fibrosis, even when traditional markers such as creatinine remain within normal or borderline ranges.

The results of the current study demonstrated highest levels observed in the on-dialysis stages group. These findings indicate that TIMP-2 increases prognostic with CKD severity, and its serum concentration may reflect the cumulative burden of tubular cell stress, ischemic injury, and systemic inflammation associated with end-stage renal disease (ESRD). This

observation is consistent with findings by Zhao et al. (2022), who reported that TIMP-2 levels increased proportionally with CKD stage and were highest in patients undergoing hemodialysis. Their study emphasized that TIMP-2 may be actively produced by damaged tubular cells or retained due to reduced clearance in dialysis patients **(Zhao et al., 2022).** Notably, they found that TIMP-2 was superior to some traditional markers such as serum creatinine, blood urea nitrogen (BUN), and estimated glomerular filtration rate (eGFR) in distinguishing ESRD patients from those in earlier stages **(Zhao et al., 2022).** The significant elevation of TIMP-2 in CKD patients, as shown in the present study, confirms its relevance as a non-invasive biomarker that reflects early pathological changes at the tubular level and supports its inclusion in emerging biomarker panels for CKD diagnosis and monitoring.

In the current study, no statistically significant differences were observed in serum TIMP-2 levels between different age groups, genders, or body mass index (BMI) categories among CKD patients, as shown in Tables (3-4), (3-5), and (3-6). These results suggest that TIMP-2 expression is not influenced by demographic or anthropometric factors, but rather reflects localized tubular epithelial stress related to renal pathology.

This finding aligns partially with previous work by **Van Duijl et al. (2022),** who reported no demographic influence on urinary TIMP-2 levels in healthy individuals. While the biological matrix (urine vs. serum) and populations (healthy vs. CKD) differ, the lack of association between TIMP-2 and demographic variables in both studies supports the stability of TIMP-2 expression across these factors.

The study results showed a statistically significant difference in TIMP-2 serum levels among Diabetic and non-Diabetic in table (3-9). This finding aligns with previous research indicating the critical role of TIMP-2 in diabetic nephropathy. For instance, a comprehensive review by **(Karsdal et al. 2020)** highlighted that decreased TIMP-2 levels contribute to extracellular matrix remodeling and fibrosis, key processes in the progression of diabetic kidney disease (DKD). Their findings support the hypothesis that altered TIMP-2 expression may underlie the accelerated kidney damage observed in diabetic CKD patients. According **to Kashani et al. (2013),** TIMP-2 shows potential as both a biomarker and a therapeutic target in diabetic kidney disease (DKD), highlighting its role in the disease's pathophysiology.

Furthermore, it has been suggested that the elevation of TIMP-2 in diabetic patients is more pronounced in advanced stages of kidney disease or in the presence of superimposed acute kidney injury (AKI). This finding was supported by **Kashani et al. (2013),** who demonstrated that TIMP-2 levels alongside IGFBP7—are significantly elevated in patients at high risk of AKI, particularly when overt tubular damage is present. These biomarkers serve as indicators of tubular cell cycle arrest and stress, mechanisms that are typically activated under conditions of significant tubular injury. Therefore, the tubular stress response reflected by TIMP-2 may not be uniformly activated in all diabetic patients, especially if tubular involvement is minimal or adequately controlled. This observation may explain the variability seen in biomarker expression across different clinical presentations of diabetic kidney disease.

The study results showed no statistically significant difference in serum TIMP-2 levels between CKD patients with hypertension and those without hypertension. Several studies have suggested that TIMP-2 plays a significant

role in the pathophysiology of both hypertension and chronic kidney disease by contributing to renal fibrosis and extracellular matrix remodeling (**Li et al., 2018).** However, the exact relationship between hypertension and TIMP-2 serum levels remains unclear, as the biomarker's elevation may depend more on the extent of tubular injury rather than hypertension.

In the present study, no statistically significant difference was observed in TIMP-2 levels between smokers and non-smokers with CKD. This finding aligns with previous evidence indicating that cigarette smoking may not induce a measurable increase in TIMP-2. In fact, a population-based study reported significantly lower circulating TIMP-2 concentrations in current smokers compared to non-smokers **(van Dijk et al., 2017).** This suggests that smoking might suppress certain cellular stress response pathways, potentially limiting TIMP-2 release despite the presence of subclinical tubular injury. Therefore, the absence of elevated TIMP-2 in smokers within our cohort could reflect this inhibitory effect, highlighting the need for further research specifically examining TIMP-2 dynamics in tobacco-related kidney injury.

The absence of TIMP-2 measurement in that context supports the novelty of including this biomarker in our study and highlights the gap in the literature regarding its specific behavior in smoking-related CKD.

TIMP-2 demonstrated non-significant negative correlations with serum urea levels. These weak and statistically non-significant inverse correlations suggest that the serum levels of these biomarkers are not directly influenced blood urea concentration in CKD patients. TIMP-2 suggests that these biomarkers reflect structural or cellular damage, rather than the overall uremic burden. In the current study, serum TIMP-2 levels showed significant correlations with key renal function indicators, underscoring its role as a

marker of tubular stress in chronic kidney disease. Specifically, TIMP-2 was positively correlated with serum creatinine and negatively correlated with estimated glomerular filtration rate (eGFR) Furthermore, a study by **Poniatowski et al. (2020)** examined TIMP-2 levels in patients with heart failure, with and without coexisting chronic kidney disease (CKD). They observed a positive modest correlation between TIMP-2 and serum creatinine. This suggests that TIMP-2 may reflect deteriorating renal function to some extent. However, the relatively weak association indicates that TIMP-2 might not be a strong standalone marker of glomerular filtration decline, especially in chronic and comorbid conditions such as heart failure.

In the present study, serum TIMP-2 levels did not show statistically significant correlations with most routine biochemical markers, including sodium, potassium, calcium, or vitamin D_3. However, a moderate and statistically significant positive correlation was observed with parathyroid hormone (PTH). This finding is consistent with the known interaction between bone-kidney axis hormones and protease inhibitors in CKD. Previous research has highlighted the role of PTH in regulating extracellular matrix turnover through modulation of MMPs and TIMPs. For instance, **Poniatowski et al. (2020)** reported that elevated PTH levels are associated with altered MMP-2 activity in CKD patients, suggesting that PTH may indirectly affect TIMP-2 expression as part of the renal–bone metabolic response. The lack of significant associations between TIMP-2 and routine biochemical markers supports the notion that TIMP-2 is more specifically involved in pathological signaling cascades, such as fibrosis and tubular remodeling, rather than general metabolic imbalance.

To current knowledge, no previous studies have directly examined serum TIMP-2 levels in relation to routine electrolytes (Na^+, K^+, Ca^{2+}) or 25-OH vitamin D in CKD patients. Although TIMP-2 is well established as a marker of tubular stress and extracellular matrix remodeling, it appears independent of standard biochemical or metabolic imbalances. This absence of association with sodium, potassium, calcium, or vitamin D3 in current study thus aligns with the current gap in literature, underscoring the specificity of TIMP-2 as a stress/fibrosis indicator rather than a marker influenced by electrolyte homeostasis. Although our results showed no significant correlation between TIMP-2 and biochemical parameters such as calcium or vitamin D3, this is consistent with the role of TIMP-2 as a marker of extracellular matrix remodeling and fibrosis, as described by **Radulescu et al. (2019),** rather than a marker influenced by mineral metabolism.

In current study, the diagnostic value of KIM-1 and TIMP-2 in identifying advanced CKD was assessed using receiver operating characteristic (ROC) analysis and logistic regression modeling. The results showed that both biomarkers demonstrated moderate discriminative ability in distinguishing in-dialysis CKD patients from healthy controls.

Specifically, KIM-1 exhibited an area under the ROC curve (AUC) of 0.751 ($p = 0.002$), while TIMP-2 had an AUC of 0.715 ($p = 0.002$) in differentiating dialysis patients. However, when tested between pre-dialysis patients and controls, the AUC values were lower — 0.634 for TIMP-2 ($p = 0.077$) and 0.662 for KIM-1 ($p = 0.066$) — reflecting their limited utility in detecting early-stage disease.

The current findings of moderate diagnostic performance of KIM-1 in identifying advanced CKD are further supported by (Hasan et al., 2024), who

evaluated serum KIM-1 levels in patients with persistent kidney failure, including those undergoing hemodialysis. In their study, KIM-1 achieved an excellent diagnostic performance, with an AUC of 0.937 ($p < 0.001$), a sensitivity of 87.3%, and a specificity of 95.0% at a cutoff value of 42.2 pg/mL. These findings indicate that KIM-1 is not only elevated in advanced stages of CKD but may also serve as a highly sensitive biomarker for identifying dialysis-dependent renal dysfunction. While variations in assay methods and sample types may account for the difference in AUC values, the consistency in detecting advanced renal impairment across studies reinforces the clinical relevance of KIM-1 as a tubular injury marker.

While the study by **(Hasan et al., 2024),** demonstrated excellent diagnostic performance of KIM-1 in advanced kidney failure, it did not explicitly evaluate early-stage CKD. Therefore, the current study adds valuable insight by assessing the biomarker's behavior across different CKD subgroups, including pre-dialysis patients. The lower AUC observed in early-stage patients (AUC = 0.662) may reflect the limited sensitivity of KIM-1 in detecting subclinical tubular injury during the initial phases of CKD.

Several studies have evaluated the diagnostic performance of TIMP-2 in acute kidney injury (AKI), with consistent reports of moderate to high diagnostic accuracy. For example, a study published in Minority Medicine and Science (MMS, 2022) demonstrated that TIMP-2, either alone or in combination with IGFBP7, yielded an area under the ROC curve (AUC) ranging from 0.75 to 0.86 in detecting early-stage AKI. These findings underscore the sensitivity of TIMP-2 to early tubular stress.

Although the MMS 2022 study did not focus specifically on patients with chronic kidney disease (CKD), the high AUC values reported support the

broader utility of TIMP-2 as a tubular injury biomarker. In the current study, a moderate AUC (0.715, p = 0.002) was observed for TIMP-2 in identifying advanced CKD, suggesting a potential role for this marker beyond acute injury settings.

Taken together, these findings reinforce the value of TIMP-2 as a diagnostic tool for renal stress. However, the limited availability of studies applying ROC analysis of TIMP-2 in CKD populations highlights the novelty and importance of the present research in expanding its application to chronic disease contexts.

Logistic regression analysis indicated that KIM-1 (p = 0.058) and TIMP-2 (p = 0.090) were borderline predictors of chronic kidney disease (CKD) status. Although these values did not reach the conventional threshold of statistical significance ($p < 0.05$), the trends observed suggest that both biomarkers may have predictive value when interpreted alongside other clinical indicators. This is consistent with findings by **Sabbisetti et al. (2014),** who demonstrated that KIM-1 levels are associated with CKD progression, particularly in diabetic nephropathy. Furthermore, recent studies, including **Meersch et al. (2014)** and Korean cohorts (2018), have highlighted the enhanced diagnostic utility of combining TIMP-2 with other biomarkers—such as IGFBP-7 and KIM-1—in multi-biomarker panels. These panels, as reflected in NephroCheck and other contemporary studies **(e.g., Scientific Reports, 2023; Frontiers in Medicine, 2025),** have shown superior performance in detecting both acute and chronic kidney injury. Hence, while KIM-1 and TIMP-2 alone may not provide sufficient diagnostic power, their integration within biomarker panels holds promise for improving early detection, risk stratification, and staging accuracy in CKD.

Overall, these findings highlight the strength of KIM-1 and TIMP-2 as supportive biomarkers in monitoring CKD progression, particularly in advanced stages, but also stress the need for larger-scale studies and multi-marker approaches to improve early detection strategies.

Chapter Five

Conclusion and Recommendation

5. Conclusion and Recommendations

5.1. Conclusion

- Significantly elevated serum levels of both KIM-1 and TIMP-2 were observed in CKD patients, particularly among those undergoing dialysis, suggesting their utility in identifying advanced tubular injury.

- Both biomarkers showed strong associations with renal function markers: KIM-1 and TIMP-2 positively correlated with serum creatinine and negatively with estimated glomerular filtration rate (eGFR), confirming their roles as indicators of declining renal function.

- TIMP-2 showed a moderate positive correlation with parathyroid hormone (PTH), indicating a possible link between mineral bone disorder and tubular stress in CKD.

- No statistically significant differences in biomarker levels were found across demographic variables (age, sex, BMI), nor in relation to hypertension, nephritis, or diabetes. However, KIM-1 levels were significantly elevated in smokers, reflecting sensitivity to nephrotoxic and inflammatory insults

- ROC analysis demonstrated moderate diagnostic performance for both KIM-1 and TIMP-2, particularly in distinguishing dialysis patients from controls. Logistic regression indicated borderline predictive value, suggesting limited utility as standalone diagnostics.

5.2. Recommendation

- It is recommended that KIM-1 and TIMP-2 be included as supplementary biomarkers in the routine monitoring of patients with moderate to advanced CKD, particularly those approaching or undergoing dialysis. Their strong correlation with renal function decline supports their value in tracking disease progression beyond traditional measures such as serum creatinine and eGFR.

- Future studies should explore the utility of KIM-1 and TIMP-2 for early detection of subclinical tubular damage, especially in patients with comorbidities such as diabetes, hypertension, and NSAID exposure. Their application in pre-dialysis stages may improve risk stratification and timely intervention

- Future studies should investigate the impact of pharmacological therapies commonly used in CKD patients (e.g., antihypertensives, phosphate binders, erythropoiesis-stimulating agents) on the serum concentrations of KIM-1 and TIMP-2.

- It is recommended to collect biomarker samples from the same CKD patient immediately before and after a hemodialysis session, to determine the direct effect of the dialysis process on KIM-1 and TIMP-2 levels and distinguish intrinsic disease-related changes from procedure-induced variations

- Future studies should involve larger and more diverse patient cohorts to enhance statistical power, improve subgroup analyses, and allow generalization of findings and investigate the role of environmental factors, personal lifestyle habits, and infectious agents (such as bacteria) in the progression of CKD, as these may represent additional determinants beyond traditional clinical parameters.

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And

Appendix

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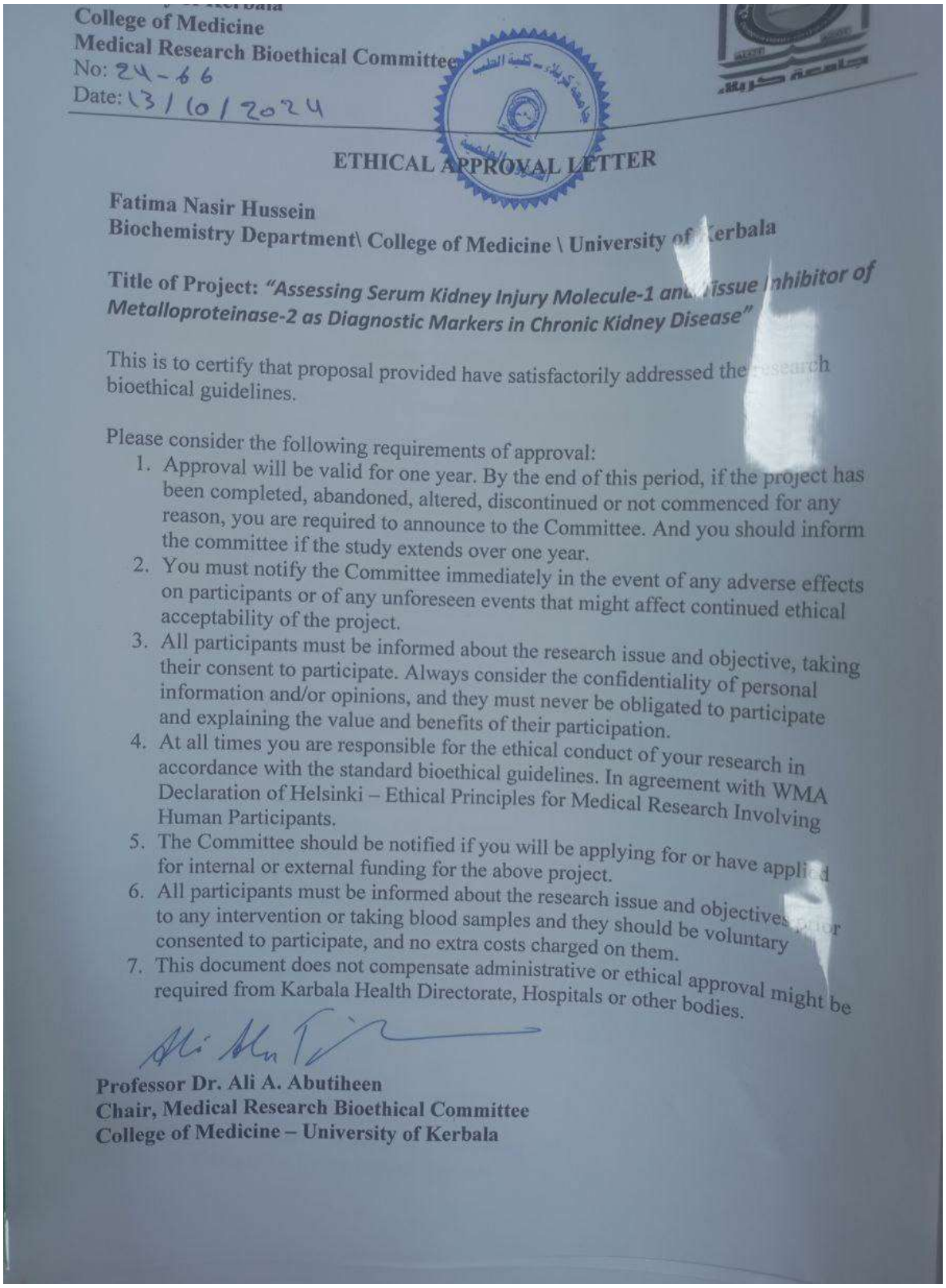

College of Medicine
Medical Research Bioethical Committee
No: 24-66
Date: 13/10/2024

ETHICAL APPROVAL LETTER

Fatima Nasir Hussein
Biochemistry Department\ College of Medicine \ University of Kerbala

Title of Project: ***"Assessing Serum Kidney Injury Molecule-1 and Tissue Inhibitor of Metalloproteinase-2 as Diagnostic Markers in Chronic Kidney Disease"***

This is to certify that proposal provided have satisfactorily addressed the research bioethical guidelines.

Please consider the following requirements of approval:

1. Approval will be valid for one year. By the end of this period, if the project has been completed, abandoned, altered, discontinued or not commenced for any reason, you are required to announce to the Committee. And you should inform the committee if the study extends over one year.
2. You must notify the Committee immediately in the event of any adverse effects on participants or of any unforeseen events that might affect continued ethical acceptability of the project.
3. All participants must be informed about the research issue and objective, taking their consent to participate. Always consider the confidentiality of personal information and/or opinions, and they must never be obligated to participate and explaining the value and benefits of their participation.
4. At all times you are responsible for the ethical conduct of your research in accordance with the standard bioethical guidelines. In agreement with WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants.
5. The Committee should be notified if you will be applying for or have applied for internal or external funding for the above project.
6. All participants must be informed about the research issue and objectives prior to any intervention or taking blood samples and they should be voluntary consented to participate, and no extra costs charged on them.
7. This document does not compensate administrative or ethical approval might be required from Karbala Health Directorate, Hospitals or other bodies.

Professor Dr. Ali A. Abutiheen
Chair, Medical Research Bioethical Committee
College of Medicine – University of Kerbala

International Journal of Environmental Sciences
ISSN: 2229-7359

Acceptance Letter

Date: 04-08-2025
Manuscript ID: IJES-156

Dear Fatima Nasir Hussein[1], Maher Abbood Mukheef[2],Dr. Ammar Majeed Mahdi3, Hashim Ali Hashim4,

I am delighted to inform you that your research paper titled "**Estimation of TIMP-2 in Patients with Chronic Kidney Disease**" has been accepted for publication in the upcoming issue of the **International Journal of Environmental Sciences.**

Congratulations on your remarkable achievement!

We would like to express our sincere gratitude for your valuable contribution.

Best regards,

Editor-in-Chief
International Journal of Environmental Sciences
ISSN: 2229-7359

KARBALA JOURNAL OF MEDICINE

No: 24
Date: August 5, 2025

Acceptance letter

To: Fatima Nasir Hussein, Maher Abbood Mukheef, Hashim Ali Hashim

We are pleased to inform you that manuscript Number (KarbalaJM-D-25-00028) entitled "Estimation of KIM-1 in Patients with Chronic Kidney Disease" has been accepted for publishing in the Karbala Journal of Medicine

Professor Ali Abdul Hussein S. AL-Janabi
Editor in chief
Karbala Journal of Medicine

Karbala Journal of Medicine (ISSN: 1990-5483) (E-ISSN: 2958-0889), https://journals.uokerbala.edu.iq/index.php/kj
Email: karbalamedj@uokerbala.edu.iq

Questionnaire

Sample no ;		
Age;		
Sex;	male	female
BMI;	Weight;	Height;
Smoking	yes	no
BP;	SBP	DBP
Physical activity;	yes	no
Diabetes mellitus;	yes	no
Family history;	yes	no
Heart failure;	yes	no
Sedentary life style;	yes	no
Hypertension;	yes	no
Excess smoking;	yes	no
Obesity;	yes	no
Duration;		
Investigations;		
Blood Urea;		
S.Creatinine;		
Sodium;		
Potassium;		
Calcium;		
Vit D3;		
KIM-1;		
TIMP-2;		
BMI;		
Medication;		

الخلاصة

مرض الكلى المزمن (CKD) هو حالة تقدمية تتميز بفقدان مستمر لا رجعه فيه بوظائف الكلى، ناجم عن أسباب متعددة تشمل داء السكري، وارتفاع ضغط الدم، والإصابة الإقفارية، والتهاب كبيبات الكلى. يُعد الكشف المبكر عن إصابة النبيبات الكلوية أمراً بالغ الأهمية لتأخير تطور المرض إلى المرحلة النهائية من القصور الكلوي (ESRD) التي تتطلب الغسيل الكلوي أو زراعة الكلى.

أُجريت هذه الدراسة من نوع الحالات والضوابط على 88 مشاركاً: 58 مريضاً بمرض الكلى المزمن (29 في المراحل قبل الغسيل، و29 يخضعون للغسيل الكلوي) و30 شخصاً سليماً كمجموعة ضابطة، تراوحت أعمارهم بين 30–70 سنة. جُمعت عينات الدم وحُللت باستخدام تقنية ELISA لقياس (KIM-1) و (TIMP-2)بالإضافة إلى مؤشرات بيوكيميائية أخرى مثل الكرياتينين في المصل، واليوريا، وتركيز الصوديوم والبوتاسيوم، والكالسيوم، وفيتامينD3 ، وبارا ثيرود هرمون (PTH). تم الحصول على الموافقة الأخلاقية من جميع المشاركين.لم تُظهر النتائج فروقاً ذات دلالة إحصائية في العمر أو مؤشر كتلة الجسم بين المجموعات الثلاث ($p > 0.05$). بلغت نسبة الذكور 60% والإناث 40% من إجمالي المشاركين، وكانت الفئة العمرية الأكثر إصابة هي ≤ 60 سنة (58.1%). إضافةً إلى ذلك، كان ارتفاع ضغط الدم أكثر الأمراض المصاحبة شيوعاً لدى مرضى(72.3%) CKD ، تلاه داء السكري من النوع الثاني (53%). وأظهر أكثر من ثلثي المرضى (66%) غياب النشاط البدني المنتظم، مما يبرز الحاجة إلى تدخلات في نمط الحياة .أظهرت الدراسة أن مستويات KIM-1 كانت مرتفعة بشكل ملحوظ لدى مرضى الغسيل الكلوي مقارنة بمرضى ما قبل الغسيل والمجموعة الضابطة($p = 0.001$) ، في حين لم تُسجل فروق ذات دلالة إحصائية بين مرضى ما قبل الغسيل والمجموعة الضابطة . كما كانت مستويات KIM-1 أعلى لدى المدخنين، وارتبطت إيجابياً مع الكرياتينين في المصل

أظهر تحليل منحنى ROC دقة تشخيصية جيدة نسبياً لمرضى الغسيل الكلوي مقابل المجموعة الضابطة ($AUC = 0.751$). أما مستويات TIMP-2 فقد كانت أعلى بشكل ملحوظ لدى مرض CKDمقارنة بالأصحاء($p = 0.004$) ، مما يشير إلى أهميتها التشخيصية المحتملة. وبمقارنة

المراحل المرضية، تبين أن مستويات TIMP-2 تزداد تدريجياً عبر المجموعات، حيث كانت الأدنى في الأصحاء، وأعلى قليلاً في مرضى ما قبل الغسيل، والأعلى في مرضى الغسيل الكلوي، مع دلالة إحصائية واضحة .(p = 0.001) كما أظهر التحليل الفرعي أن مرضى CKDالمصابين بالسكري لديهم مستويات TIMP-2 أعلى بكثير مقارنة بغير المصابين (p = 0.027).وأظهرت TIMP-2 ارتباطاً أقوى بضعف الوظيفة الكلوية مقارنة بـ KIM-1. وقد أظهر تحليل ROC قدرة تمييز متوسطة لمرضى الغسيل مقابل الأصحاء (AUC = 0.715). تشير النتائج إلى أن مؤشري KIM-1 و TIMP-2قد يكونان أدوات مفيدة للكشف عن إصابة الكلى، كما أن الجمع بينهما يُمثل نهجاً واعداً لتحسين تقييم وتشخيص المرض، نظراً لخصوصية KIM-1في كشف إصابة النبيبات القريبة، وقدرة TIMP-2 على تتبع تطور المرض عبر مراحله. إن دمج هذه المؤشرات، خاصة مع مؤشرات

أخرى، قد يعزز دقة الكشف المبكر.وتبقى هناك حاجة لإجراء دراسات طولية مستقبلية للتحقق من قيمتها التنبؤية وقابليتها للتطبيق سريرياً في فئات مرضية متنوعة.

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تقييم جزيء إصابة الكلى في المصل-1 ومثبط الأنسجة للميتالوبروتينيز-2 كعلامات تشخيصية في مرض الكلى المزمن في محافظة كربلاء

رسالة ماجستير

مقدمة الى مجلس كلية الطب\ فرع الكيمياء والكيمياء الحياتية\ جامعة كربلاء كجزء

من متطلبات نيل درجة الماجستيرفي (الكيمياء السريرية)

من قبل

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2025

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