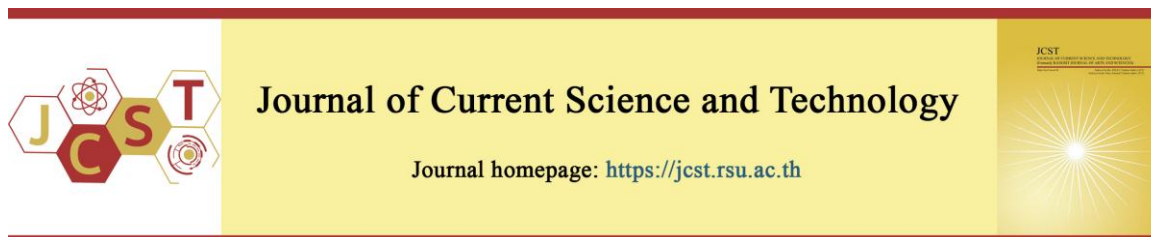


Cite this article: Karem, M. H. A., & Alqaisi, A. Q. I. (2026). Roles of *CCL2* gene expression in peripheral blood of patients with kidney disease. *Journal of Current Science and Technology*, 16(3), Article 192. <https://doi.org/10.59796/jcst.V16N3.2026.192>



Roles of *CCL2* Gene Expression in Peripheral Blood of Patients with Kidney Disease

Mohammed Hudhaifa A. Karem*, ORCID: 0009-0004-5992-3552
Amjed Qays Ibrahim Alqaisi, ORCID: 0000-0002-4759-1085

Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

*Corresponding author; E-mail: mohammad.h@ige.uobaghdad.edu.iq

Received 24 December 2025; Revised 4 March 2026; Accepted 18 March 2026; Published 25 June 2026

Abstract

Chronic kidney disease (CKD), acute kidney injury (AKI), and IgA nephropathy (IgAN) are kidney disorders that lead to progressive impairment of filtration. Standard diagnostic markers (creatinine, urea, and eGFR) remain the cornerstone of clinical detection, but they are still insufficient for early diagnosis. Recent studies highlight the potential role of genetic indicators such as *CCL2*, which are involved in inflammatory signaling and renal tissue injury by attracting monocytes and macrophages into the damaged kidney. This study was designed as a cross-sectional case-control investigation aimed at assessing fold-change expression of *CCL2* in the peripheral blood of patients with CKD ($n = 30$), AKI ($n = 10$) and IgAN ($n = 14$), compared with healthy controls ($n = 30$) after clinical diagnosis of patients with physiological markers. Creatinine, urea and eGFR exhibited highly significant differences between patients and controls, demonstrating strong diagnostic ability ($AUC > 0.90$ for all markers). Conversely, *CCL2* expression showed no significant difference between patient groups and controls, and no significant correlation with physiological markers. Mean *CCL2* fold-change were 1.707 in AKI, 1.795 in CKD, and 1.378 in IgAN, indicating only mild elevation without statistical significance. These findings contribute to the evaluation of inflammatory molecular biomarkers in renal diseases by indicating that circulating *CCL2* mRNA expression in peripheral blood does not offer additional diagnostic value beyond established physiological markers within this cohort. The results indicate that physiological markers such as creatinine, urea, and eGFR are still good indicators and highlight the limitations of blood-based transcriptional assessment of *CCL2* for reflecting renal inflammatory activity, suggesting that tissue-specific or urinary biomarkers may better represent the underlying renal pathological processes. Therefore, blood-based *CCL2* in this cohort lacks diagnostic utility, whereas tissue-based or urinary biomarkers could provide enhanced distinction.

Keywords: kidney disease, CKD, AKI, IgAN, *CCL2* gene

1. Introduction

The kidneys are complex organs that play a central role in homeostasis through the regulation of body fluids (Osborn et al., 2021). Kidney disease includes a diverse array of pathological conditions that affect kidney structure and function, encompassing both acute and possibly reversible abnormalities as well as chronic and progressive impairments (Siribamrungwong & Chaikomom, 2025). These conditions include acute kidney injury (AKI), chronic kidney disease (CKD) and IgA nephropathy (IgAN),

which involve immune-mediated injury and chronic inflammation (Al-Razak et al., 2025; Lameire et al., 2021).

These diseases cause physiological and biochemical changes that can be measured with several biomarkers. Progressive glomerular damage results in weakened renal filtration and is indicated by increasing serum creatinine, blood urea levels, and decreased estimated glomerular filtration rate (eGFR) compared with healthy individuals (Al-Razak et al., 2024; Lateef et al., 2024; Alani & Al-Lami, 2024; Barratt et al., 2025; Pattrapornpisut et al., 2021).

Unlike CKD and AKI, serum or urine biomarkers for IgAN are suggestive but nonspecific and support clinical suspicion, and renal biopsy is the only definitive diagnostic method, which is considered invasive; therefore, genetic markers are of great interest (Habas et al., 2022; Liu et al., 2023; Obrișcă et al., 2024; Tan et al., 2025; Bakdash et al., 2019; Luciano & Moeckel, 2019). In summary, patients with kidney disease usually exhibit a range of abnormal laboratory findings, including elevated creatinine, and urea levels with reduced eGFR, all of which reveal the effect of disease on renal function.

The *CCL2* gene, known as monocyte chemoattractant protein-1 (MCP-1), is a chemokine that attracts monocytes and macrophages into the damaged kidney. It is secreted by multiple cell types, such as glomerular mesangial cells, podocytes and tubular epithelial cells, in response to injury or immune stimuli. In IgAN, the accumulation of IgA immune complexes in glomeruli triggers an inflammatory condition in which *CCL2* has a significant role (Guo et al., 2025; Eardley et al., 2008). Recent transcriptomic analyses have shown *CCL2* to be a prospective biomarker in IgAN. The study conducted by (Zhou et al., 2022) concluded that *CCL2* may serve as a potential biomarker for IgAN diagnosis instead of invasive renal biopsy. Numerous investigations have examined *CCL2* protein levels (MCP-1) in urine or even renal tissue, but there are limitations in data regarding *CCL2* mRNA in peripheral blood as a prospective biomarker for renal injury. Peripheral blood gene expression analysis has advantages related to its minimal invasiveness, easier sample collection and may offer indirect insight into inflammatory pathways associated with renal disease (Baran et al., 2022). However, it remains unclear whether blood-based *CCL2* expression can reflect renal pathological processes or provide additional diagnostic value beyond established clinical markers. In this context, the present research aimed to evaluate the mRNA levels of *CCL2* in CKD, AKI, and IgAN patients compared with healthy controls, using fold change analysis of RNA expression. This study, by focusing on peripheral blood *CCL2* transcripts, tries to fill the current knowledge gap regarding whether circulating gene expression patterns can serve as a non-invasive biomarker for renal disease.

2. Objectives

1. To evaluate the serum creatinine, blood urea, and eGFR levels in CKD, AKI, and IgAN patients, compared with healthy control subjects, to assess their diagnostic performance in kidney disease.

2. To investigate the relative expression (fold-change) of the *CCL2* gene in the peripheral blood of patients with CKD, AKI, and IgAN compared with healthy controls using quantitative gene-expression analysis.

3. To investigate the clinical relevance of circulating *CCL2* expression by examining its differences among renal disease groups and its association with established renal function markers, with the aim of determining its potential utility as non-invasive biomarker for renal dysfunction.

3. Materials and Methods

Blood samples from 110 suspected patients with CKD, AKI, and IgAN, and 40 blood samples from healthy individuals were collected after primary diagnosis by a specialized doctor in Medicine City / Kidney Diseases and Transplant Center during the period from November 2024 to April 2025. Patients were classified based on clinical diagnostic criteria by analysis of serum creatinine, blood urea, and eGFR using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation, while IgAN was confirmed by renal biopsy. IgAN demonstrated dominant or co-dominant IgA deposition in the glomerular mesangium using immunofluorescence microscopy and was classified according to the Oxford classification system (MEST-C score). These pathological parameters were used to confirm the diagnosis and assess disease activity and chronicity in IgAN. Blood samples of (n = 30) CKD, (n = 14) IgAN, (n = 10) AKI, and (n = 30) healthy controls were confirmed and used in this study. The age range for all subjects is from 13 – 73 years. All participants (females and males) had no alcoholism, smoking, family history of kidney disease, thyroidal disorder, dermal problems, pregnancy, or contraceptive use for females, with the note that information was obtained through medical history interviews and review of patient medical records during clinical evaluation to ensure that participants met the exclusion criteria (Table 1).

Table 1 Baseline characteristics of the study participants by study group

Variable	AKI (n = 10)	CKD (n = 30)	IgAN (n = 14)	Control (n = 30)
Age (years), mean $\pm$ SD	50.7 $\pm$ 16.9	52.4 $\pm$ 17.4	33.8 $\pm$ 12.2	28.8 $\pm$ 6.5
Sex (Male), n (%)	7 (70%)	16 (53.3%)	7 (50%)	18 (60%)
Sex (Female), n (%)	3 (30%)	14 (46.7%)	7 (50%)	12 (40%)
Hypertension, n (%)	6 (60%)	18 (60%)	9 (64.3%)	0 (0%)
Diabetes mellitus, n (%)	1 (10%)	7 (23.3%)	2 (14.3%)	0 (0%)

The venous blood sample was collected via vein puncture using five mL disposable syringe. Whole blood was put into EDTA tubes and serum-separator (gel) tubes. About 2 – 3 mL of EDTA-anticoagulated whole blood was used for RNA extraction. EDTA tubes were immediately stored at -80°C until processing. For gene-expression analysis, total RNA was extracted from EDTA-preserved blood using a commercial RNA extraction reagent (GENEzol™ reagent-based RNA extraction system) and was then purified through binding, washing, and elution steps using spin columns according to the manufacturer's protocol. The concentration of the isolated RNA was measured using the QuantiFluor® RNA system with a Quantus™ fluorometer according to the manufacturer's protocol. Primers used were synthesized by Macrogen and prepared as working solution in nuclease-free water. The complementary DNA (cDNA) was synthesized from the extracted RNA using the RevertAid First Strand cDNA Synthesis Kit then Quantitative real-time PCR (qRT-PCR) was then performed using a two-step qRT-PCR protocol. The reaction mixture contained Luna Universal two-step reaction mix, forward and reverse primers, cDNA template and nuclease-free water in a final reaction volume of 20 μL . The primer sequences were used for *CCL2* forward (5'-AGCCACCTTCATTCCCCAAG-3') and reverse (5'-GGAGCTGGATTGGGGTTCA-3'), producing an amplicon size 248 bp, while beta-actin forward (5'-TCGAGTCGCGTCCACC-3') and reverse (5'-GGGAGCATCGTCGCCC-3'), producing an amplicon size 103 bp. Thermal cycling conditions included an initial denaturation at 95°C for 1 minute, followed by 40 cycles of denaturation at 95°C for 10 seconds and extension at 60°C for 30 seconds. Amplification specificity was assessed using melting curve analysis ($60-95^{\circ}\text{C}$). Gene expression levels were normalized using beta-actin housekeeping gene as an internal reference. Subsequently, gene expression levels were quantified as fold changes using the $\Delta\Delta\text{CT}$ method described by Livak and Schmittgen (2001). Relative gene expression was calculated using the $2^{-\Delta\Delta\text{CT}}$ method, where $\Delta\text{C}_T = \text{C}_T \text{ target gene} - \text{C}_T \text{ reference}$

gene, $\Delta\Delta\text{C}_T = \Delta\text{C}_T \text{ sample} - \Delta\text{C}_T \text{ Calibrator}$, and final fold change was expressed as $2^{-\Delta\Delta\text{CT}}$. Gel tubes were allowed to clot for 10 min at room temperature, after which serum was separated by centrifugation at 3,000 rpm for 10 min. The resulting serum was aliquoted into three plain tubes and stored at -20°C for creatinine and urea analysis. By following standard biochemical procedures concentration of creatinine and urea was measured using an automated clinical chemistry Analyzer. The kinetic Jaffe method was used for serum creatinine test. In this method, creatinine reacts with alkaline solution to create a red-orange chromophore, and increase in absorbance was recorded photometrically at 510 – 520 nm. Reagent packs (R1/R2) were loaded into the Analyzer, and samples, calibrators, and quality controls were automatically processed through dispensing, mixing, incubation, and absorbance reading steps. Creatinine concentrations were calculated automatically using calibration curve generated based on the kinetic method. Serum urea concentration was measured using enzymatic urease-glutamate dehydrogenase method. Urea is hydrolyzed by urease to create ammonia and carbon dioxide. The released ammonia reacts with α -ketoglutarate and NADH in the presence of glutamate dehydrogenase, leading to conversion of NADH to NAD^+ . The decrease in absorbance at 340 nm is proportional to the urea level in the sample. The automatic analyzer performed reagent dispensing, incubation, and photometric detection after loading the reagent pack, calibrator, control sera, and patient samples. Urea concentrations were automatically calculated based on the calibration curve, and the results were reported in mg/dL. It should be noted that all patients received treatment according to disease severity, accompanied by supportive care.

Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to test the normality of the data distribution. Categorical data were expressed as numbers and percentage, the parametric data were expressed as mean $\pm$ standard deviation, whereas the nonparametric variables were expressed as median and interquartile range (IQR: 25th percentile – 75th percentile). Kruskal-Wallis H followed by Dunn's

multiple comparisons test as post hoc test was performed for non-parametric data; meanwhile, one-way ANOVA followed by Tukey's test as post hoc test was employed for parametric data. The differences were considered significant when p -value was ≤ 0.05 . To evaluate the adequacy of the sample size, a post hoc statistical power analysis was performed based on the observed effect sizes for *CCL2* fold-change expression among study groups. The post hoc analysis indicated that, while the AKI and IgAN groups were of relatively small size, they were sufficient to identify clinically relevant differences in *CCL2* expression within the constraints of this exploratory study. The statistical analysis was performed using GraphPad Prism 10.3.1.

This study was approved by the College of Science/ Department of Biology/ research ethics committee under the Ref.: CSEC/1124/0095. Written informed consent was obtained from all adult participants, and from all participants under 18 years, consent was provided by their legal guardians. All study procedures were performed in accordance with the principles of the Declaration of Helsinki and applicable national regulations. Participant confidentiality was rigorously safeguarded, and all data were anonymized prior to analysis.

4. Results and Discussion

4.1 The Change in the Creatinine Levels

Patients with renal dysfunction showed significant increase in serum creatinine levels (Figure 1 and Table 2). The creatinine median in patients was 2.65 mg/dL (IQR: 1.545–5.255 mg/dL) compared with 0.755 mg/dL (IQR: 0.4975–0.985 mg/dL) in healthy controls ($p < 0.0001$). Patient groups showed the highest creatinine levels for acute cases (median 4.95 mg/dL), intermediate levels in CKD (median 2.93 mg/dL), and the lowest elevation in IgAN (median 0.895 mg/dL), compared with controls (median 0.755 mg/dL) (Figure 2 and Table 3). Receiver-operating characteristic (ROC) analysis showed excellent discrimination: AUC = 0.9102 ($p < 0.0001$), with 81.48% sensitivity and 100% specificity at a 1.20 mg/dL cutoff (Figure 3 and Table 4). These data demonstrate that serum creatinine differentiates between unhealthy and healthy kidneys in these groups.

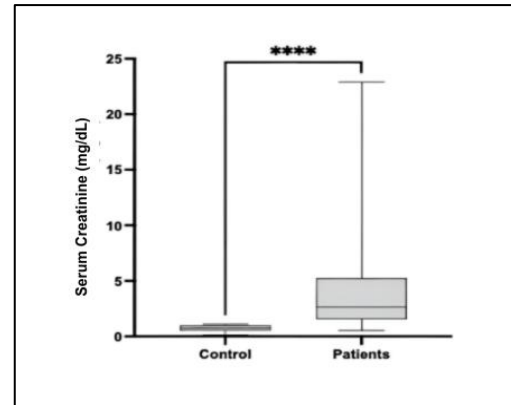


Figure 1 Serum creatinine levels in patients and control groups. Statistical significance was assessed using the Mann-Whitney test. Asterisks indicate significance levels (**** $p < 0.0001$)

Table 2 Serum creatinine levels in patients and control groups

Creatinine	Control	Patients
25% Percentile	0.4975	1.545
Median	0.7550	2.650
75% Percentile	0.9850	5.255

Table 3 Serum creatinine levels across study groups

Creatinine	Control	Acute	CKD	IgAN
25% Percentile	0.4975	3.065	1.983	0.6375
Median	0.7550	4.950	2.930	0.8950
75% Percentile	0.9850	7.150	5.255	2.123

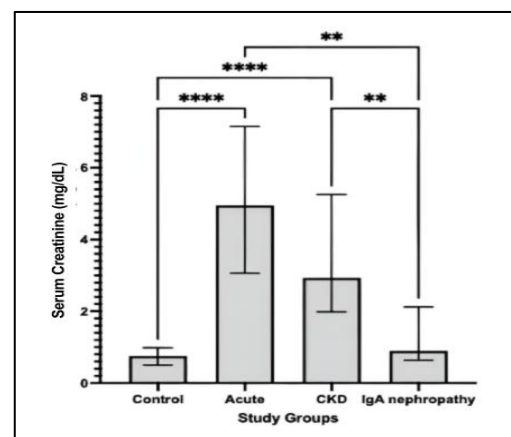


Figure 2 Serum creatinine levels across study groups. Statistical significance was assessed using the Kruskal–Wallis test followed by Dunn's multiple comparisons test. Error bars represent the median with interquartile range. Asterisks indicate significance levels (** $p < 0.01$, **** $p < 0.0001$)

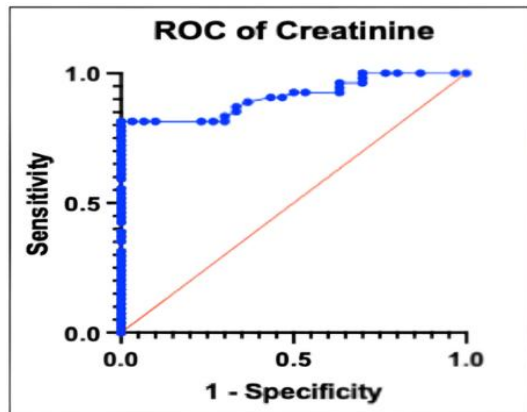


Figure 3 Receiver operating characteristic (ROC) curve showing the diagnostic accuracy of serum creatinine levels in patients with kidney disease in the study. The analysis demonstrated excellent performance with an AUC of 0.9102 ($p < 0.0001$), at a cutoff value > 1.2 mg/dL, achieving 81.48% sensitivity and 100% specificity

Table 4 Receiver operating characteristic (ROC) curve showing the diagnostic accuracy of serum creatinine levels in patients with kidney disease in the study.

Area under the ROC curve	Creatinine
Area	0.9102
Std. Error	0.03083
95% confidence interval	0.8498 to 0.9706
p -value	<0.0001
Cut off value	>1.200
Sensitivity	81.48%
Specificity	100.00%

This result is aligned with accepted renal physiology. Progressive kidney disease increases creatinine and urea and decreases eGFR (Al-Musawi et al., 2021; Brookes & Power, 2022; Provenzano et al., 2024). Creatinine remains a highly sensitive indicator of renal dysfunction, and this is confirmed by a high and statistically significant AUC (Kahindo et al., 2022). For AKI, increases ≥ 0.3 mg/dL (≥ 26.5 $\mu\text{mol/L}$) within 48 hours or an increase in serum creatinine to ≥ 1.5 times over the baseline level are necessary. Ávila et al. (2025) explained that creatinine has limitations, and its level can be affected with factors such as muscle mass, hydration, advanced age, physical activity and medications (Ávila et al., 2025). In the early stages of the kidney disease, the increase in creatinine may be delayed, while our results explain the role of creatinine in renal dysfunction, it is necessary to find newer biomarkers for earlier detection. Therefore, creatinine must be assessed with an understanding of its limitations (Pradeep et al., 2024).

4.2 The Change in the Blood Urea Levels

Urea, like creatinine, showed a significant increase in patients. The median for patients was 74.00 mg/dL (IQR: 48.75 – 133.0) versus 30.00 mg/dL (IQR: 27.50 – 36.25) in controls ($p < 0.0001$) (Figure 4 and Table 5).

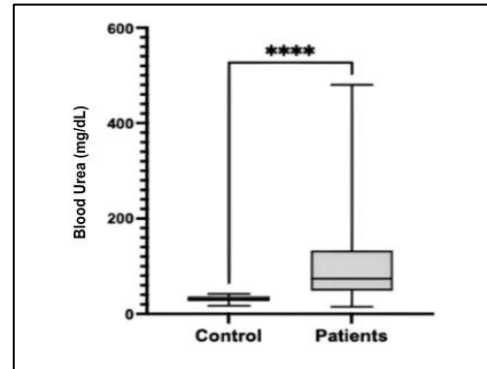


Figure 4 Blood urea levels in patients and control groups. Statistical significance was assessed using the Mann-Whitney test. Asterisks indicate significance levels (**** $p < 0.0001$)

Table 5 Blood urea levels in patients and control groups

Urea	Control	Patients
25% Percentile	27.50	48.75
Median	30.00	74.00
75% Percentile	36.25	133.0

The analysis of ROC showed AUC = 0.9247 ($p < 0.0001$), at a cutoff value > 42.00 mg/dL, with sensitivity 87.04% and specificity 100% (Figure 5 and Table 6).

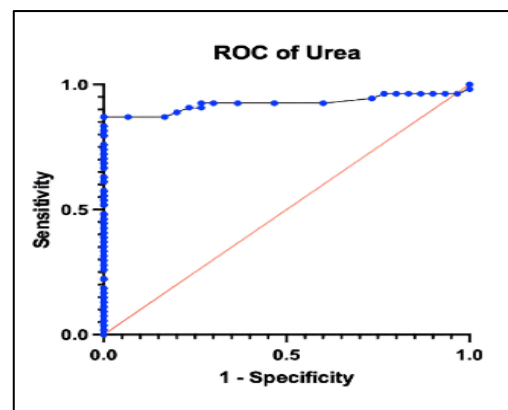


Figure 5 Receiver operating characteristic (ROC) curve showing the diagnostic accuracy of blood urea levels in patients with kidney disease in the study. The analysis demonstrated excellent performance with an AUC of 0.9247 ($p < 0.0001$), at a cutoff value > 42.00 mg/dL, achieving 87.04% sensitivity and 100% specificity.

Table 6 Receiver operating characteristic (ROC) curve showing the diagnostic accuracy of blood urea levels in patients with kidney disease in the study

Area under the ROC curve	Urea
Area	0.9247
Std. Error	0.03180
95% confidence interval	0.8624 to 0.9870
<i>p</i> -value	<0.0001
Cut off value	> 42.00
Sensitivity	87.04%
Specificity	100%

This demonstrates that blood urea has high diagnostic accuracy in distinguishing patients from healthy controls. The subgroups showed that AKI had the highest urea level (median 125.0 mg/dL), followed by CKD, which had an intermediate urea level (median 91.50 mg/dL), and finally, IgAN patients had the lowest level (median 50.0 mg/dL) compared with the control group (median 30.00 mg/dL) (Figure 6 and Table 7).

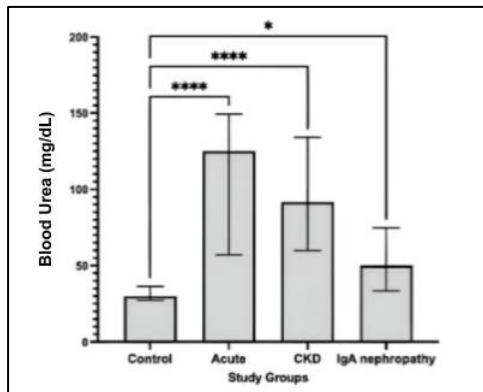


Figure 6 Blood urea levels across study groups. Statistical significance was assessed using the Kruskal–Wallis test followed by Dunn’s multiple comparisons test. Error bars represent the median with interquartile range. Asterisks indicate significance levels (**p* < 0.05, *****p* < 0.0001).

Table 7 Blood urea levels across study groups

Urea	Control	Acute	CKD	IgAN
25% Percentile	27.50	57.00	60.00	33.50
Median	30.00	125.0	91.50	50.00
75% Percentile	36.25	149.3	134.0	74.75

In a clinical context, high blood urea confirms renal impairment. Like creatinine, urea is considered a classical marker of kidney function. Increasing blood urea is mainly correlated with worse outcomes, such as cardiac and renal dysfunction, which reflect its sensitivity to filtration and catabolism (Alani & Al-

Mayaly, 2022; Younes-Ibrahim & Younes-Ibrahim, 2022). Our findings indicate that urea distinguishes patients with a sensitivity similar to creatinine, which contributes to the diagnostic value in renal injury. Blood urea can be affected by factors such as protein intake or catabolic state. It is also noted that both creatinine and urea levels increase together, as expected for patients with kidney disease. In conclusion, the results indicate that urea functions as a reliable classical indicator of renal dysfunction (Salih & Yenzeel, 2020; Lateef et al., 2024).

4.3 Estimated Glomerular Filtration Rate (eGFR)

Estimated GFR was significantly decreased in patients as compared with controls. The median eGFR in patients was 28.0 mL/min/1.73 m² (IQR: 12.33–53.88), versus the control group median of 128.0 mL/min/1.73 m² (IQR: 104.5–142.0) (*p* < 0.0001) (Figure 7 and Table 8). The analysis of ROC showed AUC = 0.9099 (*p* < 0.0001), at a cutoff value <78.65 mL/min/1.73m², with sensitivity 81.48% and specificity 100% (Figure 8 and Table 9). The subgroups showed that AKI had the lowest eGFR (median 12.9), followed by CKD, which had an intermediate eGFR level (median 25.95), whereas IgAN patients had a median eGFR of 108.5 mL/min/1.73 m² compared with the control group (median 128.0) (Figure 9 and Table 10). These data indicate that AKI causes substantial eGFR decline, whereas chronic conditions have higher eGFR.

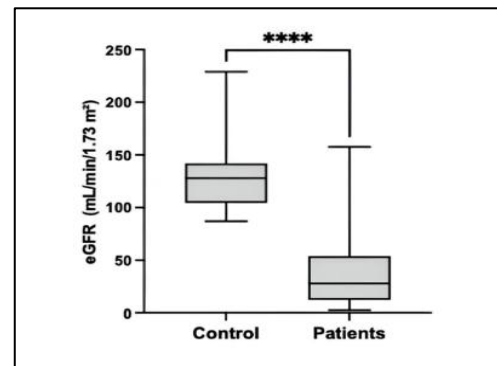


Figure 7 eGFR levels in patients and control groups. Statistical significance was assessed using the Mann-Whitney test. Asterisks indicate significance levels (*****p* < 0.0001)

Table 8 eGFR levels in patients and control groups

eGFR	Control	Patients
25% Percentile	104.5	12.33
Median	128.0	28.00
75% Percentile	142.0	53.88

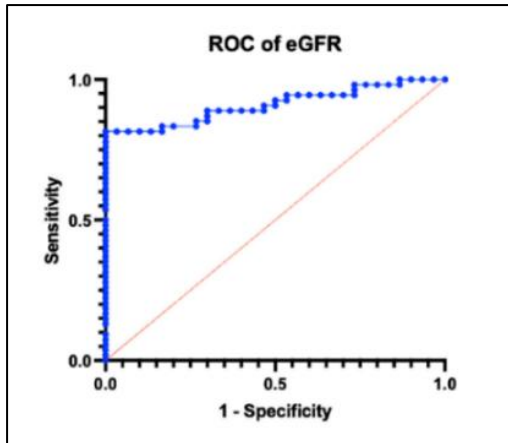


Figure 8 Receiver operating characteristic (ROC) curve showing the diagnostic accuracy of eGFR levels in patients with kidney disease. The analysis demonstrated excellent performance with an AUC of 0.9099 ($p < 0.0001$), at a cutoff value $< 78.65 \text{ mL/min/1.73 m}^2$, achieving 81.48% sensitivity and 100% specificity

Table 9 Receiver operating characteristic (ROC) curve showing the diagnostic accuracy of eGFR levels in patients with kidney disease

Area under the ROC curve	eGFR
Area	0.9099
Std. Error	0.03151
95% confidence interval	0.8481 to 0.9716
p -value	<0.0001
Cut off value	<78.65
Sensitivity	81.48
Specificity	100

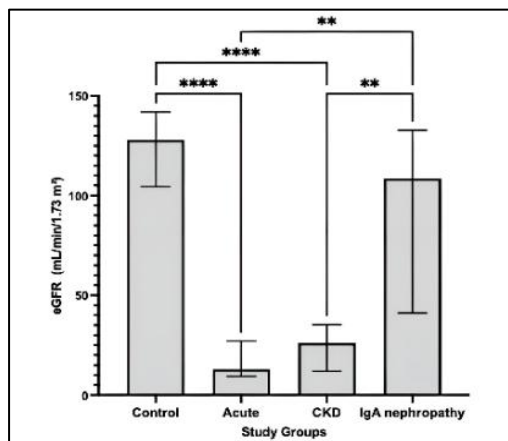


Figure 9 eGFR levels across study groups. Statistical significance was assessed using the Kruskal–Wallis test followed by Dunn’s multiple comparisons test. Error bars represent the median with the interquartile range. Asterisks indicate significance levels (** $p < 0.01$, **** $p < 0.0001$)

Table 10 eGFR levels across study groups

eGFR	Control	Acute	CKD	IgAN
Minimum	87.00	7.000	2.600	10.30
25% Percentile	104.5	9.350	11.98	41.18
Median	128.0	12.90	25.95	108.5
75% Percentile	142.0	27.08	35.40	132.8

The reduction of GFR is the hallmark of kidney dysfunction and is considered a classical indicator of renal impairment, and eGFR is often used to determine the stage of CKD. Most research indicates that lower eGFR is related to poor outcomes, for example, a reduction in eGFR can predict cardiovascular problems, end-stage renal disease, and mortality (Ibrahim et al., 2024; Ling et al., 2023; Zsom et al., 2022; Choi et al., 2022). Our findings were consistent with most relevant research. The high AUC (0.9099) shows that calculated eGFR, even though it is derived from creatinine, is still a powerful indicator of disease (Al-Jewari et al., 2021; Gao et al., 2022). In this study, eGFR and like creatinine and urea, gives expected results for patients, confirming its clinical role as a comprehensive indicator of filtration.

4.4 CCL2 Fold Change

The *CCL2* fold change showed a non-significant difference by sex and group. The median fold change for females was 1.84, greater than that of males at 0.86 ($p = 0.1334$) but still non-significant (Figure 10 and Table 11). Also, mean *CCL2* fold changes were 1.707 (AKI), 1.795 (CKD), and 1.378 (IgAN), showing no significant difference across groups (Figure 11 and Table 12).

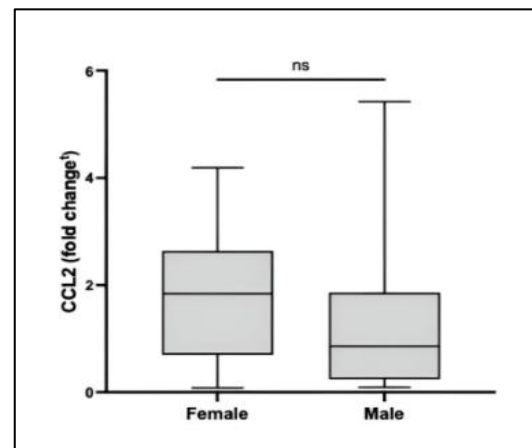


Figure 10 Fold change of *CCL2* expression stratified by sex. Statistical significance was assessed using the Mann-Whitney test. ns denotes non-significant differences

Table 11 Fold change of *CCL2* expression stratified by sex

<i>CCL2</i>	Female	Male
25% Percentile	0.7000	0.2450
Median	1.840	0.8600
75% Percentile	2.635	1.860

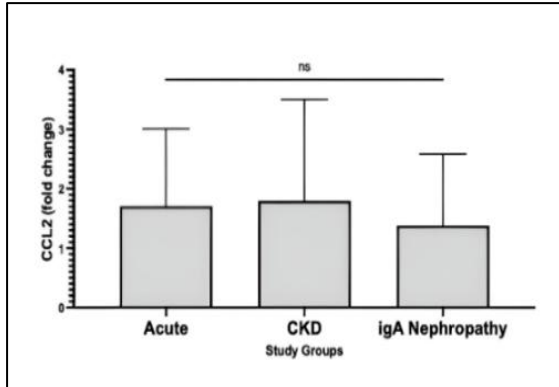


Figure 11 Fold change of *CCL2* expression across study groups. Statistical significance was assessed using one-way ANOVA test followed by Tukey's multiple comparisons test. Error bars represent mean $\pm$ standard deviation. Asterisks indicate significance levels (** $p < 0.01$, *** $p < 0.0001$)

Table 12 Fold change of *CCL2* expression across study groups

<i>CCL2</i>	Acute	CKD	IgAN
Mean	1.707	1.795	1.378
Std. Deviation	1.305	1.712	1.206
Std. Error of Mean	0.4932	0.5413	0.3344

The *CCL2* expression was slightly elevated above baseline in all patients, but it did not differentiate between kidney disease groups, suggesting that systemic *CCL2* was not highly altered by disease status. The wide standard deviation observed reflects substantial inter-patient variability in circulating *CCL2* mRNA levels, which limits the ability to detect significant differences. Possible sources of this variability may include heterogeneity in disease severity, duration of illness, ongoing treatment, genetic background and individual differences in immune responses. This result indicated that peripheral blood *CCL2* mRNA expression did not provide discriminatory diagnostic value and highlights the potential limitations of using circulating transcriptional measurements of inflammatory chemokines as biomarkers for renal disease. These findings are considered remarkable due to the role of *CCL2* in renal inflammation. Zhou et al. (2022), using a bioinformatics study, indicated that *CCL2* was one of the top three downregulated immune-related hub genes that differentiated IgAN patients from the

control group with high diagnostic accuracy. However, tissue-based transcriptome analyses in IgAN and CKD have consistently reported upregulation of *CCL2* (MCP-1) in the kidney, often correlated with damage (Delrue & Speckaert, 2025; Zheng et al., 2020). For example, Feng et al. (2018) found that mRNA for *CCL2* was markedly induced in urinary exosomes from IgAN patients as compared with control groups, and since tubulointerstitial damage is a significant histopathological risk factor for decline in renal function, urinary exosomal *CCL2* may be a useful predictive biomarker for IgAN progression (Feng et al., 2018). Several studies have shown that urinary MCP-1 protein levels strongly correlate with renal inflammation, disease severity, and progression in renal injury (Saitoh et al., 1998). This finding suggests that urinary MCP-1 protein, rather than peripheral blood mRNA, is a more informative biomarker for monitoring renal injury and prognosis. It is important to note that *CCL2* expression is closely associated with the molecular interaction between innate immune cells and endothelial cells during inflammatory responses, which facilitates leukocyte recruitment to sites of tissue injury. Therefore, the stable expression of circulating *CCL2* found in this study may reflect the localized nature of inflammatory processes within renal tissue rather than being reflected in peripheral blood. Moreover, circulating mRNA molecules generally exhibit a relatively short half-life in the blood stream due to rapid degradation by plasma ribonuclease, which may limit the stability and detectability of specific transcripts such as *CCL2* in peripheral samples (Wang & Liu, 2022). Another possible explanation is the dilution effect in peripheral blood, where transcripts derived from renal tissue may be substantially diluted by RNA derived from the larger population of circulating blood cells, thereby masking disease-specific signals. Also, *CCL2* detected in peripheral blood may primarily reflect transcriptional activity in circulating immune cells, particularly monocytes, rather than renal tissue itself. Despite no diagnostic utility being observed in this study, *CCL2* expressions could have prognostic value, particularly in tracking disease progression or therapeutic responses. Future investigations may benefit from evaluating additional pro-inflammatory mediators, particularly cytokines, to better distinguish between CKD and various types of renal failure. Overall, the results are conflicting, and our blood-based data did not show such changes. The gene expression pattern can differ between tissue and blood or vary by disease context, and this discrepancy likely

reflects compartmentalization, intrarenal *CCL2* may not translate into a measurable circulating mRNA signal. Furthermore, it is important to note that a substantial proportion of patients in this study were receiving clinical treatment according to disease severity, which may influence inflammatory gene expression levels and lead to detectable differences in circulating *CCL2* expression.

In summary, the lack of any significant alteration of *CCL2* in this study may indicate that blood *CCL2* expression does not possess diagnostic or prognostic capabilities in this context. Also, the sample size may limit the detection of subtle effects. In this investigation, *CCL2* expression in blood did not demonstrate clinical utility in this study, unlike recognizing the role in tissue function.

4.5 Spearman Correlation between *CCL2* Gene Expression and Clinical Parameters

Spearman analysis of *CCL2* fold change versus physiological kidney markers revealed no significant correlations. The correlation coefficient was $r = 0.12$ for creatinine ($p = 0.54$), $r = 0.15$ for urea ($p = 0.44$),

and $r = -0.14$ for eGFR ($p = 0.46$). The investigation of the correlation coefficient between *CCL2* and all research parameters indicated no statistically significant correlation (Table 13 and Figures 12 – 14). Practically, patients with elevated serum creatinine or reduced eGFR demonstrate no consistent variation in circulating *CCL2* mRNA compared with those with milder dysfunction.

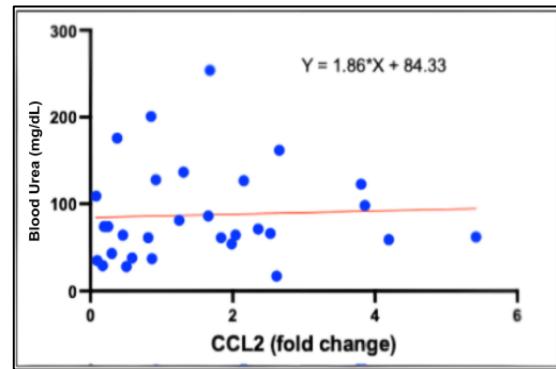


Figure 12 Scatter plots showing Spearman's correlation between fold change of *CCL2* expression and blood urea

Table 13 Spearman's correlation between fold change of *CCL2* expression and serum creatinine, urea, and eGFR

	<i>CCL2</i> vs. creatinine	<i>CCL2</i> vs. urea	<i>CCL2</i> vs. eGFR
Spearman r			
r	0.12	0.15	-0.14
95% confidence interval	-0.26 to 0.46	-0.23 to 0.48	-0.48 to 0.24
P value			
P (two-tailed)	0.54	0.44	0.46
P value summary	ns	ns	ns
Equation	$Y = 0.02 * X + 2.86$	$Y = 1.86 * X + 84.33$	$Y = -6.007 * X + 65.48$

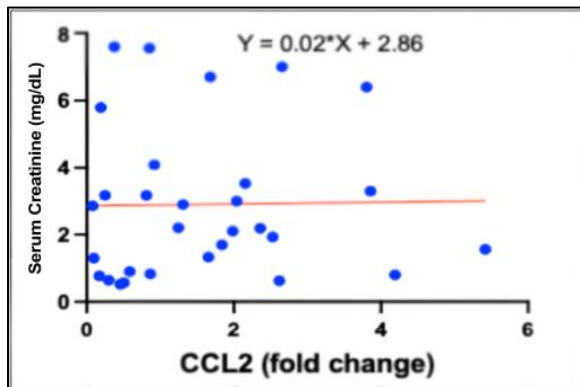


Figure 13 Scatter plots showing Spearman's correlation between fold change of *CCL2* expression and serum creatinine

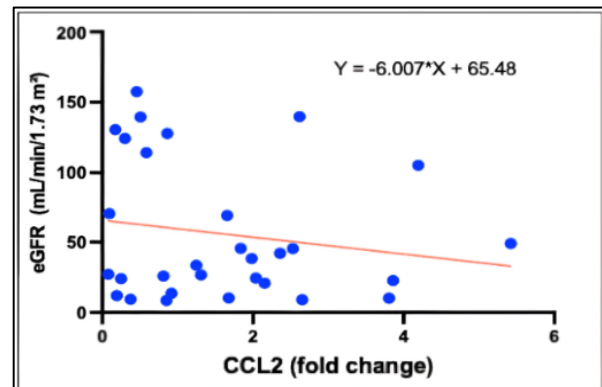


Figure 14 Scatter plots showing Spearman's correlation between fold change of *CCL2* expression and eGFR

This result differs from some tissue-based observations. In kidney biopsies from IgAN patients, elevated intrarenal *CCL2* expression is strongly associated with disease severity. For example, transcriptome information shows increased *CCL2* levels correlated with more severe proteinuria and reduced eGFR (Keskinis et al., 2025; Han et al., 2021; Feng et al., 2018).

Urinary *CCL2* (MCP-1) levels or exosomal *CCL2* mRNA reflect kidney damage in IgAN. Numerous investigations have explained that IgAN patients show increased urine MCP-1 and elevated exosomal *CCL2* mRNA, and that these markers correlate with histological activity and renal dysfunction (Lv et al., 2018; Keskinis et al., 2025; Feng et al., 2018; Duan et al., 2024). The lack of correlation in blood may indicate that systemic *CCL2* transcripts are disconnected from those intrarenal processes in the kidney. In other words, *CCL2* may be an important marker within the kidney and urine, but its expression in peripheral blood did not reflect the clinical impairment. Our findings suggest that blood *CCL2* may not be useful as an indicator for the detection of creatinine, urea, and eGFR status in all patient groups.

5. Conclusion

This study shows that classical physiological markers such as creatinine, urea, and eGFR remain reliable indicators of renal disease and exhibit strong distinction between patients and healthy groups. Peripheral blood expression of *CCL2* for CKD, AKI, and IgAN patients shows no significant difference compared with healthy control groups, nor did they correlate with renal function markers. Blood gene expression does not always reflect renal tissue expression, as *CCL2* has a mediating role in inflammation and fibrosis. The absence of correlation supports the previous result that kidney tissue and urine biomarkers are more reflective of local inflammatory activity than circulating blood mRNA. Therefore, peripheral mRNA expression may not be a sensitive diagnostic biomarker in clinical assessment of kidney disease in this cohort. Future investigations should include paired assessment of *CCL2* in blood, urine and kidney tissue, with concurrent validation at the protein levels using enzyme-linked immunosorbent assay (ELISA). In addition, a larger, multicenter cohort could help detect subtle effects and improve the generalization of findings, framing these negative results as a foundation for advancing biomarker discovery in renal disease.

6. Acknowledgements

The authors sincerely thank Dr. Adnan Abdul-Azim Al-Hassani and Dr. Talib Al-Zaidi (Nephrology & Kidney Transplantation) for their valuable contributions in clinical diagnosis, guidance, and support. The authors also acknowledge the Department of Biology, College of Science, University of Baghdad, and the Institute of Genetic Engineering and Biotechnology, University of Baghdad, for providing institutional and technical support.

7. CRediT Statement

Mohammed Hudhaifa A. Karem: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft.

Amjed Qays Ibrahim Alqaishi: Supervision, Methodology, Writing – Review & Editing.

8. Abbreviations

Abbreviation	Full Term
AKI	Acute kidney injury
AUC	Area under the curve
cDNA	complementary DNA
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease-Epidemiology collaboration
EDTA	ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
IgAN	IgA nephropathy
IQR	interquartile range
MCP-1	monocyte chemoattractant protein-1
MEST-C	Mesangial hypercellularity, Endocapillary hypercellularity, Segmental glomerulosclerosis, Tubular atrophy/interstitial fibrosis, and Crescents
qRT-PCR	Quantitative real-time PCR
ROC	Receiver operating characteristic

9. References

- Alani, H. T., & Al-Lami, M. Q. (2024). Evaluation of some biochemical and hematological parameters in patients with chronic kidney disease. *Journal of the Faculty of Medicine Baghdad*, 66(2), 154-161. <https://doi.org/10.32007/jfacmedbagdad.2269>
- Alani, M. S., & Al-Mayaly, I. K. (2022). Detection of albumin and urea in kidney failure patients by optical biosensor. *Iraqi Journal of Science*,

- 63(1), 43-52.
<https://doi.org/10.24996/ijbs.2022.63.1.5>
- Al-Jewari, K., Baban, R., & Manuti, J. (2021). Serum and urinary soluble α -Klotho levels in patients with chronic kidney disease. *Baghdad Journal of Biochemistry and Applied Biological Sciences*, 2(01), 29-38.
<https://doi.org/10.47419/bjbabs.v2i01.35>
- Al-Musawi, H. S., Al-Lami, M., & Al-Saadi, A. H. (2021). Assessment of glycemic control, renal function, and oxidative stress parameters in type 2 diabetes Mellitus Patients. *Iraqi Journal of Science*, 62(12), 4628-4638.
<https://doi.org/10.24996/ijbs.2021.62.12.4>
- Al-Razak, M. A., Al-Saadi, B., & Al Saedi, A. (2024). Evaluation of the role of two SNPs in MTHFR gene polymorphisms (rs1801133) 677 C> T and (rs 1801131) 1298A> C with homocysteine level in Iraqi patients with chronic kidney disease. *Medicina Moderna*, 31(3), 227-236.
<https://doi.org/10.31689/rmm.2024.31.3.227>
- Al-Razak, M., AlSaadi, B., & Al Saedi, A. (2025). Investigation of interleukines-4, 6 and malondialdehyde levels in serum of Iraqi patients with chronic kidney diseases. *Human Antibodies*, 33(1-2), 40-47.
<https://doi.org/10.1177/10932607251322055>
- Ávila, M., Mora Sánchez, M. G., Bernal Amador, A. S., & Paniagua, R. (2025). The metabolism of creatinine and its usefulness to evaluate kidney function and body composition in clinical practice. *Biomolecules*, 15(1), Article 41. <https://doi.org/10.3390/biom15010041>
- Bakdash, K., Schramm, K. M., Annam, A., Brown, M., Kondo, K., & Lindquist, J. D. (2019). Complications of percutaneous renal biopsy. *Seminars in Interventional Radiology*, 36(2), 97-103. <https://doi.org/10.1055/s-0039-1688422>
- Baran, J., Rogalska, M., Kuryk, L., & Staniszewska, M. (2022). Practical applications of peripheral blood mononuclear cells (PBMCs) in immunotherapy preclinical research. *Journal of Current Science and Technology*, 12(3), 592–604. <https://doi.org/10.14456/jcst.2022.45>
- Barratt, J., Mariani, L. H., Radhakrishnan, J., Rizk, D. V., Tumlin, J. A., & Lafayette, R. A. (2025). Defining disease modification in IgA nephropathy: Toward a paradigm shift in management. *Kidney International Reports*, 10(12), 4174-4187.
<https://doi.org/10.1016/j.ekir.2025.09.025>
- Brookes, E. M., & Power, D. A. (2022). Elevated serum urea-to-creatinine ratio is associated with adverse inpatient clinical outcomes in non-end stage chronic kidney disease. *Scientific Reports*, 12(1), Article 20827.
<https://doi.org/10.1038/s41598-022-25254-7>
- Choi, Y., Jacobs Jr, D. R., Shroff, G. R., Kramer, H., Chang, A. R., & Duprez, D. A. (2022). Progression of chronic kidney disease risk categories and risk of cardiovascular disease and total mortality: Coronary artery risk development in young adults cohort. *Journal of the American Heart Association*, 11(21), Article e026685.
<https://doi.org/10.1161/JAHA.122.026685>
- Delrue, C., & Speeckaert, M. M. (2025). Transcriptomic signatures in IgA nephropathy: From renal tissue to precision risk stratification. *International Journal of Molecular Sciences*, 26(20), Article 10055.
<https://doi.org/10.3390/ijms262010055>
- Duan, Z. Y., Zhang, C., Chen, X. M., & Cai, G. Y. (2024). Blood and urine biomarkers of disease progression in IgA nephropathy. *Biomarker Research*, 12(1), Article 72.
<https://doi.org/10.1186/s40364-024-00619-4>
- Eardley, K. S., Kubal, C., Zehnder, D., Quinkler, M., Lepenies, J., Savage, C. O., ... & Cockwell, P. (2008). The role of capillary density, macrophage infiltration and interstitial scarring in the pathogenesis of human chronic kidney disease. *Kidney International*, 74(4), 495-504. <https://doi.org/10.1038/ki.2008.183>
- Feng, Y., Lv, L. L., Wu, W. J., Li, Z. L., Chen, J., Ni, H. F., ... & Liu, B. C. (2018). Urinary exosomes and exosomal CCL2 mRNA as biomarkers of active histologic injury in IgA nephropathy. *The American Journal of Pathology*, 188(11), 2542-2552.
<https://doi.org/10.1016/j.ajpath.2018.07.017>
- Gao, Z., Zhu, Y., Sun, X., Zhu, H., Jiang, W., Sun, M., ... & Chang, B. (2022). Establishment and validation of the cut-off values of estimated glomerular filtration rate and urinary albumin-to-creatinine ratio for diabetic kidney disease: A multi-center, prospective cohort study. *Frontiers in Endocrinology*, 13, Article 1064665.
<https://doi.org/10.3389/fendo.2022.1064665>

- Guo, S., Zhang, Q., Guo, Y., Yin, X., Zhang, P., Mao, T., ... & Li, X. (2025). The role and therapeutic targeting of the *CCL2/CCR2* signaling axis in inflammatory and fibrotic diseases. *Frontiers in Immunology*, 15, Article 1497026. <https://doi.org/10.3389/fimmu.2024.1497026>
- Habas, E., Ali, E., Farfar, K., Errayes, M., Alfitori, J., Habas, E., ... & Elzouki, A. N. (2022). IgA nephropathy pathogenesis and therapy: Review & updates. *Medicine*, 101(48), Article e31219. <https://doi.org/10.1097/MD.00000000000031219>
- Han, S. Y., Jeong, K. H., Ihm, C. G., Kang, Y. S., & Cha, D. R. (2021). Serum interferon- γ and urinary monocyte chemoattractant peptide-1 are important factors in the pathogenesis of immunoglobulin A nephropathy. *Kidney Research and Clinical Practice*, 40(1), Article 69. <https://doi.org/10.23876/j.krcp.20.157>
- Ibrahim, R. K., Ghudhaib, K. K., & Allawi, A. A. D. (2024). Early prediction of nephropathy in Iraqi patient with diabetes type II by evaluating some relevant biochemical factors. *Baghdad Science Journal*, 21(5), 1549-1549. <https://doi.org/10.21123/bsj.2023.8008>
- Kahindo, C. K., Mukuku, O., & Mokoli, V. M. (2022). Diagnostic value of serum creatinine versus urinary neutrophil gelatinase-associated lipocalin (uNGAL) in acute kidney injury in resource-limited settings. *Urology & Nephrology Open Access Journal*, 10(1), 20-23. <https://doi.org/10.15406/unoaj.2022.10.00318>
- Keskinis, C., Moysidou, E., Stai, S., Christodoulou, M., Lioulios, G., Vamvakas, S. S., ... & Stangou, M. (2025). Prognostic value of urinary biomarkers in proteinuria progression in IgA nephropathy patients treated with budesonide. *Medicina*, 61(5), Article 807. <https://doi.org/10.3390/medicina61050807>
- Lameire, N. H., Levin, A., Kellum, J. A., Cheung, M., Jadoul, M., Winkelmayer, W. C., ... & Srisawat, N. (2021). Harmonizing acute and chronic kidney disease definition and classification: Report of a kidney disease: Improving Global Outcomes (KDIGO) consensus conference. *Kidney International*, 100(3), 516-526. <https://doi.org/10.1016/j.kint.2021.06.028>
- Lateef, A. S., Alkubaisi, M. R., AL-Hitti, I. K., AL-Rawi, K. F., Freah, Y. T., Khalaf, A. J., ... & Dawood, M. H. (2024). Evaluation of serum lipid profile and renal function levels in Iraqi patients with chronic kidney disease (CKD). *Iraqi Journal of Science*, 65(4), 1877-1885. <https://doi.org/10.24996/ij.2024.65.4.8>
- Ling, S., Xu, G., Zaccardi, F., Khunti, K., & Brunskill, N. J. (2023). Kidney function and long-term risk of end-stage kidney disease and mortality in a multiethnic population. *Kidney International Reports*, 8(9), 1761-1771. <https://doi.org/10.1016/j.ekir.2023.06.014>
- Liu, Y., Gong, Y., & Xu, G. (2023). The role of mononuclear phagocyte system in IgA nephropathy: Pathogenesis and prognosis. *Frontiers in Immunology*, 14, Article 1192941. <https://doi.org/10.3389/fimmu.2023.1192941>
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta CT$ method. *Methods*, 25(4), 402-408. <https://doi.org/10.1006/meth.2001.1262>
- Luciano, R. L., & Moeckel, G. W. (2019). Update on the native kidney biopsy: Core curriculum 2019. *American Journal of Kidney Diseases*, 73(3), 404-415. <https://doi.org/10.1053/j.ajkd.2018.10.011>
- Lv, L. L., Feng, Y. E., Wen, Y. I., Wu, W. J., Ni, H. F., Li, Z. L., ... & Liu, B. C. (2018). Exosomal *CCL2* from tubular epithelial cells is critical for albumin-induced tubulointerstitial inflammation. *Journal of the American Society of Nephrology*, 29(3), 919-935. <https://doi.org/10.1681/ASN.2017050523>
- Obrișcă, B., Mocanu, V., Jurubiță, R., Vrabie, A., Berechet, A., Lujinschi, Ș., ... & Ismail, G. (2024). Histological reappraisal of IgA nephropathy: The role of glomerular pattern of injury and mesangial complement deposition. *BMC Nephrology*, 25(1), Article 145. <https://doi.org/10.1186/s12882-024-03577-z>
- Osborn, J. W., Tyshynsky, R., & Vulchanova, L. (2021). Function of renal nerves in kidney physiology and pathophysiology. *Annual*

- Review of Physiology*, 83(1), 429-450.
<https://doi.org/10.1146/annurev-physiol-031620-091656>
- Pattrapornpisut, P., Avila-Casado, C., & Reich, H. N. (2021). IgA nephropathy: Core curriculum 2021. *American Journal of Kidney Diseases*, 78(3), 429-441.
<https://doi.org/10.1053/j.ajkd.2021.01.024>
- Pradeep, U., Chiwhane, A., Acharya, S., Daiya, V., Kasat, P. R., Sachani, P., ... & Bedi, G. N. (2024). A comprehensive review of advanced biomarkers for chronic kidney disease in older adults: Current insights and future directions. *Cureus*, 16(9), Article e70413.
<https://doi.org/10.7759/cureus.70413>
- Provenzano, M., Hu, L., Abenavoli, C., Cianciolo, G., Coppolino, G., De Nicola, L., ... & Baraldi, O. (2024). Estimated glomerular filtration rate in observational and interventional studies in chronic kidney disease. *Journal of Nephrology*, 37(3), 573-586. <https://doi.org/10.1007/s40620-024-01887-x>
- Saitoh, A., Suzuki, Y., Takeda, M., Kubota, K., Itoh, K., & Tomino, Y. (1998). Urinary levels of monocyte chemoattractant protein (MCP)-1 and disease activity in patients with IgA nephropathy. *Journal of Clinical Laboratory Analysis*, 12(1), 1-5.
[https://doi.org/10.1002/\(SICI\)1098-2825\(1998\)12:1%3C1::AID-JCLA1%3E3.0.CO;2-F](https://doi.org/10.1002/(SICI)1098-2825(1998)12:1%3C1::AID-JCLA1%3E3.0.CO;2-F)
- Salih, S. S., & Yenzeel, J. H. (2020). Evaluation of thyroid hormones and some biochemical variables in patients with chronic kidney disease. *Iraqi Journal of Science*, 61(5), 985-992.
<https://doi.org/10.24996/ij.s.2020.61.5.6>
- Siribamrungwong, M., & Chaikommon, K. (2025). Clinical characteristics and risk factors associated with acute kidney injury among hospitalized primary COVID-19 infected patients. *Chulalongkorn Medical Journal*, 69(6), Article 5.
<https://doi.org/10.56808/2673-060X.5724>
- Tan, L., Jin, L. H., Wang, Y. Q., Chen, W., & Wen, Q. (2025). Clinical features, pathological characteristics, and prognosis of patients with IgA nephropathy complicated with nephrotic syndrome. *Scientific Reports*, 15(1), Article 1804.
<https://doi.org/10.1038/s41598-025-86081-0>
- Wang, C., & Liu, H. (2022). Factors influencing degradation kinetics of mRNAs and half-lives of microRNAs, circRNAs, lncRNAs in blood in vitro using quantitative PCR. *Scientific Reports*, 12(1), Article 7259.
<https://doi.org/10.1038/s41598-022-11339-w>
- Younes-Ibrahim, M. S., & Younes-Ibrahim, M. (2022). Biomarkers and kidney diseases: A brief narrative review. *Journal of Laboratory And Precision Medicine*, 7, 1-16. <https://doi.org/10.21037/jlpm-22-1>
- Zheng, Y., Lu, P., Deng, Y., Wen, L., Wang, Y., Ma, X., ... & Tang, F. (2020). Single-cell transcriptomics reveal immune mechanisms of the onset and progression of IgA nephropathy. *Cell Reports*, 33(12), Article 108525.
<https://doi.org/10.1016/j.celrep.2020.108525>
- Zhou, X., Wang, N., Zhang, Y., & Yu, P. (2022). Expression of CCL2, FOS, and JUN may help to distinguish patients with IgA nephropathy from healthy controls. *Frontiers in Physiology*, 13, Article 840890.
<https://doi.org/10.3389/fphys.2022.840890>
- Zsom, L., Zsom, M., Salim, S. A., & Fülöp, T. (2022). Estimated glomerular filtration rate in chronic kidney disease: a critical review of estimate-based predictions of individual outcomes in kidney disease. *Toxins*, 14(2), Article 127.
<https://doi.org/10.3390/toxins14020127>