GMR
The Original

IDENTIFICATION OF MOLECULAR MARKERS AND MICRORNAS ASSOCIATED WITH DISEASE SEVERITY IN CHRONIC KIDNEY DISEASE PATIENTS WITH LIVER DYSFUNCTION

Dr. Boya Koushik Kumar[1*], Dr. Vijay Nagaonkar[2], Dr. Sushama K Jotkar[3], Dr. Rajesh J. Khyalappa[4]

[1]Junior Resident, Department of General Medicine, D.Y.Patil Medical College, Kolhapur, Maharashtra, India. D.Y Patil Education Society (Deemed to be University) Kolhapur.
[2]Associate Professor, Department of General Medicine, D.Y. Patil Medical College, Kolhapur, Maharashtra, India. D.Y. Patil Education Society (Deemed to be University) Kolhapur.
[3]Professor and HOD, Department of General Medicine, D.Y. Patil Medical College, Kolhapur, Maharashtra, India. D.Y. Patil Education Society (Deemed to be University) Kolhapur
[4]Dean and Professor, Department of General Medicine, D.Y. Patil Medical College, Kolhapur, Maharashtra, India. D.Y. Patil Education Society (Deemed to be University) Kolhapu
*Corresponding Author: Dr. Boya Koushik Kumar, Email ID: boyakoushikkumar@gmail.com

ABSTRACT

Background*:* Chronic kidney disease (CKD) is a progressive and irreversible disorder frequently associated with liver dysfunction, resulting in increased morbidity and poor clinical outcomes. Conventional renal and hepatic biomarkers may not adequately reflect the molecular mechanisms underlying disease progression. Molecular biomarkers and microRNAs (miRNAs) have emerged as promising tools for evaluating renal injury, fibrosis, inflammation, and disease severity in CKD patients with concomitant liver dysfunction.

Methods*:* This experimental cross-sectional study was conducted in the Department of General Medicine, Dr. D. Y. Patil Medical College, Hospital and Research Institute, Kolhapur. Sixty adult CKD patients with liver dysfunction and twenty healthy controls were enrolled. Clinical, hematological, renal, and liver function parameters were assessed. Plasma levels of Kidney Injury Molecule-1 (KIM-1), Neutrophil Gelatinase-Associated Lipocalin (NGAL), and Matrix Metalloproteinase-9 (MMP-9) were measured. Expression of miR-21, miR-29, and miR-122 was quantified using quantitative real-time PCR. Biomarker levels were compared between groups and across CKD stages, and correlations with estimated glomerular filtration rate (eGFR), serum creatinine, blood urea, and liver function tests were analyzed.

Results*:* CKD patients with liver dysfunction demonstrated significantly elevated KIM-1 (417.27 vs. 209.08 pg/mL), NGAL (447.27 vs. 210.05 pg/mL), MMP-9 (2311.52 vs. 1632.56 pg/mL), and miR-21 expression (2.71 vs. 1.29-fold), while miR-29 expression was significantly reduced (0.38 vs. 1.09-fold) compared with controls (all $p<0.001$). These biomarkers exhibited progressive stage-wise alterations with advancing CKD severity. MiR-29 showed the strongest correlation with eGFR ($r=+0.974$, $p<0.001$) and serum creatinine ($r=-0.916$, $p<0.001$), followed by KIM-1, NGAL, MMP-9, and miR-21. MiR-122 demonstrated no significant differences between groups or correlations with renal or hepatic parameters. None of the evaluated biomarkers showed significant association with liver function tests.

Conclusion*:* KIM-1, NGAL, MMP-9, miR-21, and particularly miR-29 are strongly associated with CKD severity in patients with liver dysfunction. Among these, miR-29 emerged as the most sensitive molecular marker for disease staging and progression. These biomarkers may serve as valuable non-invasive tools for risk stratification, monitoring, and precision management of CKD patients with concomitant liver dysfunction.

KEYWORDS: Chronic Kidney Disease; Liver Dysfunction; KIM-1; NGAL; MMP-9; microRNA-21; microRNA-29; microRNA-122; Biomarkers; Renal Fibrosis; Disease Severity.

INTRODUCTION

Chronic kidney disease (CKD) is a advanced as well as irretrievable disorder considered by a steady decline in renal function over time, eventually foremost to end-stage renal illness if left untreated. It represents a main global health problem due to its rising prevalence, significant morbidity, as well as strong association with multiple comorbidities. Among these, liver dysfunction is an important yet often under-recognized complication in patients with CKD. This coexistence arises from a complex interplay of systemic inflammation, metabolic disturbances, oxidative stress, and altered hemodynamics. The kidney and liver are closely interconnected through shared roles in metabolism, detoxification, and immune regulation. Consequently, dysfunction in one organ can significantly influence the

function of the other, leading to a cycle of progressive damage, poorer clinical outcomes, and limited therapeutic options. Understanding the molecular mechanisms underlying this interaction is therefore essential for improving diagnosis, prognostication, and management strategies. [1,2]

In recent years, molecular biomarkers have gained prominence as valuable tools for evaluating disease progression and severity in complex multisystem disorders such as CKD. These biomarkers deliver vision into fundamental pathophysiological procedures including inflammation, fibrosis, and cellular injury, which are not sufficiently captured by conservative biochemical constraints. In CKD participants with liver dysfunction, the identification of reliable molecular markers can facilitate initial recognition, risk stratification, as well as targeted therapeutic interventions. Among the important biomarkers, Kidney Injury Molecule-1 (KIM-1) as well as Neutrophil Gelatinase-Associated Lipocalin (NGAL) are engrained indicators of renal tubular injury, while Matrix Metalloproteinase-9 (MMP-9) possess a importantpart in extracellular matrix remodeling and fibrosis. These markers collectively reflect key pathological processes involved in both renal and hepatic injury, making them relevant candidates for assessing disease severity in this patient population. [2,3]

MicroRNAs (miRNAs) have emerged as a novel class of molecular regulators with considerable diagnostic and prognostic potential. These small, non-coding RNA molecules, roughly 18–25 nucleotides in length, control gene appearance at the post-transcriptional level by compulsory to target messenger RNAs, subsequent in translational repression or else deprivation. MiRNAs are complicated in numerous biological procedures like cell propagation, apoptosis, inflammation, and fibrosis mechanisms that are central to the pathogenesis of both CKD and liver disease. Notably, miRNAs are steady in movement and can be noticed in body fluids like serum and plasma, making them promising non-invasive biomarkers. Among these, miR-21 and miR-29 are closely related with fibrotic pathways, whereas miR-122, a liver-specific miRNA, possess a vital part in hepatic metabolism and injury. Vicissitudes in the expression of these miRNAs might be linked to disease progression and severity in both renal and hepatic disorders. [4,5]

The pathophysiological link between CKD and liver dysfunction is multifactorial. CKD contributes to systemic inflammation, accretion of uremic toxins, oxidative stress, and metabolic dysregulation, all of which can adversely affect liver function. Conversely, liver dysfunction can accelerate renal injury through impaired detoxification, altered protein synthesis, and hormonal imbalances. This bidirectional relationship creates a vicious cycle that exacerbates damage in both organs. Molecular markers such as KIM-1 and NGAL reflect ongoing renal injury, while MMP-9 is associated with tissue remodeling and fibrosis, a common pathway in chronic organ damage. Similarly, miRNAs like miR-21 and miR-29 regulate fibrotic processes, and miR-122 reflects hepatic involvement, thereby providing a comprehensive molecular perspective of dual-organ dysfunction. [6,7]

Fibrosis is a key mechanism underlying the progression of both CKD and liver disease. It involves extreme deposition of extracellular matrix components arbitrated through activation of fibroblasts and pro-fibrotic gesturing pathways, predominantly converting development factor-beta (TGF-β). MMP-9 plays a critical part in matrix remodeling and fibrosis, and its dysregulation contributes to pathological tissue changes. In parallel, miR-21 is known to promote fibrosis, whereas miR-29 generally acts as an anti-fibrotic regulator by inhibiting collagen synthesis. The imbalance between these molecular factors can accelerate disease progression. Inflammation as well as oxidative stress further underwrite to cellular wound and organ dysfunction, reinforcing the importance of these biomarkers in assessing disease severity. [8,9]

Metabolic dysregulation also plays a significant role in the interaction between CKD and liver dysfunction. CKD is frequently associated with abnormalities in lipid and glucose metabolism, including insulin resistance and dyslipidemia. Liver dysfunction can exacerbate these disturbances due to impaired metabolic processing. MiR-122, a liver-specific microRNA, is closely linked to lipid metabolism and hepatocellular integrity, and its altered expression serves as an important indicator of hepatic dysfunction. The combined assessment of molecular markers and miRNAs can therefore provide valuable insights into the metabolic and structural alterations occurring in these patients. [10,11]

The integration of molecular biomarkers and miRNAs into clinical evaluation holds significant promise for advancing precision medicine in patients with CKD and liver dysfunction. These markers can enhance risk stratification, enable early diagnosis, and guide individualized therapeutic strategies. Furthermore, they might assist as potential goals for novel therapeutic interventions and as surrogate endpoints in clinical research. Thus, the study of selected molecular markers such as KIM-1, NGAL, and MMP-9 and also miRNAs miR-21, miR-29, and miR-122 is vital for improving our sympathetic of illness mechanisms and optimizing patient outcomes in this complex clinical condition. [12,13]

The goal of this investigation was to measure the identification of molecular markers and microrn as accompanying by illness severity in chronic kidney disease participants through liver dysfunction.

METHODOLOGY

An observational, cross-sectional study was conducted at the Department of General Medicine, Dr. D.Y. Patil Medical College, Hospital, and Research Institute in Kolhapur on CKD patients with liver dysfunction. Participants were recruited from both outpatient (OPD) and inpatient (IPD) departments. After getting ethical approval from the Institutional Ethics Committee study was started. The study was conducted for a total duration of 18 months. Participants fulfilling the inclusion and exclusion criteria were selected for the study. The minimum essential sample size was 60 patients. An additional 20 controls were included for comparative analysis.

Inclusion Criteria (in points): Adults aged ≥18 years identified through CKD (stages 1–5 as per KDIGO guidelines), Concurrent liver dysfunction confirmed by elevated liver enzymes (AST/ALT ≥1.5× ULN) or imaging evidence of hepatic pathology, Willingness to provide informed consent, Availability of complete clinical records, including eGFR, serum creatinine, and liver function tests.

Exclusion Criteria (in points): Acute kidney injury or acute liver failure, History of organ transplantation, Active infections (e.g., HBV, HCV, HIV), Immunosuppressive therapy within the last 6 months, Alcohol or substance abuse disorder.

Study Procedure

Sample Collection: Venous blood (5 mL) was drawn into EDTA tubes, inverted gently, and centrifuged at 1500 × g for 10 minutes to isolate plasma.
RNA Isolation: Overall RNA was removed by means of a commercial kit (e.g., Qiagen miRNeasy), followed by DNase treatment to remove genomic DNA.
cDNA Synthesis: Reverse transcription was achieved by means of Superscript IV, with spike-in controls for miRNA normalization.
qPCR Analysis: TaqMan assays quantified target miRNAs and mRNAs. Housekeeping genes (GAPDH, U6 snRNA) served as endogenous controls.

RESULT

The CKD with liver disease group had a higher median age compared to controls (52.5 [IQR: 40.0–64.3] vs 42.0 [IQR: 32.5–58.3]) and a higher mean age (52.2 ± 17.2 years vs 44.1 ± 16.0 years). The largest proportion of participants in both groups belonged to the 50–59year age category. Participants aged 60 years and above were more frequent in the CKD+liver cohort associated to controls. However, the alteration in age distribution between the two cohort was not statistically important (Mann–Whitney U=758.5, p=0.0791).

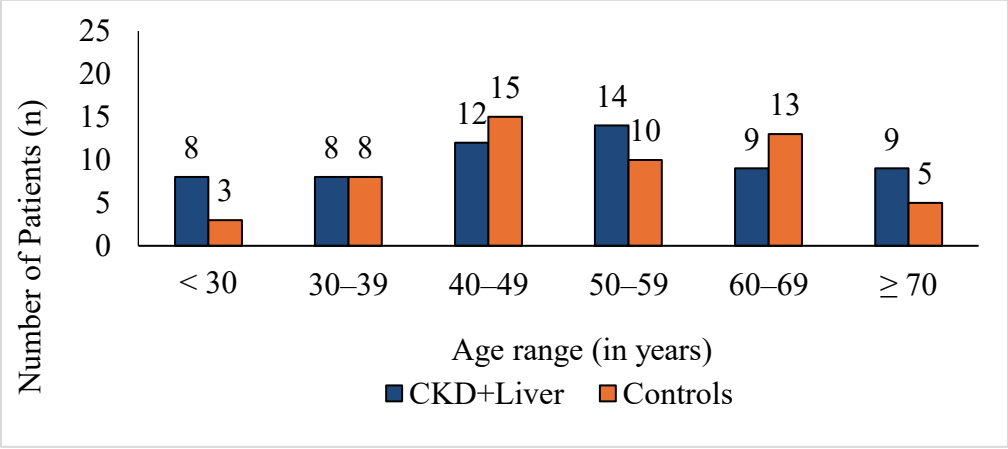

Figure 1: Age distribution for CKD+Liver group (left) and Control group (Right)

Table 1: Gender Distribution

Sex	CKD+Liver (n=60)	Control (n=20)	Chi-square (p-value)
Male	38 (63.3%)	12 (60.0%)	χ^2=0.000, p=1.000 (ns)
Female	22 (36.7%)	8 (40.0%)	

Gender distribution was comparable between the two groups (p=1.000). Male predominance observed in both groups.

Table 2: CKD Stage Distribution (CKD+Liver Group)

'CKD Stage	n	Percentage (%)	eGFR Range (KDIGO)	Median eGFR [IQR]
Stage 2	3	5.0%	60–89 mL/min/1.73m²	61.0 [60.0–68.5]
Stage 3a	10	16.7%	45–59 mL/min/1.73m²	49.0 [46.5–50.8]
Stage 3b	15	25.0%	30–44 mL/min/1.73m²	33.0 [32.5–36.0]
Stage 4	21	35.0%	15–29 mL/min/1.73m²	21.0 [16.0–26.0]
Stage 5	11	18.3%	<15 mL/min/1.73m²	12.0 [10.5–14.0]'
Total	60	100%		

The majority of patients were in CKD Stage 4 (35.0%), followed by Stage 3b (25.0%) and Stage 5 (18.3%), indicating that the recruited population had predominantly advanced CKD. Controls were classified as "No CKD" (n=20).

Table 3: Haematological Parameters

Parameter	CKD+Liver Median [IQR]	Control Median [IQR]	p-value	Sig.
Hb% (g/dL)	10.10 [8.58–11.12]	13.65 [13.17–15.35]	<0.001	***
WBC (cells/mm³)	9430 [4862–11200]	6610 [5855–7910]	0.046	*
Platelets (cells/mm³)	173500 [140750–240000]	253500 [211250–321000]	<0.001	***

CKD patients exhibited significantly lower haemoglobin (p less than 0.001) and also platelet counts (p less than 0.001) associated to controls, consistent with anaemia of chronic kidney illness as well as thrombocytopaenia associated with chronic liver disease. WBC counts were marginally elevated in CKD patients (p = 0.046), reflecting low-grade systemic inflammation.

Table 4: Liver Function Parameters

Parameter	CKD+Liver Median [IQR]	Control Median [IQR]	p-value	Sig.
SGOT (U/L)	46.48 [26.54–63.62]	17.74 [14.92–23.55]	<0.001	***
SGPT (U/L)	39.10 [25.04–53.80]	21.86 [17.93–25.19]	<0.001	***
Total Bilirubin (mg/dL)	2.25 [1.65–2.97]	0.61 [0.46–0.86]	<0.001	***
Direct Bilirubin (mg/dL)	1.44 [1.05–2.23]	0.15 [0.12–0.21]	<0.001	***
Albumin (g/dL)	3.34 [2.79–3.52]	4.19 [4.03–4.35]	<0.001	***
Total Proteins (g/dL)	5.71 [5.17–6.42]	6.99 [6.78–7.28]	<0.001	***
Alk. Phosphatase (U/L)	111.88 [99.05–135.96]	78.65 [67.86–89.92]	<0.001	***

All LFT parameters were meaningfully abnormal in the CKD+Liver cohort (p less than 0.001 for all). Elevated transaminases (SGOT, SGPT), hyper bilirubinaemia, hypo albuminaemia, and elevated alkaline phosphatase confirm the diagnosis of liver dysfunction in the patient cohort and validate the clinical inclusion criteria.

Table 5: Renal Function Parameters

Parameter	CKD+Liver Median [IQR]	Control Median [IQR]	p-value	Sig.
Blood Urea (mg/dL)	77.25 [57.45–99.44]	20.62 [17.27–25.96]	<0.001	***
Serum Creatinine (mg/dL)	2.38 [1.83–3.96]	0.76 [0.67–0.84]	<0.001	***
eGFR (mL/min/1.73m²)	28.50 [15.75–37.75]	106.00 [99.00–117.25]	<0.001	***

CKD patients had markedly elevated blood urea and creatinine with severely reduced eGFR, confirming advanced renal impairment in the patient group. The eGFR values did not overlap at all with controls (U = 0.0), reflecting complete separation of renal function between the two populations.

Table 6: Molecular Marker Levels

Marker	CKD+Liver Median [IQR]	Control Median [IQR]	p-value	Sig.
KIM-1 (pg/mL)	417.27 [352.97–572.55]	209.08 [172.90–228.84]	<0.001	***
NGAL (pg/mL)	447.27 [355.75–587.86]	210.05 [176.35–235.96]	<0.001	***
MMP-9 (pg/mL)	2311.52 [1925.49–2586.76]	1632.56 [1511.62–1741.34]	<0.001	***

All three molecular markers were meaningfully elevated in the CKD+Liver cohort (p < 0.001 for all). KIM-1 and NGAL showed approximately 2-fold elevations, while MMP-9 showed a 1.4-fold elevation. These results support the

diagnostic usefulness of these indicators in distinguishing CKD patients with liver dysfunction from healthy individuals.

Table 7: MicroRNA Expression Levels

miRNA	CKD+Liver Median [IQR]	Control Median [IQR]	p-value	Sig.
miR-21 (Fold Change)	2.71 [2.16–3.57]	1.29 [1.16–1.40]	<0.001	***
miR-29 (Fold Change)	0.38 [0.30–0.49]	1.09 [1.02–1.17]	<0.001	***
miR-122 (Fold Change)	3.66 [3.10–4.26]	3.65 [3.21–3.91]	0.718	ns

miR-21 was meaningfully upregulated, miR-29 was meaningfully downregulated in CKD+Liver patients vs controls. miR-122, a liver-specific miRNA, showed no significant difference between groups.

MOLECULAR MARKERS AND miRNAs ACROSS CKD STAGES

To evaluate whether biomarker levels varied significantly with CKD disease stage (Stages 2, 3a, 3b, 4, and 5), a Kruskal-Wallis H test was performed.

To evaluate whether biomarker levels varied significantly with CKD disease stage, a Kruskal-Wallis H test was performed across Stages 3a, 3b, 4, and 5 (n=57). Stage 2 (n=3) was retained in the descriptive table for completeness but was excluded from the Kruskal-Wallis analysis due to insufficient subgroup representation ($n < 6$), which precludes reliable non-parametric comparison.

Table 8: Kruskal-Wallis Analysis Across CKD Stages

Biomarker	H statistic	p-value	Stage 2 Med. [IQR]	Stage 3a Med. [IQR]	Stage 3b Med. [IQR]	Stage 4 Med. [IQR]
KIM-1 (pg/mL)	49.0	<0.001***	268.8 [263.9–272.6]	320.5 [298.4–348.4]	384.2 [345.9–402.1]	509.2 [439.5–610.5]
NGAL (pg/mL)	50.6	<0.001***	298.6 [285.6–302.3]	337.6 [308.3–344.7]	390.9 [348.3–442.7]	515.8 [437.6–582.5]
MMP-9 (pg/mL)	49.1	<0.001***	1735.8 [1571.6–1875.0]	1889.8 [1843.6–1922.1]	2030.0 [1824.5–2218.4]	2463.8 [2327.8–2599.0]
miR-21 (FC)	50.2	<0.001***	1.90 [1.78–2.00]	2.10 [1.94–2.21]	2.47 [2.14–2.61]	3.16 [2.72–3.61]
miR-29 (FC)	51.5	<0.001***	0.71 [0.68–0.77]	0.59 [0.56–0.61]	0.46 [0.43–0.48]	0.32 [0.30–0.36]
miR-122 (FC)	4.6	0.328 (ns)	3.08 [2.62–3.41]	3.40 [3.21–3.65]	3.36 [2.88–4.29]	4.06 [3.53–4.50]
eGFR (mL/min)	51.5	<0.001***	61.0 [60.0–68.5]	49.0 [46.5–50.8]	33.0 [32.5–36.0]	21.0 [16.0–26.0]

Biomarker	Stage 5 Median [IQR]	Trend
KIM-1 (pg/mL)	680.3 [559.1–723.4]	Progressive increase →
NGAL (pg/mL)	676.5 [617.0–705.8]	Progressive increase →
MMP-9 (pg/mL)	2749.3 [2681.9–2923.9]	Progressive increase →
miR-21 (FC)	3.98 [3.58–4.23]	Progressive increase →
miR-29 (FC)	0.27 [0.24–0.29]	Progressive decrease ←
eGFR (mL/min)	12.0 [10.5–14.0]	Progressive decrease ←

FC = Fold Change relative to housekeeping reference.

Effect Size:

Effect sizes were computed as η^2 (eta-squared) by means of the formula $\eta^2 = (H - k + 1) / (n - k)$, where H = Kruskal-Wallis H statistic, k = number of cohort (4 stages, excluding Stage 2), and n = total observations (57). Benchmarks: η^2 less than 0.01 = negligible; 0.01–0.06 = small; 0.06–0.14 = moderate; > 0.14 = large.

Levels of KIM-1 and NGAL progressively increased from CKD Stage 2 to Stage 5. The Kruskal–Wallis H test (performed across Stages 3a–5, n=57; Stage 2 excluded due to n=3) demonstrated significant overall differences across CKD stages for both biomarkers (KIM-1: H=49.0, p<0.001; NGAL: H=50.6, p<0.001). miR-21 expression also showed progressive upregulation from Stage 3a (median fold change = 2.10) to Stage 5 (median fold change = 3.98), with significant differences across stages (H=50.2, p<0.001). In contrast, miR-29 expression progressively declined with advancing CKD, decreasing from a 0.590-fold change in Stage 3a to 0.270-fold in Stage 5 (H=51.5, p<0.001). MMP-9 levels demonstrated a stepwise increase from Stage 3a (median 1889.8 pg/mL) to Stage 5 (median 2749.3 pg/mL), with significant variation across CKD stages (H=49.1, p<0.001).

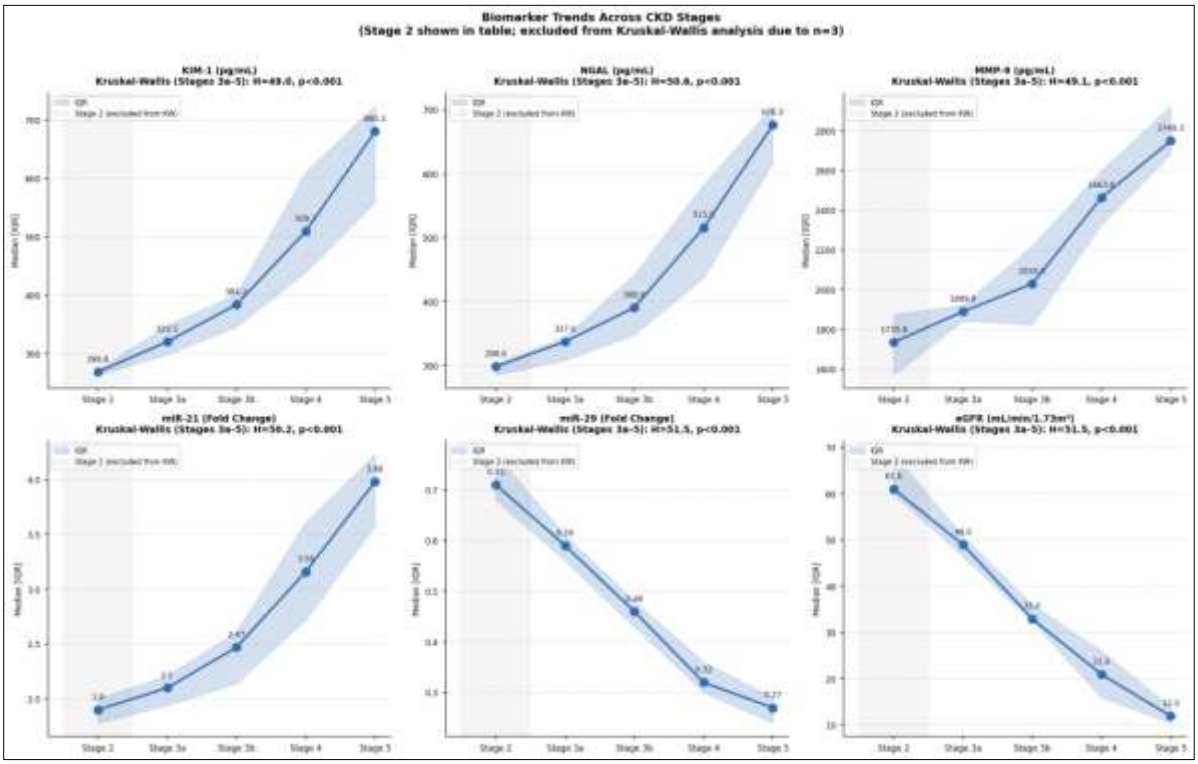

Figure 2: Trend of biomarker levels across CKD Stages 3a–5 (Kruskal-Wallis analysis). Stage 2 is displayed in the table for reference but was excluded from statistical analysis (n=3). Shaded bands represent IQR.

Pairwise Mann-Whitney U tests through Bonferroni correction were achieved to classify which stage pairs differed significantly.

Post-hoc pairwise comparisons showed that comparisons involving Stage 2 could not be assessed because of inadequate subgroup representation. Between Stages 3a and 3b, most biomarkers were not significantly different, while miR-29 and eGFR presented exceedingly important alterations (p less than 0.001). Contrasts between Stage 3a and Stage 4 demonstrated highly significant differences across all biomarkers and eGFR ($p<0.001$). Stage 3a versus Stage 5 comparisons also showed significant differences for all markers, with eGFR demonstrating the highest level of significance (p less than 0.001). Similarly, Stage 3b versus Stage 4 and Stage 3b versus Stage 5 comparisons revealed significant differences across most biomarkers, particularly miR-29 and eGFR. Between Stages 4 and 5, KIM-1 and MMP-9 did not show significant differences, whereas NGAL, miR-21, miR-29, and eGFR remained significantly different between the two stages.

CORRELATION OF MOLECULAR MARKERS/miRNAs WITH CLINICAL PARAMETERS

Spearman rank correlations were computed between biomarkers and eGFR as the primary indicator of CKD severity (n=60, CKD+Liver group).

Table 10: Correlation with eGFR (Renal Function Indicator)

Biomarker	Spearman r	p-value	Sig.	Interpretation
KIM-1 (pg/mL)	−0.902	<0.001	***	Strong negative correlation
NGAL (pg/mL)	−0.821	<0.001	***	Strong negative correlation
MMP-9 (pg/mL)	−0.819	<0.001	***	Strong negative correlation
miR-21 (FC)	−0.835	<0.001	***	Strong negative correlation
miR-29 (FC)	+0.974	<0.001	***	Strong positive correlation
miR-122 (FC)	−0.140	0.287	ns	No significant correlation
Serum Creatinine	−0.930	<0.001	***	Expected inverse reference
Blood Urea	−0.372	0.004	**	Moderate negative correlation

Correlation analysis presented that miR-29 had a strong positive association with eGFR, with its expression progressively decreasing as renal function declined. KIM-1 demonstrated a strong negative correlation through eGFR,

with levels increasing as renal function worsened. Serum creatinine also presented a strong negative correlation through eGFR and served as the reference marker for comparison with the novel biomarkers. Similarly, miR-21 exhibited a strong negative association through eGFR, with progressive upregulation observed across advancing CKD stages. NGAL demonstrated a strong negative association with eGFR, with levels increasing alongside CKD progression. MMP-9 also showed a strong negative correlation with eGFR, with progressively higher levels seen in advanced disease stages. Blood urea demonstrated a moderate negative correlation with eGFR. In contrast, miR-122 did not demonstration any important association through eGFR.

Table 11: Correlation with Renal Parameters (Creatinine and Blood Urea)

Biomarker	vs Creatinine r	p	vs Blood Urea r	p	Sig.	Interpretation
KIM-1	+0.953	<0.001	+0.392	0.002	***/***	Strong positive correlation with creatinine and moderate positive correlation with blood urea; both statistically significant
NGAL	+0.862	<0.001	+0.690	<0.001	***/**	Strong positive correlations with both creatinine and blood urea
MMP-9	+0.847	<0.001	+0.434	<0.001	***/***	Strong positive correlation with creatinine and moderate positive correlation with blood urea
miR-21	+0.894	<0.001	+0.712	<0.001	***/***	Strong positive correlations with both renal function markers
miR-29	−0.916	<0.001	−0.323	0.012	***/*	Strong negative correlation with creatinine and weak-to-moderate negative correlation with blood urea
miR-122	+0.090	0.492	+0.167	0.203	ns	No statistically significant correlation with either creatinine or blood urea

KIM-1 showed the strongest correlation with creatinine (r=+0.953), making it an excellent surrogate renal injury marker. miR-29 inversely correlates with creatinine (r=−0.916) consistent with its role as a fibrosis suppressor that is progressively silenced.

Table 12: Correlation with Liver Function Tests

Biomarker	SGOT r (p)	SGPT r (p)	Tot. Bil r (p)	Alk. Phos r (p)	Albumin r (p)	Significant
KIM-1	+0.128 (0.33)	−0.019 (0.88)	+0.050 (0.71)	+0.022 (0.87)	−0.072 (0.58)	None
NGAL	+0.020 (0.88)	−0.078 (0.55)	+0.063 (0.63)	+0.001 (0.99)	−0.004 (0.98)	None
MMP-9	+0.078 (0.55)	+0.070 (0.59)	+0.061 (0.64)	+0.051 (0.70)	+0.032 (0.81)	None
miR-21	+0.029 (0.82)	−0.073 (0.58)	+0.089 (0.50)	−0.020 (0.88)	−0.039 (0.77)	None
miR-29	−0.087 (0.51)	+0.048 (0.72)	+0.060 (0.65)	+0.022 (0.87)	−0.020 (0.88)	None
miR-122	−0.051 (0.70)	+0.185 (0.16)	+0.060 (0.65)	+0.202 (0.12)	−0.039 (0.77)	None

Notably, none of the molecular markers or miRNAs showed significant correlation with liver function assessments (SGOT, SGPT, bilirubin, alkaline phosphatase, albumin) in the CKD+Liver group. This indicates these biomarkers primarily reflect renal injury severity rather than hepatic functional impairment, and their elevation in CKD is predominantly driven by the degree of renal dysfunction.

Table 13: Correlation of Molecular Biomarkers with Hepatic Parameters

‘Biomarker	Total Bilirubin r (sig)	ALT (SGPT) r (sig)	AST (SGOT) r (sig)	Albumin r (sig)	Significant
KIM-1	0.050 (ns)	-0.019 (ns)	0.128 (ns)	-0.072 (ns)	**None**
NGAL	0.063 (ns)	-0.078 (ns)	0.020 (ns)	-0.004 (ns)	**None**
MMP-9	0.061 (ns)	0.070 (ns)	0.078 (ns)	0.032 (ns)	**None**
miR-21	0.089 (ns)	-0.073 (ns)	0.029 (ns)	-0.039 (ns)	**None**
miR-29	0.060 (ns)	0.048 (ns)	-0.087 (ns)	-0.020 (ns)	**None**
miR-122	0.060 (ns)	0.185 (ns)	-0.051 (ns)	-0.039 (ns)	**None’**

Correlation analysis presented no statistically important associations between any of the evaluated biomarkers and liver function parameters, including total bilirubin, ALT (SGPT), AST (SGOT), and serum albumin (all p>0.05). KIM-1, NGAL, MMP-9, miR-21, miR-29, and miR-122 demonstrated only weak correlation coefficients with all liver function markers, and none reached statistical significance.

DIAGNOSTIC AND PROGNOSTIC POTENTIAL OF BIOMARKERS

Table 14: Summary of Biomarker Performance

Biomarker	CKD vs Control	Stage Staging	Strongest Correlation
KIM-1	↑ ($p<0.001$)	H=44.9***	eGFR r=−0.902
NGAL	↑ ($p<0.001$)	H=38.2***	Creatinine r=+0.862
MMP-9	↑ ($p<0.001$)	H=36.8***	Creatinine r=+0.847
miR-21	↑ ($p<0.001$)	H=37.6***	Creatinine r=+0.894
miR-29	↓ ($p<0.001$)	H=52.1***	eGFR r=+0.974
miR-122	ns	ns	None

Based on the correlation analysis, miR-29 demonstrated the strongest correlation with eGFR (r=+0.974, p less than 0.001), stronger even than serum creatinine, suggesting it as the most sensitive molecular marker for grading CKD severity in patients with concomitant liver dysfunction. KIM-1 also showed excellent performance (r=−0.902 with eGFR, r=+0.953 with creatinine).

All five biomarkers (KIM-1, NGAL, MMP-9, miR-21, miR-29) showed significant stepwise changes across CKD stages on Kruskal-Wallis testing (all $p<0.001$), supporting their combined utility in disease staging. Post-hoc analysis showed the most consistent differentiation between Stage 3a and Stage 4, and between Stage 3b and Stage 5.

The absence of significant correlation between any marker and liver function tests (SGOT, SGPT, bilirubin, albumin) suggests that in CKD patients, these markers reflect renal rather than hepatic disease activity. Further study incorporating dedicated hepatic fibrosis markers (e.g., TIMP-1, procollagen III, hyaluronic acid, FIB-4 index) would help delineate the hepatic component.

ROC Curve Analysis

Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic utility of NGAL, KIM-1, miR-21, and miR-29 in identifying severe CKD and distinguishing CKD with liver dysfunction patients from healthy controls. Table 13 summarises the AUC, optimal cut-off values, sensitivity, and specificity for each biomarker.

Table 15: ROC Analysis - Diagnostic Performance of Biomarkers

Biomarker	Comparison	AUC	Optimal Cut-off	Sensitivity	Specificity
NGAL	Severe CKD (Stage 4–5) vs. Non-severe CKD	0.900	457.4 pg/mL	81.2%	89.3%
KIM-1	Advanced Renal Dysfunction (Stage 4–5) vs. Non-severe CKD	0.967	434.6 pg/mL	84.4%	100.0%
miR-21	CKD+Liver Dysfunction vs. Healthy Controls	1.000	1.750 fold change	100.0%	100.0%
miR-29	CKD+Liver Dysfunction vs. Healthy Controls	1.000	≤0.840 fold change	100.0%	100.0%

NGAL demonstrated an AUC of 0.900 for identifying severe CKD (Stage 4–5) versus non-severe CKD, with an optimal cut-off of 457.4 pg/mL yielding a sensitivity of 81.2% and specificity of 89.3%. KIM-1 showed a higher AUC of 0.967 for discriminating advanced renal dysfunction (Stage 4–5), with a cut-off of 434.6 pg/mL providing 84.4% sensitivity and 100.0% specificity. Both miR-21 and miR-29 achieved perfect discrimination between CKD with liver dysfunction patients and healthy controls, with AUC of 1.000 each. For miR-21, a fold-change cut-off of 1.750 identified all CKD patients with 100% sensitivity and specificity. For miR-29, a fold-change threshold of ≤0.840 similarly achieved perfect separation, reflecting the consistent and marked downregulation of miR-29 in the patient group.

DISCUSSION

The goal of the current investigation was to classify and assess molecular markers and microRNAs associated with disease severity in patients with chronic kidney disease (CKD) and liver dysfunction, and to determine their relationship with renal function, hepatic parameters, and CKD stage progression. Conventional haematological, renal, and liver function parameters were evaluated along with KIM-1, NGAL, MMP-9, miR-21, miR-29, and miR-122. The study demonstrated that KIM-1, NGAL, MMP-9, miR-21, and miR-29 were significantly associated with CKD severity, while miR-29 showed the strongest relationship with eGFR and creatinine, suggesting its potential role as a

sensitive molecular staging marker. These findings support the use of non-invasive molecular biomarkers for monitoring disease progression and risk stratification in CKD patients with liver dysfunction.

Age and Gender Distribution

The CKD with liver dysfunction group was older than controls, with a median age of 52.5 years [IQR: 40.0–64.3] versus 42.0 years [IQR: 32.5–58.3], although the difference was not statistically significant (U=758.5, p=0.0791). Most CKD+Liver patients belonged to the 50–59 years age group, and older age categories were more common among cases. This pattern supports the chronic and progressive nature of combined liver-kidney dysfunction. Similar observations regarding renal involvement in chronic liver disease populations were reported by Iovanescu V *et al*. [14], while Eren Z and Kantarcı G emphasized the importance of accurate renal assessment in chronic liver disease [15].

Gender distribution was comparable between groups, with males accounting for 63.3% of CKD+Liver patients and 60.0% of controls (χ^2=0.000, p=1.000). Therefore, the significant biochemical, molecular, and microRNA differences observed were likely related to disease status rather than sex distribution. This supports previous evidence that liver-kidney interaction remains clinically important independent of demographic variables [16].

CKD Stage Distribution

Most patients had moderate-to-advanced CKD, with Stage 4 being the most common (35.0%), followed by Stage 3b (25.0%), Stage 5 (18.3%), Stage 3a (16.7%), and Stage 2 (5.0%). Overall, 78.3% of participants belonged to Stage 3b or higher. Median eGFR values declined progressively from 61.0 mL/min/1.73m² in Stage 2 to 12.0 mL/min/1.73m² in Stage 5, validating disease classification and providing a framework for biomarker assessment. Francoz C *et al*. highlighted the importance of biomarkers such as NGAL and KIM-1 in chronic liver disease-associated renal dysfunction [17], while Asrani SK *et al*. described these markers as promising tools for renal injury detection [18].

Haematological Parameters

Haemoglobin was significantly lower in CKD+Liver patients than controls (10.10 vs. 13.65 g/dL, p<0.001), indicating a substantial burden of anaemia. This likely reflects reduced erythropoietin production, chronic inflammation, uraemia, nutritional deficiencies, and hepatic dysfunction. Carmona A *et al*. reported inflammatory microRNA alterations in advanced CKD, supporting the role of systemic inflammation in disease progression [19].

WBC count was significantly higher in CKD+Liver patients (9,430 vs. 6,610 cells/mm³, p=0.046), suggesting chronic low-grade inflammation. CKD and liver dysfunction both contribute to inflammatory activation through uraemic toxins, oxidative stress, immune dysregulation, and cytokine imbalance. Similar inflammatory associations have been reported by Carmona A *et al*. [19] and Cisilotto J *et al*. [20].

Platelet count was significantly reduced in CKD+Liver patients (173,500 vs. 253,500 cells/mm³, p<0.001). This thrombocytopaenia likely reflects impaired thrombopoietin production, hypersplenism, chronic inflammation, and advanced liver disease. Similar liver-related renal involvement has been documented by Iovanescu V *et al*. [14].

Liver and Renal Function Parameters

Liver function tests were markedly abnormal in CKD+Liver patients. SGOT, SGPT, total bilirubin, direct bilirubin, and alkaline phosphatase were significantly elevated, whereas albumin and total protein were significantly reduced (all p<0.001). Although Zhang Y *et al*. demonstrated that miR-122 increases with liver injury severity [21], the present study found no significant miR-122 alteration despite marked biochemical abnormalities.

Renal function parameters confirmed severe renal impairment. Blood urea and serum creatinine were significantly elevated, while eGFR was markedly reduced (all p<0.001). Eren Z and Kantarcı G noted limitations of creatinine-based renal assessment in chronic liver disease [15], and Francoz C *et al*. emphasized the value of novel biomarkers such as NGAL and KIM-1 [17]. In the present study, conventional renal parameters established CKD severity, while molecular markers provided additional evidence of tubular injury and disease progression.

Molecular Biomarkers

KIM-1

KIM-1 was significantly elevated in CKD+Liver patients (417.27 vs. 209.08 pg/mL, p<0.001) and increased progressively across CKD stages. It showed a strong negative correlation with eGFR (r=−0.902, p<0.001) and a strong positive correlation with creatinine (r=+0.953, p<0.001). These findings support previous reports identifying KIM-1 as an important marker of renal tubular injury [17,18].

NGAL
NGAL was significantly elevated in CKD+Liver patients (447.27 vs. 210.05 pg/mL, $p<0.001$) and increased progressively with disease stage. Strong correlations were observed with eGFR ($r=-0.821$), creatinine ($r=+0.862$), and blood urea ($r=+0.690$) (all $p<0.001$). These findings are consistent with studies by Elshemy EE *et al*. [22] and Francoz C *et al*. [17], which identified NGAL as a valuable marker of renal injury in chronic liver disease.

MMP-9
MMP-9 was significantly elevated in CKD+Liver patients (2311.52 vs. 1632.56 pg/mL, $p<0.001$) and increased progressively across CKD stages. Significant correlations with eGFR ($r=-0.819$), creatinine ($r=+0.847$), and blood urea ($r=+0.434$) indicate its association with extracellular matrix remodelling and progressive renal fibrosis. These findings align with evidence linking molecular changes to fibrotic progression in CKD [23].

MicroRNA Expression
miR-21
miR-21 was significantly upregulated in CKD+Liver patients (2.71 vs. 1.29 fold change, $p<0.001$) and increased progressively with advancing CKD stage. It showed strong correlations with eGFR ($r=-0.835$), creatinine ($r=+0.894$), and blood urea ($r=+0.712$). Schauerte C *et al*. demonstrated the profibrotic role of miR-21 in chronic renal injury [24], while Martín-Taboada M *et al*. highlighted its role in fibrotic signalling within the adipo-hepato-renal axis [25]. The present findings support its role as a marker of CKD progression and fibrosis.

miR-29
miR-29 was significantly downregulated in CKD+Liver patients (0.38 vs. 1.09 fold change, $p<0.001$) and progressively declined across CKD stages. It demonstrated the strongest association with disease severity, showing a very strong positive correlation with eGFR ($r=+0.974$) and a strong negative correlation with creatinine ($r=-0.916$). Donderski R *et al*. also reported altered miR-29 expression in CKD-associated fibrosis [23]. The present findings suggest that miR-29 is the most sensitive molecular marker of CKD severity and fibrotic progression.

miR-122
Unlike the other biomarkers, miR-122 showed no significant difference between CKD+Liver patients and controls ($p=0.718$), no stage-wise variation ($p=0.328$), and no significant correlation with renal or hepatic parameters. Although Zhang Y *et al*. reported its association with liver injury severity [21], the present study suggests that miR-122 may be more relevant in specific hepatocellular injury states than in CKD-associated liver dysfunction.

Correlation Analysis and Clinical Significance
Most biomarkers demonstrated strong associations with renal function. KIM-1, NGAL, MMP-9, and miR-21 correlated negatively with eGFR and positively with creatinine, whereas miR-29 showed the strongest positive correlation with eGFR and negative correlation with creatinine. Similar relationships between circulating miRNAs and renal function have been reported by Kumar A *et al*. [26].

Notably, none of the evaluated biomarkers showed significant correlations with liver function tests. Even miR-122 failed to correlate with SGOT, SGPT, bilirubin, alkaline phosphatase, or albumin. These findings indicate that KIM-1, NGAL, MMP-9, miR-21, and miR-29 primarily reflect renal injury and CKD severity rather than hepatic biochemical impairment [21,20].

Diagnostic and Prognostic Potential
KIM-1, NGAL, MMP-9, miR-21, and miR-29 successfully distinguished CKD patients with liver dysfunction from healthy controls and demonstrated progressive stage-related changes. KIM-1 and NGAL reflected tubular injury, MMP-9 represented extracellular matrix remodelling, miR-21 indicated profibrotic activity, and miR-29 emerged as the strongest molecular staging marker. These observations support previous findings regarding the utility of molecular biomarkers and microRNAs in renal risk assessment and fibrosis monitoring [24,23,18].

Overall, the study demonstrates that miR-29, KIM-1, NGAL, MMP-9, and miR-21 have significant diagnostic and prognostic value in CKD patients with liver dysfunction, with miR-29 showing the greatest potential as a sensitive indicator of disease severity and progression.

CONCLUSION
The present study concluded that patients with chronic kidney disease (CKD) and liver dysfunction exhibited significant haematological, hepatic, renal, molecular, and microRNA alterations compared with healthy controls. Although the CKD with liver dysfunction group had a higher median age than controls (52.5 vs. 42.0 years), the

difference was not statistically significant (p=0.0791), and gender distribution was comparable between groups (p=1.000). The study population predominantly represented advanced CKD, with Stage 4 being the most common stage (35.0%), followed by Stage 3b (25.0%) and Stage 5 (18.3%). A progressive decline in eGFR from 61.0 mL/min/1.73m² in Stage 2 to 12.0 mL/min/1.73m² in Stage 5 confirmed increasing renal severity.

CKD patients with liver dysfunction showed significant anaemia, thrombocytopaenia, and inflammatory tendency, with reduced haemoglobin and platelet counts and increased WBC counts. Liver dysfunction was evident through significantly elevated SGOT, SGPT, bilirubin, and alkaline phosphatase levels, along with reduced albumin and total proteins (all $p<0.001$). Renal impairment was also marked, with significantly elevated blood urea and serum creatinine and markedly reduced eGFR compared with controls (all $p<0.001$).

Molecular-marker analysis demonstrated significantly elevated KIM-1, NGAL, and MMP-9 levels, indicating increased renal tubular injury, inflammatory activation, and extracellular matrix remodeling. MicroRNA profiling revealed significant miR-21 upregulation and miR-29 downregulation (both $p<0.001$), whereas miR-122 showed no significant difference between groups (p=0.718). Stage-wise analysis showed progressive increases in KIM-1, NGAL, MMP-9, and miR-21, while miR-29 progressively declined with advancing CKD stage. Significant stage-wise differences were observed for all these biomarkers ($p<0.001$), whereas miR-122 remained non-significant.

Correlation analysis identified miR-29 as the strongest molecular marker of CKD severity, showing a very strong positive correlation with eGFR ($r=+0.974$) and a strong negative correlation with serum creatinine ($r=-0.916$). KIM-1 also demonstrated excellent performance, with strong correlations with creatinine ($r=+0.953$) and eGFR ($r=-0.902$). NGAL, MMP-9, and miR-21 similarly showed strong associations with renal function. Importantly, none of the biomarkers or microRNAs correlated significantly with liver function tests, indicating that their alterations primarily reflected renal disease severity rather than hepatic biochemical impairment.

Therefore, KIM-1, NGAL, MMP-9, miR-21, and especially miR-29 are promising biomarkers for identifying and grading CKD severity in patients with liver dysfunction. Among these, miR-29 emerged as the most sensitive molecular staging marker, whereas miR-122 showed no meaningful diagnostic, staging, or correlation utility in the present cohort.

REFERENCES

1. Goren Y, Kushnir M, Zafrir B, Tabak S, Lewis BS, Amir O. Serum levels of microRNAs in patients with heart failure. Eur J Heart Fail. 2012;14(2):147-154.
2. Chen C, Lu X, Yan S, Zhu H, Ma Y, Wang D, *et al*. miR-122 regulates hepatitis B virus replication through cyclin G1–modulated p53 pathway. J Virol. 2011;85(17):8917-8929.
3. Li S, Zhu J, Zhang W, Chen Y, Zhang K, Popescu LM, *et al*. Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. Circulation. 2011;124(2):175-184.
4. Zuo L, Liu Y, Chen Q, Li X, Ni T, Ma Y, *et al*. Plasma microRNAs characterizing hepatocellular carcinoma in patients with chronic hepatitis B. J Clin Oncol. 2011;29(4):478-486.
5. Neal CS, Michael MZ, Pimlott LK, Yong TY, Li JY, Gleadle JM. Circulating microRNA expression is reduced in chronic kidney disease. Nephrol Dial Transplant. 2011;26(11):3794-3802.
6. Wang B, Jha JC, Hagiwara S, McClelland AD, Jandeleit-Dahm K, Thomas MC, *et al*. Transforming growth factor-β1–mediated renal fibrosis is dependent on the regulation of transforming growth factor receptor 1 translation by RNA-binding protein human antigen R. J Biol Chem. 2015;290(52):31107-31119.
7. Marquez RT, Bandyopadhyay S, Wendlandt EB, Keck K, Hoffer BA, Icardi MS, *et al*. Correlation between microRNA expression levels and clinical parameters associated with chronic hepatitis C viral infection in humans. Lab Invest. 2010;90(12):1727-1736.
8. Xu J, Wu C, Che X, Wang L, Yu D, Zhang T, *et al*. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. Mol Carcinog. 2011;50(2):136-142.
9. Chung AC, Huang XR, Meng X, Lan HY. miR-192 mediates TGF-β/Smad3-driven renal fibrosis. J Am Soc Nephrol. 2010;21(8):1317-1325.
10. Ho J, Ng KH, Rosen S, Dostal A, Gregory RI, Kreidberg JA. Podocyte-specific loss of functional microRNAs leads to rapid glomerular and tubular injury. J Am Soc Nephrol. 2008;19(11):2069-2075.
11. Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, *et al*. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology. 2011;53(1):209-218.
12. Ben-Dov IZ, Tan YC, Morozov P, Wilson PD, Rennert H, Blumenfeld JD, *et al*. Urine microRNA as potential biomarkers of autosomal dominant polycystic kidney disease progression: A pilot study. Eur J Hum Genet. 2014;22(6):784-790.

13. Wang J, Gao Y, Ma M, Li M, Zou D, Yang J, *et al*. Effect of miR-155 on renal fibrosis by regulating epithelial-to-mesenchymal transition in diabetic nephropathy. Kidney Blood Press Res. 2018;43(2):297-309.
14. Iovanescu V, Streba C, Alexandru D, Constantinescu A, Vere C, Rogoveanu I, *et al*. Particularities of Renal Manifestations in Chronic Viral Hepatitis. Curr Health Sci J. 2015;41:227-32.
15. Eren Z, Kantarcõ G. Assessment of Renal Functions in Patients with Liver Disease: Which One is Correct? 2010.
16. Wijarnpreecha K, Thongprayoon C, Scribani M, Ungprasert P, Cheungpasitporn W. Noninvasive fibrosis markers and chronic kidney disease among adults with nonalcoholic fatty liver in USA. Eur J Gastroenterol Hepatol. 2017;30:404–10.
17. Francoz C, Nadim M, Durand F. Kidney biomarkers in cirrhosis. J Hepatol. 2016;65(4):809-24.
18. Asrani S, Shankar N, da Graca B, Nadim M, Cárdenas A. Role of Novel Kidney Biomarkers in Patients With Cirrhosis and After Liver Transplantation. Liver Transpl. 2021;28.
19. Carmona A, Guerrero F, Jimenez MJ, Ariza F, Agüera M, Obrero T, *et al*. Inflammation, Senescence and MicroRNAs in Chronic Kidney Disease. Front Cell Dev Biol. 2020;8.
20. Cisilotto J, do Amaral AE, Rosolen D, Rode M, Silva AH, Winter E, *et al*. MicroRNA profiles in serum samples from Acute-On-Chronic Liver Failure patients and miR-25-3p as a potential biomarker for survival prediction. Sci Rep. 2020;10.
21. Zhang Y, Jia Y, Zheng R, Guo Y, Wang Y, Guo H, *et al*. Plasma microRNA-122 as a biomarker for viral-, alcohol-, and chemical-related hepatic diseases. Clin Chem. 2010;56(12):1830-8.
22. Elshemy EE, Omar H, Ahmed A, Eltaweel E. Serum and urinary neutrophil gelatinase-associated lipocalin in chronic liver disease patients and its value in detecting renal impairment. Sci J Al-Azhar Med Fac Girls. 2020;4:42–51.
23. Donderski R, Szczepanek J, Naruszewicz N, Naruszewicz R, Tretyn A, Skoczylas-Makowska N, *et al*. Analysis of profibrogenic microRNAs (miRNAs) expression in urine and serum of chronic kidney disease (CKD) stage 1–4 patients and their relationship with proteinuria and kidney function. Int Urol Nephrol. 2021;54:937–47.
24. Schauerte C, Hübner A, Rong S, Wang S, Shushakova N, Mengel M, *et al*. Antagonism of profibrotic microRNA-21 improves outcome of murine chronic renal allograft dysfunction. Kidney Int. 2017;92(3):646-56.
25. Martín-Taboada M, Vila-Bedmar R, Medina-Gómez G. From Obesity to Chronic Kidney Disease: How Can Adipose Tissue Affect Renal Function? Nephron. 2021;145:609–13.
26. Kumar A, Priyadarshini G, Parameswaran S, Ramesh A, Rajappa M. Evaluation of MicroRNA 145 and MicroRNA 155 as Markers of Cardiovascular Risk in Chronic Kidney Disease. Cureus. 2024;16.