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SERUM MICRORNA AS POTENTIAL BIOMARKER FOR ASSESSING SEVERITY IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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ABSTRACT

Background*:* Chronic kidney disease (CKD) is a progressive disorder characterized by irreversible decline in renal function and increased risk of cardiovascular morbidity and mortality. Conventional biomarkers such as serum creatinine, blood urea, and estimated glomerular filtration rate (eGFR) have limitations in accurately reflecting ongoing molecular injury and disease severity. MicroRNAs (miRNAs), particularly miRNA-21 and miRNA-29, have emerged as promising non-invasive biomarkers due to their involvement in renal fibrosis, inflammation, and disease progression.

Methods*:* A case-control cohort study was conducted in the Department of Medicine and Intensive Care Unit at Dr. D. Y. Patil Medical College, Hospital and Research Institute, Kolhapur, over a period of 18 months. A total of 127 participants were enrolled, including 98 CKD patients and 29 healthy controls. Patients were categorized according to CKD stage based on eGFR. Serum miRNA-21 and miRNA-29 expression levels were measured and correlated with renal function parameters including eGFR, serum creatinine, and serum urea. Statistical analysis was performed to assess differences between groups and correlations with disease severity.

Results: CKD patients demonstrated significantly reduced eGFR (16.86 ± 13.56 vs. 104.90 ± 20.05 mL/min/1.73 m²; p=0.001) and significantly elevated serum creatinine (5.54 ± 2.68 vs. 0.79 ± 0.18 mg/dL; p=0.001) and serum urea (109.61 ± 41.84 vs. 32.65 ± 15.56 mg/dL; p=0.001) compared with controls. Mean serum miRNA-21 expression was significantly higher in CKD patients than controls (4.42 ± 1.43 vs. 1.45 ± 0.29; p<0.001), while mean serum miRNA-29 expression was significantly lower (0.25 ± 0.11 vs. 0.99 ± 0.13; p<0.001). Stage-wise analysis showed progressive elevation of miRNA-21 from 2.38 ± 0.98 in Stage 2 to 5.33 ± 0.95 in Stage 5 CKD, whereas miRNA-29 progressively declined from 0.63 ± 0.01 in Stage 2 to 0.18 ± 0.03 in Stage 5 CKD (p=0.001). miRNA-21 showed strong negative correlation with eGFR (r = -0.806, p<0.001) and positive correlations with serum creatinine (r = 0.953, p<0.001) and serum urea (r = 0.831, p<0.001). Conversely, miRNA-29 demonstrated a strong positive correlation with eGFR (r = 0.995, p<0.001) and negative correlations with serum creatinine (r = -0.784, p<0.001) and serum urea (r = -0.702, p<0.001).

Conclusion*:* Serum miRNA-21 and miRNA-29 are significantly associated with CKD severity. miRNA-21 acts as a positive marker of disease progression, while miRNA-29 behaves as an inverse marker reflecting preserved renal function. These findings support the potential utility of serum miRNA-21 and miRNA-29 as non-invasive biomarkers for assessing disease severity and monitoring progression in patients with chronic kidney disease.

KEYWORDS: Chronic kidney disease, CKD, microRNA-21, microRNA-29, biomarker, eGFR, serum creatinine, renal fibrosis, disease severity.

INTRODUCTION

Chronic kidney disease (CKD) is a global health burden with rising prevalence and significant morbidity and mortality. It is characterized by a gradual and irreversible decline in renal function, ultimately leading to end-stage kidney disease (ESKD) requiring dialysis or transplantation. The global prevalence of CKD is estimated to affect 9–13% of the population, with diabetes mellitus and hypertension being the leading etiological factors. Despite advances in renal replacement therapies, CKD is associated with poor clinical outcomes and a high risk of cardiovascular complications. Traditional diagnostic and prognostic measures, such as serum creatinine, estimated glomerular filtration rate (eGFR), and proteinuria, are often limited by low sensitivity and specificity, as they reflect renal damage only after substantial

nephron loss has occurred. This creates an urgent need for novel, sensitive biomarkers that can detect kidney injury at an earlier stage and assess disease severity with greater precision [1,2].

MicroRNAs (miRNAs), a class of small, non-coding RNA molecules of approximately 18–25 nucleotides, have recently emerged as promising candidates for biomarker discovery. They are highly conserved, regulate post-transcriptional gene expression, and play critical roles in cellular processes such as apoptosis, proliferation, differentiation, and fibrosis. Importantly, miRNAs exhibit remarkable stability in serum and other body fluids due to their encapsulation within extracellular vesicles or binding to proteins, making them suitable for non-invasive biomarker applications. In CKD, dysregulated miRNA expression has been linked to key pathogenic mechanisms, including glomerulosclerosis, tubulointerstitial fibrosis, inflammation, and vascular calcification. Studies have demonstrated that circulating miRNAs not only reflect kidney injury but also correlate with disease severity, suggesting their potential utility in both diagnosis and prognosis [3,4].

The role of inflammation and fibrosis in CKD progression highlights the mechanistic significance of miRNAs. For instance, miR-21 has been shown to promote renal fibrosis by targeting metabolic pathways and enhancing transforming growth factor-β (TGF-β)-mediated signaling, which is central to the development of glomerulosclerosis. Similarly, miR-29 family members are known to regulate extracellular matrix deposition and their downregulation has been implicated in progressive renal scarring. In addition, miR-192 has been reported to influence TGF-β signaling and collagen expression in diabetic nephropathy, underscoring the importance of miRNAs in disease-specific pathways. The consistent association between these molecules and fibrotic processes in the kidney strengthens the argument that serum miRNA signatures could serve as biomarkers for assessing CKD severity and progression [5,6].

Another advantage of using serum miRNAs as biomarkers lies in their early detectability. Traditional measures such as eGFR decline or proteinuria often manifest late, whereas miRNA dysregulation can precede overt renal dysfunction. For example, circulating levels of miR-21 and miR-155 have been found elevated in patients with early CKD, suggesting that miRNA profiling could enable earlier diagnosis and timely interventions. Moreover, dynamic changes in miRNA levels have been reported to correlate with CKD stages, offering the possibility of disease severity stratification and monitoring therapeutic responses [7].

Recent advances in high-throughput technologies, such as next-generation sequencing (NGS) and quantitative real-time polymerase chain reaction (qRT-PCR), have facilitated large-scale profiling of serum miRNAs. These methods provide sensitive and specific quantification, allowing researchers to identify unique miRNA signatures associated with CKD. Notably, studies have reported distinct panels of serum miRNAs that differentiate CKD patients from healthy controls, and even among CKD stages. For instance, one study identified a panel including miR-21, miR-210, and miR-423 as potential markers for renal function decline, demonstrating diagnostic accuracy superior to traditional biochemical markers. These findings underscore the translational potential of miRNAs into clinical practice as tools for patient risk stratification [8].

The clinical implications of adopting serum miRNAs as biomarkers are profound. They offer the potential to personalize CKD management by enabling early detection, accurate staging, and prediction of disease progression. This could guide therapeutic interventions, such as initiating Reno protective therapies or adjusting treatment intensity based on miRNA profiles. Additionally, since miRNAs regulate multiple pathways, their measurement could provide mechanistic insights into the heterogeneity of CKD, helping to distinguish between different etiologies and responses to therapy. Ultimately, integrating serum miRNAs into clinical practice may help shift the paradigm of CKD management from reactive to proactive care [10,11].

MEHODOLOGY

A case-control cohort studywas conducted in the Ward and Intensive Care Unit (ICU) of the Medicine Department at Dr. D. Y. Patil Medical College, Hospital, and Research Institute, Kolhapur. The purposive sampling technique was used. Duration of the study was 18 months. The study was started after getting ethical approval from Institutional Ethics Committee. The study subjects were selected according to exclusion and inclusion criteria. The sample size was 124.

Inclusion Criteria: Patients clinically diagnosed with chronic kidney disease, Patients representing all stages of CKD severity: mild, moderate, and severe, as determined by estimated glomerular filtration rate (eGFR) and serum creatinine, Patients with documented structural or functional abnormalities of the kidneys persisting for more than 3 months, Patients with eGFR<60 mL/min/1.73 m^2 for more than 3 months, Patients who provided written informed consent after counselling, in English or Marathi, Patients whose clinical and laboratory profiles also included relevant liver function assessments (AST, ALT, bilirubin) and other standard renal markers.

Exclusion Criteria: Patients with a history of renal transplantation, Patients receiving immunosuppressive therapy, Patients with a recent episode of acute kidney injury, Patients diagnosed with severe co-existing systemic conditions such as malignancies or autoimmune disorders, Patients unwilling to provide consent or withdraw from participation at any point during the study.

RESULT

Comparison of Clinical and Demographic Characteristics Between CKD and Control Groups

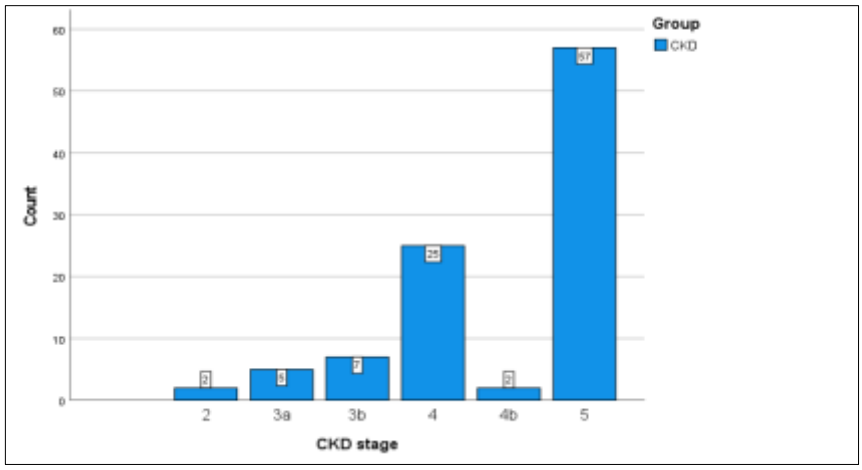

Figure 1: Distribution of CKD Stages Among Study Participants

Among the study population n=29 controls and n= 98 were CKD patients were enrolled. Among which the Stage 5 CKD was the most common category, observed in 57 (58.2%) participants, followed by Stage 4 in 25 (25.5%) participants. No control participants belonged to any CKD stage category.

Table 1: Demographic Characteristics of Study Participants

Variable	Category	CKD N (%) Mean ± SD	Control N (%) Mean ± SD	P value
Age Group	18–40	15 (15.3%)	9 (31.0%)	0.157
	41–60	49 (50.0%)	11 (37.9%)	
	>60	34 (34.7%)	9 (31.0%)	
Age		53.96 ± 13.91	49.24 ± 16.51	0.12
Sex	Female	39 (39.8%)	8 (27.6%)	0.232
	Male	59 (60.2%)	21 (72.4%)	

Among 127 study participants, 98 belonged to the CKD group and 29 to the control group. The mean age of participants in the CKD group was 53.96 ± 13.91 years, whereas the control group had a mean age of 49.24 ± 16.51 years. Participants with CKD demonstrated a comparatively higher mean age than controls. However, no statistical significance in mean age In the CKD group, 49 (50.0%) participants were aged 41–60 years, followed by 34 (34.7%) aged above 60 years and 15 (15.3%) aged 18–40 years. In the control group, 11 (37.9%) participants were aged 41–60 years, while 9 (31.0%) each belonged to the 18–40 years and >60years categories. Male participants predominated in both groups, accounting for 59 (60.2%) in the CKD group and 21 (72.4%) in the control group. No statistically significant association was observed between age group or sex distribution and study groups ($p>0.05$).

Description: Comorbidity assessment demonstrated a statistically significant difference between CKD and control groups ($p<0.001$). Type 2 diabetes mellitus with hypertension was the most common comorbidity among CKD patients, observed in 49 (50.0%) participants, compared to 4 (13.8%) in controls. Hypertension alone was identified in 34 (34.7%) CKD patients and 3 (10.3%) controls. Only 4 (4.1%) participants in the CKD group had no comorbidities compared to 20 (69.0%) in the control group.

Description: Addiction patterns did not differ significantly between the study groups ($p=0.090$). The majority of participants reported no addiction, accounting for 65 (66.3%) CKD patients and 26 (89.7%) controls. Reduced appetite was significantly more common among CKD patients, affecting 33 (33.7%) participants compared to only 2 (6.9%)

controls (p=0.005). Similarly, reduced sleep was observed in 56 (57.1%) CKD patients, whereas only 2 (6.9%) participants in the control group reported reduced sleep, demonstrating a statistically significant association (p<0.001).

Pallor was present in 33 (33.7%) CKD patients and 5 (17.2%) controls, although the association was not statistically significant (p=0.157). Oedema was significantly more common among CKD patients, affecting 29 (29.6%) participants, whereas none of the control participants had oedema (p=0.001).

Table 2: Comparison of miRNA-21 Fold Change Between CKD and Control Groups

Variable	Group	N	Mean ± SD
miRNA-21 Fold Change	Control	29	1.45 ± 0.29
	CKD	98	4.42 ± 1.43

Description:
The mean miRNA-21 fold change was markedly elevated in the CKD group (4.42 ± 1.43) compared to the control group (1.45 ± 0.29). The observed increase in miRNA-21 expression among CKD patients suggests significant upregulation of this biomarker in individuals with chronic kidney disease (p <0.001)

Table 3: Comparison of miRNA-29 Fold Change Between CKD and Control Groups

Variable	Group	N	Mean ± SD
miRNA-29 Fold Change	Control	29	0.99 ± 0.13
	CKD	98	0.25 ± 0.11

The mean miRNA-29fold change was substantially lower in the CKD group (0.25 ± 0.11) compared to the control group (0.99 ± 0.13). The reduction in miRNA-29 expression among CKD patients indicates significant downregulation of this biomarker in chronic kidney disease.

Table 4: Comparison of Biochemical and Hematological Parameters Between CKD and Control Groups

Variable	Control (Mean ± SD)	CKD (Mean ± SD)	P Value
eGFR	104.90 ± 20.05	16.86 ± 13.56	0.001
Serum Creatinine	0.79 ± 0.18	5.54 ± 2.68	0.001
Serum Urea	32.65 ± 15.56	109.61 ± 41.84	0.001
Serum Albumin	3.79 ± 0.74	3.60 ± 1.12	0.38
Hb%	12.29 ± 2.76	10.10 ± 2.55	0.001
WBC	6872.40 ± 3409.62	6233.22 ± 3990.79	0.43
Platelet Count	260413.79 ± 123643.07	237021.28 ± 93356.75	0.27

Comparison of biochemical and hematological parameters demonstrated significant renal dysfunction among CKD patients. The mean eGFR was markedly lower in the CKD group (16.86 ± 13.56) compared to controls (104.90 ± 20.05) (p=0.001). Serum creatinine and serum urea levels were significantly elevated among CKD patients, with mean values of 5.54 ± 2.68 and 109.61 ± 41.84, respectively, compared to 0.79 ± 0.18 and 32.65 ± 15.56 in controls (p=0.001 for both). Hemoglobin levels were significantly reduced in CKD patients (10.10 ± 2.55) compared to controls (12.29 ± 2.76) (p=0.001). Serum albumin, WBC count, and platelet count did not demonstrate statistically significant differences between the study groups (p>0.05).

Table 5: Comparison of miRNA-21 Fold Change Across CKD Stages

Stage (CKD)	Mean	Standard Deviation	P Value
Control	1.45	0.29	0.001
Stage 2	2.38	0.98	
Stage 3	2.61	0.63	
Stage 4	3.47	0.92	
Stage 5	5.33	0.95	

miRNA-21fold change demonstrated a progressive increase across advancing CKD stages. The control group exhibited a mean miRNA-21 expression of 1.45 ± 0.29, while Stage 2 CKD patients showed a mean value of 2.38 ± 0.98. Expression levels further increased in Stage 3 (2.61 ± 0.63), Stage 4 (3.47 ± 0.92), and Stage 5 CKD (5.33 ± 0.95). Overall comparison between stages was statistically significant (p=0.001), indicating progressive upregulation of miRNA-21 with worsening renal disease severity.

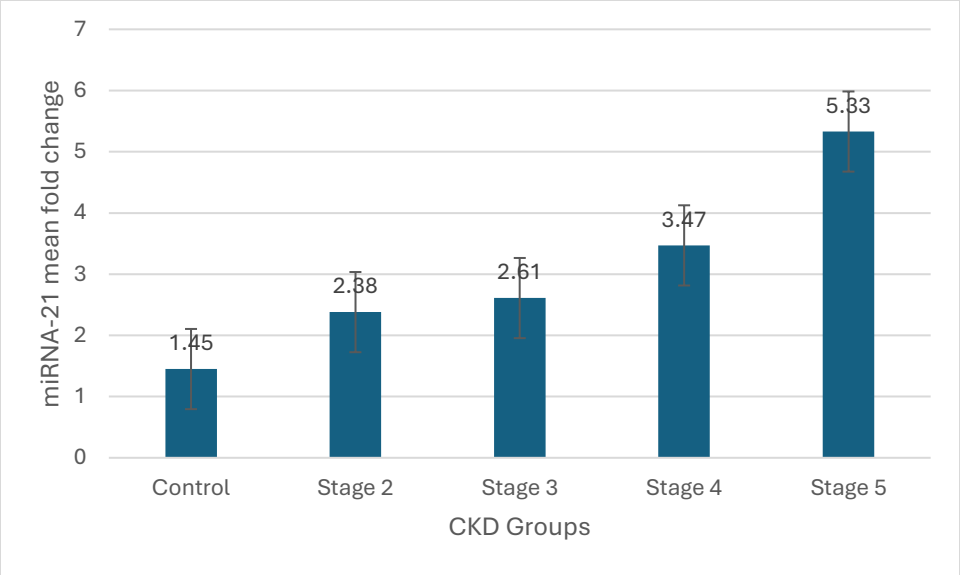

Figure 2: Comparison of miRNA-21 Fold Change Across CKD Stages

Table 6: Pairwise Comparison of miRNA-21 Fold Change Across CKD Stages

(I) Stage (CKD)	(J) Stage (CKD)	Mean Difference (I–J)	Std. Error	P value
Control	2.00	-0.93000	0.59428	1.000
Control	3.00	-1.16083*	0.27902	0.001
Control	4.00	-2.01815*	0.21739	<0.001
Control	5.00	-3.87596*	0.18541	<0.001
2.00	3.00	-0.23083	0.62085	1.000
2.00	4.00	-1.08815	0.59570	0.702
2.00	5.00	-2.94596*	0.58479	<0.001
3.00	4.00	-0.85731*	0.28202	0.029
3.00	5.00	-2.71513*	0.25818	<0.001
4.00	5.00	-1.85782*	0.18991	<0.001

Pairwise comparison analysis demonstrated significantly higher miRNA-21 expression in advanced CKD stages. Compared to controls, Stage 3, Stage 4, and Stage 5 CKD showed significantly elevated miRNA-21fold change ($p \leq 0.001$). Stage 5 CKD demonstrated the highest increase in expression compared to controls, with a mean difference of -3.87596. Significant differences were also observed between Stage 3 and Stage 4 ($p=0.029$), Stage 3 and Stage 5 ($p<0.001$), and Stage 4 and Stage 5 ($p<0.001$), indicating progressive elevation of miRNA-21 with CKD progression.

Table 7: Comparison of miRNA-29 Fold Change Across CKD Stages

Stage (CKD)	Mean	Standard Deviation	P Value
Control	0.99	0.13	0.001
Stage 2	0.63	0.01	
Stage 3	0.45	0.07	
Stage 4	0.27	0.05	
Stage 5	0.18	0.03	

miRNA-29fold change demonstrated a progressive decline across advancing CKD stages. The control group exhibited a mean expression of 0.99 ± 0.13, which decreased to 0.63 ± 0.01 in Stage 2 CKD. Expression levels further declined in Stage 3 (0.45 ± 0.07), Stage 4 (0.27 ± 0.05), and Stage 5 CKD (0.18 ± 0.03). Overall comparison between stages was statistically significant ($p=0.001$), indicating marked downregulation of miRNA-29 with worsening CKD severity.

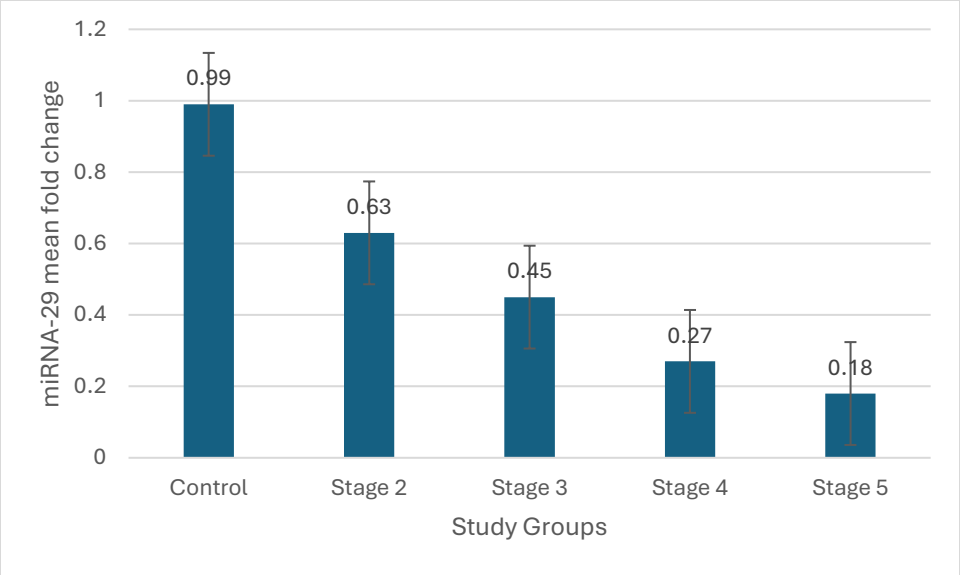

Figure 3: Comparison of miRNA-29 Fold Change Across CKD Stages

Table 8: Pairwise Comparison of miRNA-29 Fold Change Across CKD Stages

(I) Stage (CKD)	(J) Stage (CKD)	Mean Difference (I–J)	Std. Error	P value
Control	2.00	0.35655*	0.05302	<0.001
Control	3.00	0.53239*	0.02489	<0.001
Control	4.00	0.72137*	0.01939	<0.001
Control	5.00	0.80199*	0.01654	<0.001
2.00	3.00	0.17583*	0.05539	0.019
2.00	4.00	0.36481*	0.05315	<0.001
2.00	5.00	0.44544*	0.05217	<0.001
3.00	4.00	0.18898*	0.02516	<0.001
3.00	5.00	0.26961*	0.02303	<0.001
4.00	5.00	0.08062*	0.01694	<0.001

Pairwise comparison analysis revealed significant reductions in miRNA-29 expression across advancing CKD stages. Compared to controls, all CKD stages demonstrated significantly lower miRNA-29fold change ($p<0.001$). Significant differences were also identified between Stage 2 and Stage 3 ($p=0.019$), Stage 2 and Stage 4 ($p<0.001$), Stage 2 and Stage 5 ($p<0.001$), Stage 3 and Stage 4 ($p<0.001$), Stage 3 and Stage 5 ($p<0.001$), and Stage 4 and Stage 5 ($p<0.001$), indicating progressive downregulation of miRNA-29 with disease advancement.

Table 9: Correlation of miRNA-21 and miRNA-29 Fold Change with eGFR

		eGFR	Serum Creatinine	Serum urea	Serum albumin	miRNA21_FoldChange
Serum Creatinine	Pearson Correlation	-.768**	--			
	Sig. (2-tailed)	.000				
Serum urea	Pearson Correlation	-.666**	.637**	--		
	Sig. (2-tailed)	.000	.000			
Serum albumin	Pearson Correlation	.095	-.066	-.062	--	
	Sig. (2-tailed)	.333	.488	.517		
miRNA21_FoldChange	Pearson Correlation	-.806**	.953**	.831**	-.059	--
	Sig. (2-tailed)	.000	.000	.000	.530	
miRNA29_FoldChange	Pearson Correlation	.995**	-.784**	-.702**	.120	-.821**
	Sig. (2-tailed)	.000	.000	.000	.204	.000

Pearson's correlation analysis demonstrated significant associations between renal function parameters and circulating microRNA expression levels. Estimated glomerular filtration rate (eGFR) exhibited a strong and statistically significant negative correlation with serum creatinine ($r = -0.768$, $p < 0.001$), indicating that declining renal filtration capacity was associated with elevated creatinine levels. Similarly, eGFR showed a significant negative correlation with serum urea ($r = -0.666$, $p < 0.001$), suggesting worsening renal function with increasing blood urea concentration. Serum creatinine and serum urea demonstrated a strong positive correlation ($r = 0.637$, $p < 0.001$), reflecting the close physiological relationship between these conventional renal biomarkers in assessing kidney dysfunction. Serum albumin, however, did not demonstrate any statistically significant correlation with eGFR, serum creatinine, or serum urea ($p > 0.05$), indicating that albumin levels remained relatively independent of the measured renal functional parameters in the present cohort.

miRNA-21fold change showed a very strong positive correlation with serum creatinine ($r = 0.953$, $p < 0.001$) and serum urea ($r = 0.831$, $p < 0.001$), while demonstrating a strong negative correlation with eGFR ($r = -0.806$, $p < 0.001$). These findings indicate that increased miRNA-21 expression was significantly associated with worsening renal impairment. No significant association was observed between miRNA-21fold change and serum albumin levels ($r = -0.059$, $p = 0.530$). In contrast, miRNA-29fold change demonstrated an almost perfect positive correlation with eGFR ($r = 0.995$, $p < 0.001$), suggesting that higher miRNA-29 expression was strongly associated with preserved renal function. Additionally, miRNA-29 showed significant negative correlations with serum creatinine ($r = -0.784$, $p < 0.001$), serum urea ($r = -0.702$, $p < 0.001$), and miRNA-21fold change ($r = -0.821$, $p < 0.001$). These findings imply that miRNA-29 may exhibit a protective or inverse relationship with renal dysfunction, in contrast to the pathogenic association observed with miRNA-21. No statistically significant correlation was identified between miRNA-29fold change and serum albumin ($r = 0.120$, $p = 0.204$).

ROC Analysis

Table 10: Diagnostic Performance of Serum miRNA-21 and miRNA-29 for Differentiating CKD Patients from Healthy Controls

Biomarker	AUC (95% CI)	Cut-off Value	Sensitivity (%)	Specificity (%)	P value
miRNA-21	0.994	> 2.22	95.9	100.0	<0.001
miRNA-29	1.000	< 0.64	100.0	100.0	<0.001
Combined miRNA-21 + miRNA-29	0.997	> 0.604*	98.0	100.0	<0.001

*Predicted probability derived from binary logistic regression model incorporating both miRNA-21 and miRNA-29.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of serum miRNA-21 and miRNA-29 in distinguishing CKD patients from healthy controls. Serum miRNA-21 demonstrated excellent diagnostic accuracy with an area under the curve (AUC) of 0.994. A cutoff value of >2.22 yielded a sensitivity of 95.9% and specificity of 100.0%. Serum miRNA-29 demonstrated outstanding discriminatory ability with an AUC of 1.000, achieving both 100.0% sensitivity and 100.0% specificity at a cutoff value of <0.64. Furthermore, a combined logistic regression model incorporating both miRNA-21 and miRNA-29 demonstrated an AUC of 0.997 with sensitivity of 98.0% and specificity of 100.0%, indicating excellent overall diagnostic performance for identifying CKD patients ($p<0.001$).

DISCUSSION

The aim of the present study was to evaluate serum microRNA, particularly miRNA-21 and miRNA-29, as potential non-invasive biomarkers for assessing disease severity in patients with chronic kidney disease and to compare their expression with clinical, demographic, biochemical, hematological, and renal function parameters between CKD patients and healthy controls. Chronic kidney disease is a progressive disorder associated with gradual decline in renal function, increased comorbidity burden, anemia, fluid retention, reduced quality of life, and risk of progression to end-stage renal disease. Conventional markers such as eGFR, serum creatinine, and serum urea are routinely used for diagnosis and monitoring; however, these markers mainly reflect established functional impairment and may not fully represent underlying molecular injury, fibrosis, inflammation, or disease progression. Therefore, identification of reliable serum biomarkers that can reflect CKD severity may improve early assessment and risk stratification. In the present study, miRNA-21 was evaluated as a potential positive severity marker, as its expression increased from 1.45 ± 0.29 in controls to 4.42 ± 1.43 in CKD patients and progressively increased up to 5.33 ± 0.95 in Stage 5 CKD. Similarly, miRNA-29 was assessed as a potential inverse severity marker, as its expression decreased from 0.99 ± 0.13 in controls to 0.25 ± 0.11 in CKD patients and further declined to 0.18 ± 0.03 in Stage 5 CKD. The significance of this study lies in its integrated evaluation of serum microRNAs with CKD stage, eGFR, serum creatinine, serum urea, and clinical characteristics. The strong association of miRNA-21 and miRNA-29 with renal function parameters suggests that these biomarkers may provide additional molecular insight beyond routine biochemical tests. Hence, this

study may contribute to the development of non-invasive, severity-based biomarker approaches for better monitoring, prognostication, and future personalized management of patients with chronic kidney disease.

Distribution of Study Groups and CKD Stages

The present study included 127 participants, of whom 98 (77.2%) were CKD patients and 29 (22.8%) were controls. Among CKD patients, advanced disease predominated, with Stage 5 CKD being the most common category, observed in 57 (58.2%) patients, followed by Stage 4 in 25 (25.5%) patients, Stage 3b in 7 (7.1%), Stage 3a in 5 (5.1%), Stage 2 in 2 (2.0%), and Stage 4b in 2 (2.0%). This distribution indicates that most CKD participants had moderate-to-severe or end-stage renal dysfunction, making the cohort suitable for assessing biomarkers related to disease severity. The predominance of Stage 5 CKD is important because advanced renal disease is commonly associated with greater biochemical abnormality, symptom burden, fibrosis, and molecular dysregulation. Beltrami *et al*. emphasized that CKD requires better non-invasive biomarkers because kidney biopsy is invasive and carries a 3% risk of major complications, while urine protein quantification may lack sufficient predictive power [12]. Similarly, Liu *et al*. compared controls, CKD Stage 1, and CKD Stage 5 patients and found more pronounced miRNA dysregulation in advanced CKD, with 42 differentially expressed miRNAs in CKD5 compared with controls and 70 differentially expressed miRNAs between CKD1 and CKD5 [13]. Thus, the present predominance of Stage 5 CKD supports evaluation of miRNA-21 and miRNA-29 as severity-related biomarkers.

eGFR

Estimated glomerular filtration rate was markedly reduced in CKD patients compared with controls. The mean eGFR among controls was 104.90 ± 20.05, whereas CKD patients had a mean eGFR of 16.86 ± 13.56, and this difference was statistically significant ($p=0.001$). This confirms severe impairment of renal filtration among CKD patients and is consistent with the stage distribution, where Stage 5 CKD accounted for 57 (58.2%) cases and Stage 4 for 25 (25.5%) cases. eGFR is the central marker used for CKD staging, and its marked reduction in the present study validates the clinical classification of the CKD group. Brigant *et al*. reported significant associations between circulating miRNAs and eGFR, with miR-126 showing a Pearson correlation coefficient of -0.52 ($p=0.002$), indicating that decreased kidney function was linked with altered miRNA expression [69]. Fujii *et al*. also showed that individuals in the lowest serum miR-126 tertile had the greatest eGFR decline, with a mean reduction of -3.18 mL/min/1.73 m² and a 3.85-fold increased risk of developing CKD over five years [14]. In the present study, eGFR showed a strong negative correlation with miRNA-21 ($r=-0.806$, $p<0.001$) and an almost perfect positive correlation with miRNA-29 ($r=0.995$, $p<0.001$), indicating that both miRNAs were strongly associated with renal filtration status and CKD severity.

Serum Creatinine

Serum creatinine was significantly elevated among CKD patients compared with controls, confirming impaired renal excretory function. The mean serum creatinine level was 5.54 ± 2.68 in CKD patients, while controls had a mean value of 0.79 ± 0.18, with a statistically significant difference ($p=0.001$). Serum creatinine also showed a strong negative correlation with eGFR ($r=-0.768$, $p<0.001$), indicating that increasing creatinine was associated with declining renal filtration. In addition, serum creatinine demonstrated a very strong positive correlation with miRNA-21fold change ($r=0.953$, $p<0.001$) and a significant negative correlation with miRNA-29fold change ($r=-0.784$, $p<0.001$). These findings indicate that miRNA-21 increased and miRNA-29 decreased as creatinine rose, supporting their role as severity-related biomarkers. Beltrami *et al*. emphasized that conventional markers such as serum creatinine and albuminuria may not fully capture disease progression and that non-invasive RNA-based diagnostics could supplement current CKD markers [12]. Aitbaev *et al*. further noted that creatinine clearance may become abnormal only after 40–50% nephron loss, whereas miRNA changes may appear earlier [74]. In the present study, creatinine was already markedly elevated, reflecting established CKD, but its strong association with miRNA-21 and miRNA-29 suggests that these biomarkers may provide additional molecular information beyond conventional renal function testing.

Serum Urea

Serum urea was markedly elevated in CKD patients compared with controls. The mean serum urea level was 109.61 ± 41.84 in CKD patients and 32.65 ± 15.56 in controls, with a statistically significant difference ($p=0.001$). Serum urea showed a significant negative correlation with eGFR ($r=-0.666$, $p<0.001$) and a strong positive correlation with serum creatinine ($r=0.637$, $p<0.001$), confirming its relationship with declining renal excretory capacity. Importantly, serum urea also showed strong positive correlation with miRNA-21fold change ($r=0.831$, $p<0.001$) and significant negative correlation with miRNA-29fold change ($r=-0.702$, $p<0.001$). These findings suggest that increasing uremic burden was associated with upregulation of miRNA-21 and downregulation of miRNA-29. Trionfini *et al*. reviewed the biological role of miRNAs in CKD and reported that miR-21 and miR-192 were pro-fibrotic miRNAs consistently upregulated in CKD patients, whereas miR-29 and miR-200 families were downregulated [17]. Zhao *et al*. similarly

described miR-21 as 3–4 times higher in CKD tissues compared with healthy controls, while miR-29 was typically downregulated and associated with enhanced extracellular matrix deposition [18]. The present findings are consistent with this pattern, as higher serum urea was associated with higher miRNA-21 and lower miRNA-29 expression, indicating worsening renal impairment and molecular dysregulation.

miRNA-21 Fold Change Between CKD and Controls

Serum miRNA-21fold change was markedly elevated in CKD patients compared with controls. The mean miRNA-21fold change was 4.42 ± 1.43 in CKD patients, whereas controls showed a mean value of 1.45 ± 0.29, with statistical significance ($p<0.001$). This indicates strong upregulation of miRNA-21 among CKD patients and supports its potential role as a non-invasive biomarker of CKD. Trionfini *et al*. described miR-21 and miR-192 as pro-fibrotic miRNAs consistently upregulated in CKD, while miR-29 and miR-200 families were downregulated [68]. Wonnacott *et al*. reported that urinary miR-21 abundance increased by more than 2.5-fold in patients with biopsy-proven interstitial fibrosis compared with controls ($p<0.01$) [19]. Zhao *et al*. similarly reported that miR-21 expression was 3–4 times higher in CKD tissues compared with healthy controls [73]. Aitbaev *et al*. also noted that serum miR-21 may be elevated up to 4-fold in CKD patients and linked to fibrotic signaling pathways [74]. In comparison, the present study found miRNA-21 expression approximately three times higher in CKD patients than controls, increasing from 1.45 ± 0.29 to 4.42 ± 1.43. This consistency with earlier evidence supports miRNA-21 as a biomarker reflecting renal injury, fibrosis, and CKD severity.

miRNA-29 Fold Change Between CKD and Controls

Serum miRNA-29fold change was substantially lower among CKD patients compared with controls. The mean miRNA-29fold change was 0.25 ± 0.11 in CKD patients, whereas controls showed a mean value of 0.99 ± 0.13. This marked reduction indicates significant downregulation of miRNA-29 in chronic kidney disease. miRNA-29 is commonly regarded as an anti-fibrotic miRNA family involved in regulation of extracellular matrix deposition, and its reduction may indicate progressive renal fibrosis and loss of protective molecular regulation. Trionfini *et al*. reported that miR-29 and miR-200 families have anti-fibrotic effects and are downregulated in CKD disease states, with reduced miR-29a correlating with increased interstitial fibrosis ($p<0.05$) [17]. Wonnacott *et al*. also stated that reduction in urinary miR-29c was associated with worse eGFR and higher albuminuria, suggesting loss of anti-fibrotic signaling [19]. Zhao *et al*. reported that miR-29 is typically downregulated in CKD and contributes to enhanced extracellular matrix deposition [18]. Prkacin *et al*. similarly highlighted that miR-29 and miR-200 families are downregulated in CKD, contributing to extracellular matrix deposition and renal fibrosis [20]. The present findings strongly align with these observations, as miRNA-29 decreased from 0.99 ± 0.13 in controls to 0.25 ± 0.11 in CKD patients, supporting its role as an inverse biomarker of CKD severity.

Stage-Wise miRNA-21 Expression

Stage-wise analysis demonstrated progressive elevation of miRNA-21fold change with advancing CKD severity. Mean miRNA-21 was 1.45 ± 0.29 in controls, 2.38 ± 0.98 in Stage 2, 2.61 ± 0.63 in Stage 3, 3.47 ± 0.92 in Stage 4, and 5.33 ± 0.95 in Stage 5 CKD. The overall difference across stages was statistically significant ($p=0.001$). Pairwise analysis showed significantly higher miRNA-21 expression in Stage 3, Stage 4, and Stage 5 compared with controls, with $p\leq0.001$. Significant differences were also observed between Stage 3 and Stage 4 ($p=0.029$), Stage 3 and Stage 5 ($p<0.001$), and Stage 4 and Stage 5 ($p<0.001$). Liu *et al*. found that miRNA dysregulation increased with CKD severity, reporting 20 differentially expressed miRNAs in CKD1 and 42 in CKD5 compared with controls, along with 70 differentially expressed miRNAs between CKD1 and CKD5 [13]. Although Liu *et al*. identified miR-483-5p and miR-363-3p rather than miRNA-21, their findings support stage-dependent miRNA dysregulation. Aitbaev *et al*. reported that miR-21 may rise up to 4-fold in CKD serum samples [16], and Zhao *et al*. described miR-21 expression as 3–4 times higher in CKD tissues [18]. The present increase from 1.45 ± 0.29 in controls to 5.33 ± 0.95 in Stage 5 supports miRNA-21 as a severity-associated marker.

Stage-Wise miRNA-29 Expression

Stage-wise comparison showed progressive decline of miRNA-29fold change with advancing CKD severity. Mean miRNA-29 expression was 0.99 ± 0.13 in controls, 0.63 ± 0.01 in Stage 2, 0.45 ± 0.07 in Stage 3, 0.27 ± 0.05 in Stage 4, and 0.18 ± 0.03 in Stage 5 CKD. The overall comparison was statistically significant ($p=0.001$). Pairwise comparison revealed that all CKD stages had significantly lower miRNA-29 expression than controls ($p<0.001$). Significant differences were also observed between Stage 2 and Stage 3 ($p=0.019$), Stage 2 and Stage 4 ($p<0.001$), Stage 2 and Stage 5 ($p<0.001$), Stage 3 and Stage 4 ($p<0.001$), Stage 3 and Stage 5 ($p<0.001$), and Stage 4 and Stage 5 ($p<0.001$). This consistent decline suggests that miRNA-29 may be highly sensitive to CKD severity. Trionfini *et al*. reported that miR-29 has anti-fibrotic effects and is downregulated in CKD disease states [17]. Wonnacott *et al*. found that reduced urinary miR-29c was associated with worseeGFR and higher albuminuria [19]. Zhao *et al*. also stated that miR-29 downregulation contributes to extracellular matrix deposition in CKD [18]. The present decline

from 0.99 ± 0.13 in controls to 0.18 ± 0.03 in Stage 5 CKD closely supports these previous observations and suggests that miRNA-29 may serve as an inverse severity marker in CKD.

Correlation of miRNA-21 with Renal Parameters

miRNA-21fold change showed strong associations with renal function parameters in the present study. It demonstrated a strong negative correlation with eGFR ($r=-0.806$, $p<0.001$), indicating that miRNA-21 increased as renal filtration declined. It also showed a very strong positive correlation with serum creatinine ($r=0.953$, $p<0.001$) and a strong positive correlation with serum urea ($r=0.831$, $p<0.001$), suggesting that higher miRNA-21 expression was associated with worsening biochemical renal dysfunction. No significant association was observed between miRNA-21 and serum albumin ($r=-0.059$, $p=0.530$). Brigant *et al*. reported significant associations between circulating miRNAs and eGFR, with miR-126 showing a Pearson correlation coefficient of -0.52 ($p=0.002$), supporting the concept that circulating miRNAs are linked to kidney function [15]. Trionfini *et al*. also reported that elevated miR-21 and reduced miR-29a correlated with increased interstitial fibrosis ($p<0.05$) [68]. Zhao *et al*. described miR-21 as positively correlated with tubulointerstitial fibrosis and 3–4 times higher in CKD tissues compared with controls [18]. In the present study, miRNA-21 was elevated in CKD patients at 4.42 ± 1.43 compared with 1.45 ± 0.29 in controls and showed strong correlations with eGFR, creatinine, and urea. These findings support miRNA-21 as a marker of renal injury and CKD severity.

Correlation of miRNA-29 with Renal Parameters

miRNA-29fold change showed a strong and opposite correlation pattern compared with miRNA-21. It demonstrated an almost perfect positive correlation with eGFR ($r=0.995$, $p<0.001$), indicating that higher miRNA-29 expression was associated with preserved renal function. It also showed significant negative correlations with serum creatinine ($r=-0.784$, $p<0.001$) and serum urea ($r=-0.702$, $p<0.001$), meaning that miRNA-29 expression declined as conventional markers of renal dysfunction increased. In addition, miRNA-29 showed a significant negative correlation with miRNA-21 ($r=-0.821$, $p<0.001$), supporting an inverse relationship between these two biomarkers. No significant correlation was found with serum albumin ($r=0.120$, $p=0.204$). Wonnacott *et al*. reported that reduced urinary miR-29c was associated with worseeGFR and higher albuminuria [19], which closely supports the present strong positive association between miRNA-29 and eGFR. Trionfini *et al*. reported that miR-29 downregulation was associated with increased interstitial fibrosis ($p<0.05$) [17]. Prkacin *et al*. also highlighted that reduced miR-29 contributes to extracellular matrix deposition and renal fibrosis [20]. In the present study, miRNA-29 decreased from 0.99 ± 0.13 in controls to 0.18 ± 0.03 in Stage 5 CKD and strongly correlated with renal function. These findings support miRNA-29 as a protective or inverse biomarker of CKD severity.

The present ROC analysis demonstrated excellent diagnostic performance of both miRNA-21 and miRNA-29 for differentiating CKD patients from healthy controls. The exceptionally high AUC values suggest strong discriminatory capacity; however, these findings should be interpreted cautiously and validated in larger multicentric cohorts with independent external validation datasets.

CONCLUSION

This study concludes that serum miRNA-21 and miRNA-29 are strongly associated with CKD severity. miRNA-21 appears to behave as a positive severity marker, increasing with declining eGFR and rising creatinine and urea, whereas miRNA-29 appears to behave as an inverse severity marker, decreasing with worsening renal function. These findings support the potential role of serum miRNA-21 and miRNA-29 as non-invasive biomarkers for assessing severity in patients with chronic kidney disease, especially when interpreted alongside conventional renal function parameters.

REFERENCES

1. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, Hobbs FDR. Global prevalence of chronic kidney disease – A systematic review and meta-analysis. PLoS One. 2016;11(7):e0158765. doi:10.1371/journal.pone.0158765. PMID: 27383068; PMCID: PMC4934905.
2. Stevens PE, Levin A; Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group Members. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: Improving Global Outcomes 2012 clinical practice guideline. Ann Intern Med. 2013;158(11):825–30. doi:10.7326/0003-4819-158-11-201306040-00007. PMID: 23732715.
3. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014;15(8):509–24. doi:10.1038/nrm3838. PMID: 25027649.
4. Szeto CC, Kwan BC, Lai KB, Lai FM, Choi PC, Wang G, Chow KM, Li PK. Micro-RNA expression in the urinary sediment of patients with chronic kidney diseases. Dis Markers. 2012;33(3):137–44. doi:10.1155/2012/842764. PMID: 22960330; PMCID: PMC3810778.

5. Chau BN, Xin C, Hartner J, Ren S, Castano AP, Linn G, Li J, Tran PT, Kaimal V, Huang X, Chang AN, Li S, Kalra A, Grafals M, Portilla D, MacKenna DA, Orkin SH, Duffield JS. MicroRNA-21 promotes fibrosis of the kidney by silencing metabolic pathways. SciTransl Med. 2012;4(121):121ra18. doi:10.1126/scitranslmed.3003205. PMID: 22344696.
6. Krupa A, Jenkins R, Luo DD, Lewis A, Phillips A, Fraser D. Loss of microRNA-192 promotes fibrogenesis in diabetic nephropathy. J Am SocNephrol. 2010;21(3):438–47. doi:10.1681/ASN.2009050530. PMID: 20056746; PMCID: PMC2831862.
7. Glowacki F, Savary G, Gnemmi V, Buob D, Van der Hauwaert C, Lo-Guidice JM, Bouyé S, Hazzan M, Pottier N, Perrais M, Aubert S, Cauffiez C. Increased circulating miR-21 levels are associated with kidney fibrosis. PLoS One. 2013;8(2):e58014. doi:10.1371/journal.pone.0058014. PMID: 23469132; PMCID: PMC3585177.
8. 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):3794–802. doi:10.1093/ndt/gfr485. PMID: 21891774.
9. Kato M, Arce L, Natarajan R. MicroRNAs and their role in progressive kidney diseases. Clin J Am SocNephrol. 2009;4(7):1255–66. doi:10.2215/CJN.00520109. PMID: 19581401; PMCID: PMC2709515.
10. Lv LL, Cao Y, Liu D, Xu M, Liu H, Tang RN, Ma KL, Liu BC. Isolation and quantification of microRNAs from urinary exosomes/microvesicles for biomarker discovery. Int J Biol Sci. 2013;9(10):1021–31. doi:10.7150/ijbs.6100. PMID: 24250247; PMCID: PMC3831115.
11. Levey AS, Coresh J. Chronic kidney disease. Lancet. 2012;379(9811):165–80. doi:10.1016/S0140-6736(11)60178-5. PMID: 21840587.
12. Beltrami C, Clayton A, Phillips A, Fraser D, Bowen T. Analysis of urinary microRNAs in chronic kidney disease. BiochemSoc Trans. 2012;40(4):875–9. doi:10.1042/BST20120090.
13. Liu X, Wang W, Bai Y, Zhang H, Zhang S, He L, *et al*. Identification of a genome-wide serum microRNA expression profile as potential noninvasive biomarkers for chronic kidney disease using next-generation sequencing. J Int Med Res. 2020;48(12):1–12. doi:10.1177/0300060520969481
14. Brigant B, Metzinger-Le Meuth V, Massy Z, McKay N, Liabeuf S, Pelletier M, *et al*. Serum microRNAs are altered in various stages of chronic kidney disease: a preliminary study. Clin Kidney J. 2016;10(1):30–7. doi:10.1093/ckj/sfw060.
15. Fujii R, Yamada H, Tsuboi Y, Ando Y, Munetsuna E, Yamazaki M, *et al*. Association between circulating microRNAs and changes in kidney function: a five-year prospective study among Japanese adults without CKD. ClinChimActa. 2021;519:58–64. doi:10.1016/j.cca.2021.07.002.
16. Aitbaev KA, Murkamilov IT, Fomin VV. Circulating microRNAs as potential biomarkers of chronic kidney disease. TerArkh. 2019;91(6):131–6. Russian. doi:10.26442/00403660.2019.06.000046. PMID: 36471608.
17. Trionfini P, Benigni A, Remuzzi G. MicroRNAs in kidney physiology and disease. Nat Rev Nephrol. 2015;11(1):23–33. doi:10.1038/nrneph.2014.202. PMID: 25385286.
18. Zhao H, Ma S-X, Shang Y-Q, Zhang H-Q, Su W. MicroRNAs in chronic kidney disease. ClinChimActa. 2019;491:59–65. doi:10.1016/j.cca.2019.01.008.
19. Prkacin I, Cavric G, Basic-Jukic N. MicroRNA in kidney disease. BANTAO J. 2016;14(1):8–10. doi:10.1515/bj-2016-0002.
20. Prkacin I, Cavric G, Basic-Jukic N. MicroRNA in kidney disease. BANTAO J. 2016;14(1):8–10. doi:10.1515/bj-2016-0002.