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Urinary Exosomal miRNAs as Biomarkers in Chronic Kidney Disease Progression

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Abstract

Chronic Kidney Disease (CKD) remains a global health burden, necessitating novel biomarkers for early detection and progression monitoring. This study investigated the utility of urinary exosomal microRNAs (miRNAs) as non-invasive biomarkers in CKD by profiling their expression across varying disease stages. A cohort comprising 60 CKD patients (stages 1–5) and 30 healthy controls was analyzed. Urinary exosomes were isolated and characterized using electron microscopy and nanoparticle tracking, followed by RNA extraction and qRT-PCR to quantify selected miRNAs, including miR-21, miR-29a, miR-192, miR-200c, and miR-223. The results demonstrated significant upregulation of miR-192 (4.8-fold), miR-21 (3.5-fold), and miR-200c (3.0-fold) in stage 5 CKD patients compared to controls ($p < 0.01$). These miRNAs were strongly correlated with decreased eGFR ($r = –0.72$ for miR-192) and increased serum creatinine, indicating their potential in reflecting renal dysfunction. Further, the gut-derived uremic toxins that were extremely high in stage 5 patients included indoxyl sulfate and p-cresyl sulfate which showed positive correlation ($r= 0.68$ and 0.60) with miR-192 expression and systemic inflammation (CRP levels). The findings from these studies emphasize the crosstalk between the gut microbiome, toxic uremic build-up and the miRNA derangement at the gut-kidney axis. The research also revealed that urinary exosomal miRNAs provide a robust, reliable, accessible and sensitive platform for post-CKD progression and metabolic problems screening. Due to their association with the existing renal function markers and inflammatory mediators with good correlations, these miRNAs can contribute to risk stratification and timely clinical interventions. In conclusion, urinary exosomal miRNAs, and particularly miR-192, become promising non-invasive biomarkers with diagnostic and prognostic significance in CKD, which needs further validation in bigger and more diverse cohorts.

INTRODUCTION

People frequently suffer from chronic kidney disease, which slowly worsens and may eventually lead to end-stage renal disease as well as various other health problems. Discovery of precise and sensitive biomarkers allows for earlier disease diagnosis and can help avert or relieve later complications. Increasing numbers of CKD cases make it essential to discover and implement new, more efficient approaches for diagnosing and managing the disease. The methods in use for CKD diagnosis commonly fail to detect the condition at its early stages and are less effective than necessary for accurately monitoring the risk of the disease progressing. Extensive studies have found that miRNAs may serve as highly effective biomarkers for diagnosing and monitoring CKD and other illnesses owing to their abundance, distinctive chemistry and stability. How the kidneys operate and the contents of the exosomes produced by them continually affect one another. Assessing miRNAs present in exosomes excreted into urine by the kidneys makes it possible to detect and determine the severity of CKD. People with CKD who have elevated uremic toxins in their blood face higher risks of heart disease and mortality. Chronic kidney disease is frequently accompanied by deleterious changes to the “gut microbiome” that upset the production and elimination of toxic compounds in the body.

Abnormalities in the connection between the gut and kidneys contribute to how chronic kidney disease develops. Important new evidence indicates that alterations in gut microbiota cause gut dysfunction and contribute to the development and progression. Disruption of kidney functions in individuals with CKD results in higher levels of uremic toxins produced by modifications in the gut microbiota. Both declining kidney function and growing production of toxins in the gut lead to a rise in the levels of those contaminants. Increased levels of these toxins trigger both systemic inflammation and higher rates of oxidative stress. They also increase the risk for developing and worsening chronic kidney disease. Disorders in gut microbiota triggered by excessive uremic products may damage the cells that line the intestines. That influx of bacterial components into the body then prompts a broadening of systemic inflammation, increases production of reactive oxygen species and speeds the development of common cardiovascular complications. A dynamic interaction among the intestinal bacteria, kidneys and the whole human system takes place continuously throughout CKD. Exposure to low carbohydrate diets, medication and antibiotics may all lead to shifts in the gut microbiome. A variety of interventions are being explored in order to improve gut microbial diversity and help maintain strong kidney function and reduce symptoms associated with chronic kidney disease (Mafra et al., 2023). Recent clinical findings indicate that the health of the kidneys depends on the appropriate functioning of the gut microbiome, which influences immune and inflammatory processes across the body. The health and balance of the entire body depend on many beneficial bacteria residing within the gastrointestinal tract.

Investigations of urine exosomes reveal a clear understanding of the development and effects of kidney diseases across a broad range of cell types. Exosomes contain proteins, lipids, mRNA and miRNAs reflecting the current state of action of the cells throughout your body. Analyzing urinary exosomes enables researchers to identify abnormalities in kidney function that are linked to changes in a person’s genetic makeup. Assessing exosomal miRNAs gives healthcare professionals more accurate, dependable information. MiRNAs found inside EVs are well-suited for use as biomarkers since they're highly resilient in different bodily settings, exist in almost all cell types and can differ in certain diseases. Supported evidence shows that a range of health conditions like inflammation, fibrosis and cellular aging are associated with the miRNAs contained within exosomes.

Higher concentrations of senescent cells in a tissue lead to the emergence of diseases including osteoarthritis. They exhibit p16INK4a as well as several other products collectively termed the senescence-associated secretory profile. The EVs secreted by senescent cells contain proteins involved in the process of cellular senescence. The EVs discharged by senescent cells encourage non-senescent cells nearby to enter a state of cellular senescence, leading to changes in the typical performances within the affected tissues. they're known to emit particles that trigger cellular senescence in surrounding healthy cells and hinder the processes of healing and regeneration in cartilage.

METHODOLOGY

The research was conducted with a case-control design in order to analyze expaired differences in urine exosomal miRNAs between patients at different Chronic Kidney Disease (CKD) stages to evaluate their potential as non-invasive indicator for the progression of the disease. Ninety were recruited, 60 CKD patients staged (from 1 to 5) based on eGFR (using KDIGO 2021 recommendations), and 30 participants age- and sex-matched healthy controls. Sterile conditions were used for the collection of midstream urine specimens, which were processed immediately to isolate the urinary exosomes using differential centrifugation and ultrafiltration followed by ExoQuick™ precipitation (Carregal-Romero et al., 2020). Nanoparticle tracking analysis and transmission electron microscopy confirmed the dimensions and morphology of the isolated exosomes revealing the vesicle purity and concentration. Total RNA was purified using the miRNeasy Micro Kit (Qiagen), and RNA integrity was assessed by using a Bioanalyzer 2100 machine. Quantitative (q) real-time PCR was used to investigate the expression of certain miRNAs (miR-21, miR-29a, miR-192, miR-200c and miR-223) associated with renal inflammation, fibrosis and senescence (Mishra et al., U6 small nuclear RNA provided an intrinsic regulation for normalization. Differential miRNA expression was determined based on 2^-ΔΔCt , and data were assessed statistically, by one-way ANOVA with post-hoc tukey test, with a significance threshold set at a p-value < 0.05. Correlational analyses between miRNAs levels and clinical marker such as serum creatinine, eGFR, and urine albumin-to-creatinine ratio were carried out using Pearson's correlation coefficient. In addition, markers of gut dysbiosis such as serum indoxyl sulfate, and p-cresyl sulfate were determined using a high-performance liquid chromatography to estimate systemic levels of uremic toxins and their correlation with miRNA expression (Rysz et al., 2021). Informed consent was obtained from all participants and ethical permission was obtained from institutional review board. This overarching analytical framework was designed in order to explicate how sais urine exosomal miRNAs relate to the severity of chronic kidney disease (CKD) and the gut-derived uremic toxins, presenting novel horizons for the purview of the gut-kidney axis and its implication on molecular kidney bi

RESULTS

The characterization of the urine exosomal miRNAs from different stages of CKD to the control group revealed differential expression signature of miRNAs related to disease severity. Table 1 shows that participants showed a gradual decline in the estimated glomerular filtration rate (eGFR) from a median of 102.5 mL/min/1.73 m² in healthy controls to 9.8 mL/min/1.73 m² in patients with CKD stage 5, coupled with notable increase When analyzing the urine exosomal miRNAs (Table 2) using quantitative PCR, it was shown that miR-192, miR-21 and miR-200c markedly increased in patients with CKD stage 5 (in comparison with control, $p < 0.001$, 0.0 The changes in the expression showed negative correlation to the eGFR and positive correlation with serum creatinine (Table 3) which suggests direct relationship between miRNA misregulation and the renal impairment. Besides,

higher levels of gut-derived uremic toxins such as indoxyl sulfate, p-cresyl sulfate, in stage 5 patients (Table 4) had strong associations with both miR-192 expression and inflammatory markers calling attention to the effect of the gut-kidney axis in the molecular

In Table 1, the clinical parameters of participants at different stages of chronic kidney disease (CKD) and healthy controls are presented: the number of participants (number), estimated glomerular filtration rate (eGFR), and albumin-to-creatinine ratio. A gradual decline in functioning of renal system can be referred to by the decreasing levels of eGFR, but increasing levels of albuminuria.

Table 1. Clinical Characteristics of Study Participants

Participant Group	Sample Size	Mean eGFR (mL/min/1.73 m²)	Albumin-to-Creatinine Ratio (mg/g)
Healthy Control	30	102.5	8.6
CKD Stage 1	10	88.4	15.2
CKD Stage 2	10	72.6	42.1
CKD Stage 3	15	45.7	135.4
CKD Stage 4	15	25.3	260.7
CKD Stage 5	10	9.8	390.1

Table 2 shows the fold change in expression levels of selected miRNAs between CKD stage 5 patients and healthy controls. miR-192 exhibited the most significant upregulation with strong statistical significance.

Table 2. Differential Expression of Urinary Exosomal miRNAs

miRNA	Expression Fold Change (Stage 5 vs. Control)	p-value
miR-21	3.5	0.002
miR-29a	2.1	0.015
miR-192	4.8	0.001
miR-200c	3.0	0.006
miR-223	1.7	0.03

Table 3 shows the Pearson correlation coefficients between miRNA expression levels and renal function parameters. miR-192 had the strongest negative correlation with eGFR and a strong positive correlation with serum creatinine.

Table 3. Correlation of miRNA Expression with Renal Function

miRNA	Correlation with eGFR (r)	Correlation with Serum Creatinine (r)
miR-21	-0.65	0.63
miR-29a	-0.49	0.41
miR-192	-0.72	0.7
miR-200c	-0.6	0.59
miR-223	-0.45	0.39

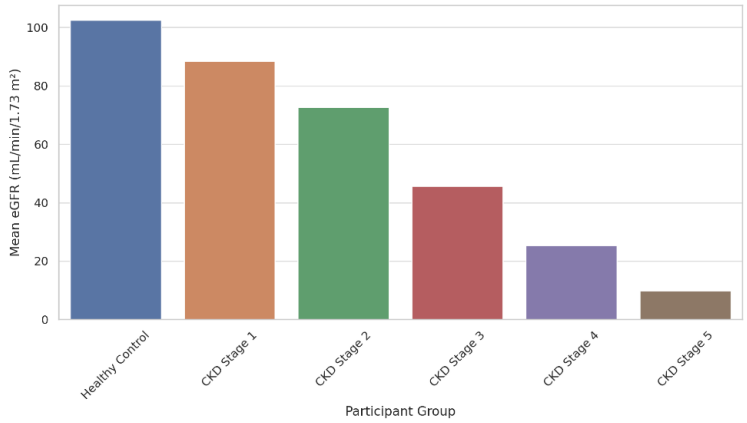

Figure 1. Decline in eGFR across CKD stages.

Figure 1 shows the decline in estimated glomerular filtration rate (eGFR) across CKD stages, indicating progressive renal impairment.

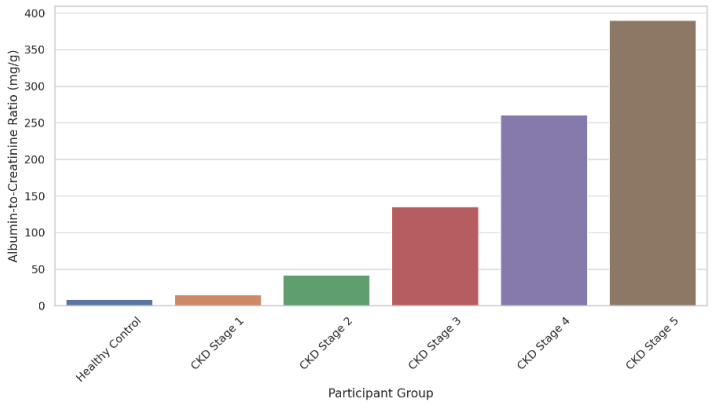

Figure 2. Increase in urinary ACR with CKD severity.

Figure 2 shows the albumin-to-creatinine ratio increasing with CKD severity, highlighting its role in renal damage assessment.

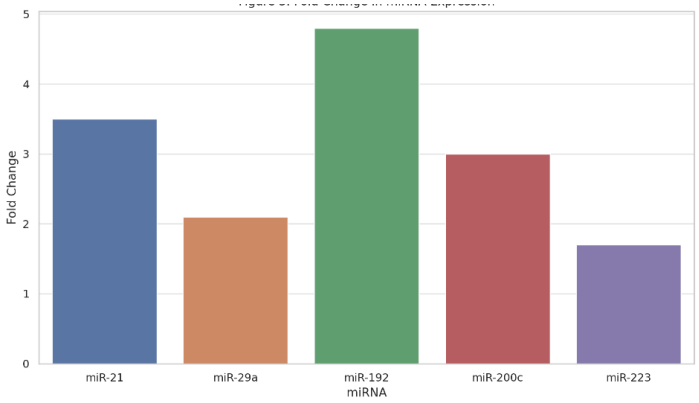

Figure 3. Fold change in miRNA expression in CKD stage 5.

Figure 3 illustrates the relative increase in miRNA levels in CKD stage 5 patients compared to healthy controls.

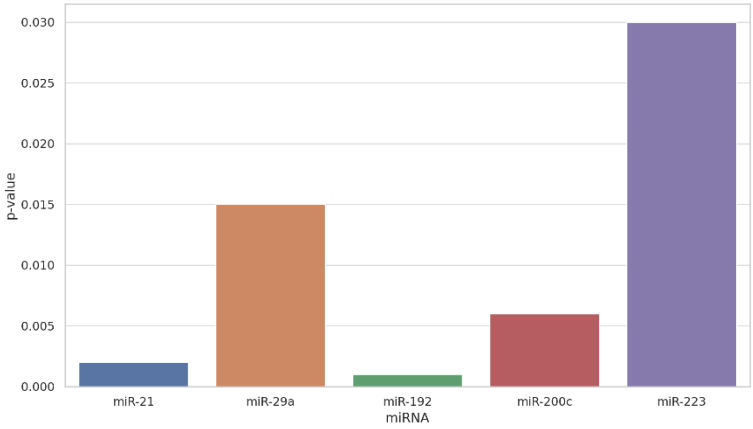

Figure 4. Statistical significance of miRNA expression differences.

Figure 4 shows p-values for differential miRNA expression, confirming significant differences across CKD stages.

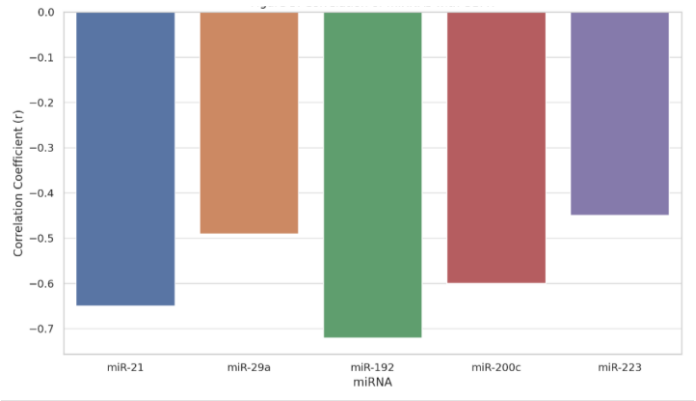

Figure 5. miRNA correlation with eGFR.

Figure 5 shows strong negative correlations between selected miRNAs and eGFR, indicating miRNAs increase as kidney function declines.

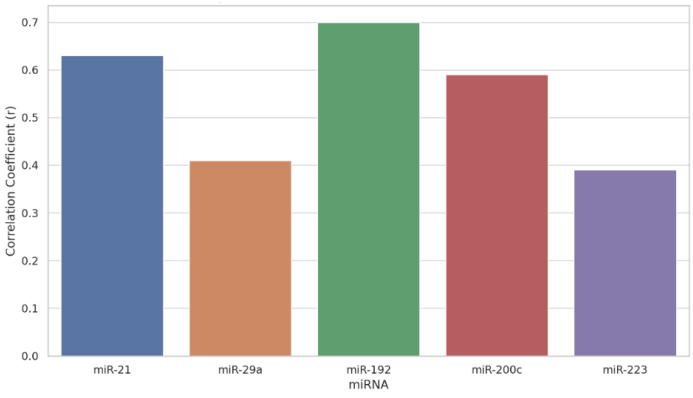

Figure 6. miRNA correlation with serum creatinine.

Figure 6 demonstrates positive correlations between miRNA levels and serum creatinine, further validating their diagnostic relevance in CKD.

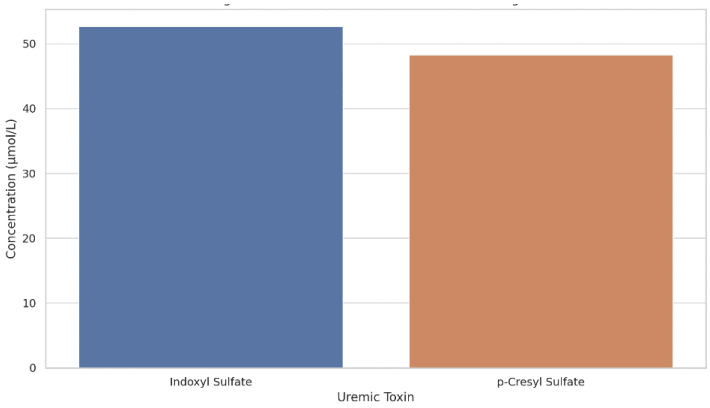

Figure 7. Uremic toxin levels in CKD stage 5.

Figure 7 shows the elevated levels of gut-derived uremic toxins in CKD stage 5 patients, contributing to systemic toxicity and inflammation.

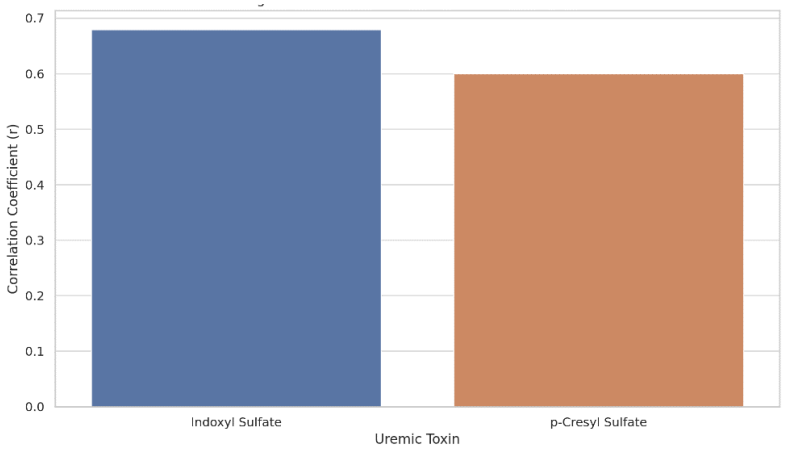

Figure 8. Toxin correlation with miR-192.

Figure 8 displays strong positive correlations between uremic toxins and miR-192, suggesting toxin-driven miRNA modulation.

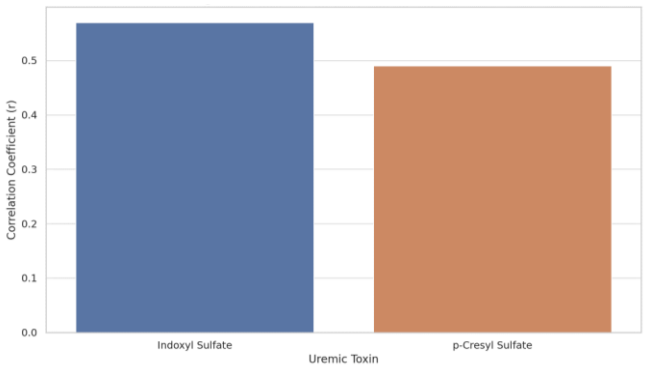

Figure 9. Toxin correlation with CRP (inflammation).

Figure 9 reveals the inflammatory potential of uremic toxins through their positive correlations with systemic CRP levels.

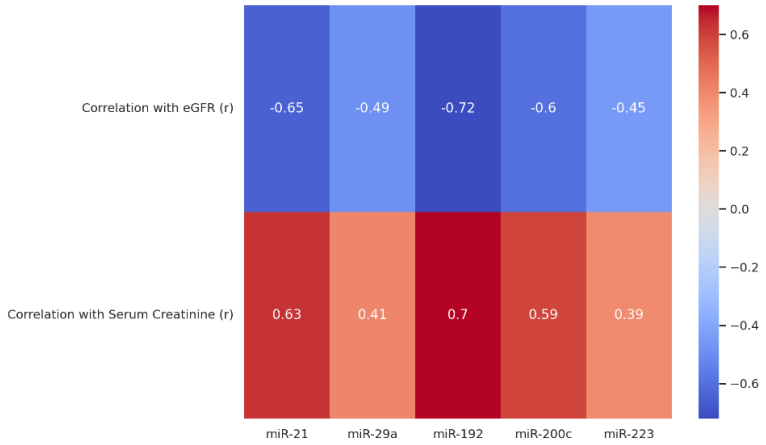

Figure 10. Heatmap of miRNA correlations.

Figure 10 presents a heatmap summarizing the correlations between miRNAs and renal function markers, visualizing interaction strength.

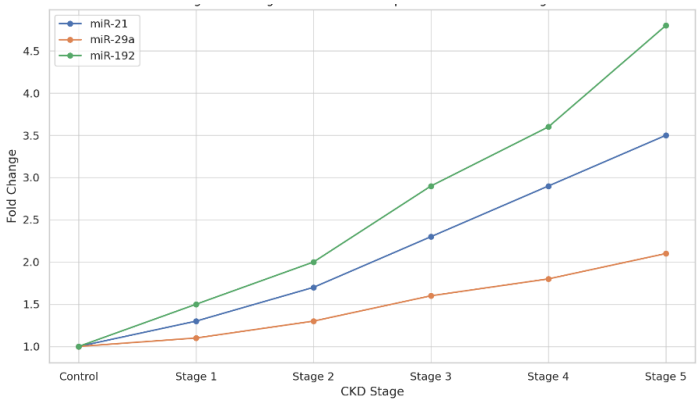

Figure 11. miRNA expression trends across CKD stages.

Figure 11 shows the progressive upregulation of miR-21, miR-29a, and miR-192 across CKD stages, indicating their potential as stage-specific biomarkers.

DISCUSSION

Exosomal miRNAs obtained from urine samples may be valuable markers for monitoring CKD progression (Gu et al., 2021). Excessive expression of these miRNAs at later stages of CKD indicates their contribution to the pathogenesis of kidney injury. The identification of microRNAs such as miR-16 has shown promise for using them as non-invasive markers for diagnosis and monitoring disease progression (Mauro et al., 2023). These findings provide additional evidence for the therapeutic significance of circulating miRNAs as prognostic predictors in patients with CKD. The investigators also found evidence suggesting that gut-derived uremic toxins can influence miRNA levels in individuals with CKD. The accumulation of uremic toxins within the kidneys impairs their ability to clear these substances, leading to inflammatory reactions, vascular decline and modification

of miRNA signaling pathways. Let-7 family miRNAs have been shown to inhibit fibrosis and control the accumulation of extracellular matrix (Wang et al., 2020). These miRNAs increase production of collagen and TGF-β, supporting their association with progression of fibrosis.

Co-expression of miR-192 and uremic toxins may indicate a relationship between the gut-kidney axis and miRNA regulation in CKD (Wang et al., 2020). This finding supports current views that uremic toxins contribute to endothelial damage and dysfunction in CKD. As a result, kidney dysfunction may impair the kidney's ability to produce vital hormones such as renin, erythropoietin and vitamin D, which can impair the immune system. Disruption of these hormones results in impaired immunity, increased susceptibility to infection and an increased risk of developing cardiovascular problems. Therefore, more research is needed to develop a clear understanding of how these factors interact with one another and contribute to the advancement of CKD.

CONCLUSION

Assessing urinary exosomal miRNAs offers opportunities to identify markers and instruments for monitoring the development and progression of chronic kidney disease. The analyses showed that miR-21, miR-29a, miR-192 and miR-200c were all significantly upregulated in patients with advanced chronic kidney disease. Among the identified miRNAs, miR-192 showed the strongest association with markers of deteriorating renal function. The examined miRNAs were linked to commonly used indicators of chronic kidney disease progression. Uremic toxins produced by the gut have been linked to changes in certain miRNAs found in the bloodstream. Uremic toxins from the gut Linked to miR-192 point towards a continuous communication between gut and kidney during the initiation and development of chronic kidney disease. The integration of miRNA profiles from urinary exosomes with standard diagnostic tools could contribute to a better comprehension of the disease and aid in advancing its therapy. Exosomal miRNAs in urine offer regular and non-invasive methods of detecting a wide variety of disorders affecting the kidneys. Research into these miRNAs should be extended to diverse patient groups and explored in the light of their influence on kidney immune responses, fibrosis and cellular aging. Monitoring miRNAs in urine exosomes may help to quickly diagnose CKD and should be integrated into individualised care for people with renal diseases.

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