

# miRNAs are a Novel Class of Regulatory Genes associated with Kidney Disease

## Abstract

**Introduction:** Micro RNAs have been associated with chronic kidney disease progression and mortality. However, miR 223 and miR 192 role in CKD is poorly evaluated.

**Aim:** The learning aims to find the part of miR 223 and 192 in CKD patients

**Methods:** A cross-sectional observational study that included 450 study subjects out of which, 400 were cases and 50 were controls. Biochemical parameters were assessed by ELISA and miR expression levels were evaluated by quantitative PCR.

**Results:** It was found that miR 223 and 192 were significantly increased in the advancing stages of CKD when compared to controls.

**Conclusion:** miR 192 and miR 223 can be used as diagnostic markers of CKD.

**Keywords:** *disease, progression, stages of kidney disease, expression.*

## Introduction

In the recent past, novel non-coding RNAs have been revealed taking new insights into the process of genetic factor parameters [1]. The size of the RNAs deviates them into short and long noncoding RNAs [2]. These non-coding RNAs harmfully control genetic factor appearance by deprivation of their mark mRNAs [3]. It is increasingly evident that miRNAs are expressed in usual and compulsive tissue which are intricate in kidney disease [4]. Research on microRNAs as probable biomarkers in the analysis and progression of diseases has gained traction in the current research field. These mRNAs play a crucial role in different cellular and regulatory processes like apoptosis, development, differentiation, and proliferation and also in the proper functioning of the kidney [5]. It is also obvious that various miRNAs are identified in saliva, serum, urine, and plasma. Earlier studies demonstrated that mRNAs exhibit unique pathology and can be used as potential diagnostic markers [6]. There is a growing demand to explore the part of extracellular miRNAs in the progression and growth of kidney disease i.e.; CKD [7-9]. However, most of the findings relevant to mRNA appearance in several organic solutions of CKD are unpredictable.

As the main objective of the present study is to find the connotation of miRNA with CKD.

## Materials & Methods

This cross-section observational learning was shown from 2020 to 2024 in urban and rural health centers of Narayana Medical College and Hospital Nellore, Andhra Pradesh, India Informed consent is obtained from all the subjects and they were conducted after getting approval from the institutional ethical committee. Patients with CKD are considered in the current study. Patients in the age group 18 to 55 years, having been diagnosed with enduring kidney disease according to kidney disease, result quality initiative criteria were considered for the present study. Patients with a history of epilepsy, hypertensive encephalopathy malignancies, and infections. And 5<sup>th</sup> stage of CKD patients were excluded from the present study. 5 ml of Venus blood samples were withdrawn from each subject and transferred in Serum vacuums at the sample collection center. The serum is separated using centrifugation at 3000 rpm for 13 actions at room temperature.

300- 500 µL serum sample Was used for micro-RNA extraction using a Mir easy serum/plasma kit. Extracted MIRNAs were converted into cDNA using U6 universal primer Further, each cDNA was checked for integrity using agricultural electrophoresis and nanodrop reading. Quantified cDNA was used for the quantitative expression analysis of two distinguished micro RNAs, that is, mir 223 and mir 192 in patient samples about control subjects, with real-time measurable PCR and 2 delta q technique.

## Results

A total of 450 subjects comprising 400 CKD patient roles and 50 well controls were comprised in the present learning. The patients in the study were bifurcated into four groups and healthy controls. Four groups of diversion are based on the stages of CKD Phase 1 (n=100); Stage 2(n=100), stage 3(n=100), and Stage 4 (n=100) and healthy controls (n=50). The scientific and biological parameters of the learning subjects are potted as follows. (Table 1&2).

**Table 1: Medical parameters of the study subjects**

Variable	Number of cases
Mean Age (years)	
Males	54 ± 2.3
Females	52 ± 3.5
Males	235
Females	165
Mean weight (Kg)	58
Mean Height (m)	1.64
Mean BMI (kg/m <sup>2</sup> )	24
Systolic BP (mm Hg)	120
Diastolic BP (mm Hg)	80
Family history of CKD (number)	120
Smoking (only males)	168
Chewing betel (both males and females)	98
Consumption of Alcohol (only males)	76
History of Hypertension	89
Diabetes mellitus	20
History of Malaria	55

**Table 2: Biochemical parameters of the study subjects**

Biochemical parameters (mean)	Stage 1 CKD	Stage 2 CKD	Stage 3 CKD	Stage 4 CKD	Control
Urea (mg/dL)					
Male	37	38	44	46	32
Female	38	32	42	48	30
Creatinine (mg/dl)					
Male	1.4	1.5	2.1	2.8	1.0
Female	1.32	1.46	2.5	2.7	1.1
eGFR (ml/min)					
Male	68	63	42	30	110
Female	62	62	43	31	108

Glucose (mg/g)					
Male	79	200	76	15	70
Female	77	201	74	12	43

Dual miRNAs be identified in the samples and serum echelons of miR 192 and miR223 concluded the CKD patients were brief in Figures 1& 2.

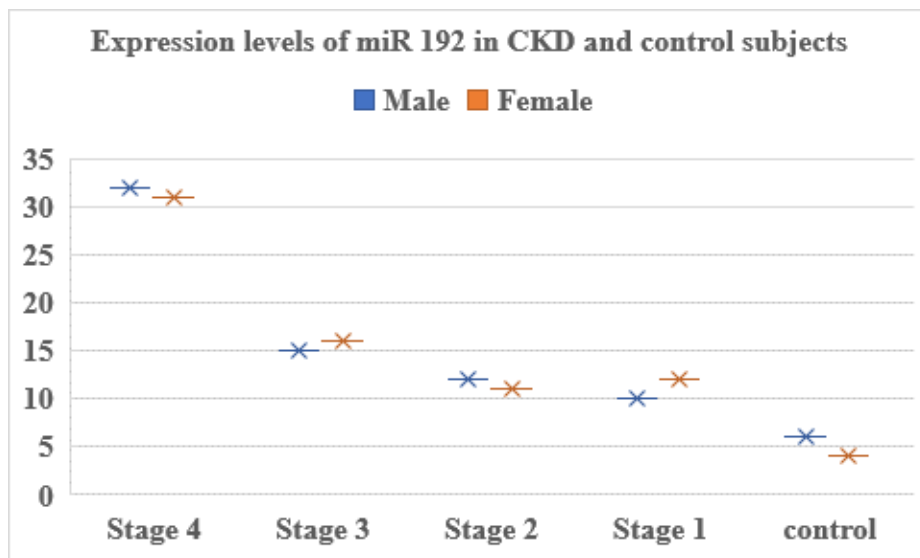


Figure 1: Expression of miR 192 in CKD of all stages

The appearance levels of miR 192 in females were higher than the males of the study.

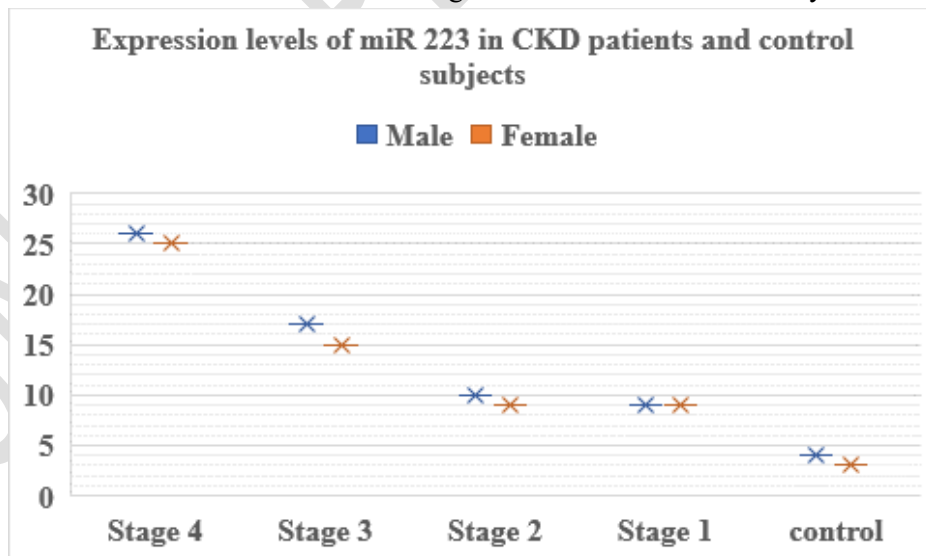


Figure 2: Expression of miR 223 in CKD of all stages

The appearance of miR 223 in males is more advanced than in the females of the CKD-affected individuals.

## Discussion

The current study proved a significant increase in mingling miR 192 and miR 223 in patients with more unadorned stages of CKD.

Glomerular purification rate estimation meanwhile the serum levels of creatinine currently remain the most suitable marker of renal function for average practice and large epidemiological studies [10]. Albuminuria, proteinuria, and seric urea levels are useful indicators of renal character once controlled for urinary creatinine. However, these are not actual complex when detecting the first phases of CKD. Some other markers have been evaluated for their prognostic value of death, cardiac complications, and kidney disease evolution without victory [11-13]. miRNAs take attention as biomarker candidates to measure kidney disease severity [14,15]. One of the main compensations of miRNAs is their serac stability which makes them appropriate as a non-invasive biomarker [16]. So, one can hope that minor RNAs might prove to be a dependable marker to be valuable in clinical practice.

Quantification of miRNA stages in blood samples is performed by quantitative PCR. Various study squads have depicted the appearance of miRNA in plasma circulation in CKD advanced stages [17, 18]. A cohort study by Chen et al proved that miR-125b, miR 145, and 155 stages declined as the disease progressed [19]. The plasma levels of cardiac miRNAs as well decline with eGFR [20].

In difference to the above studies, in the present investigation, there is a growth in the levels of miR 223 and 192 are advanced stages of CKD.

As miR-223 is measured to be a marker of tenderness, it can be used as a marker for detecting CKD [21]. As per our study observations, miR 192 and miR 223 remain as prophetic markers of CKD identification and detection. The present investigation is the first study to identify the raised levels of miRNA in CKD patients.

## Conclusion

To conclude, this is one of the rare studies to prove miRNAs be used as diagnostic markers in detecting chronic kidney disease. miR 192 and miR 223 are used as prognostic markers for identifying CKD disease progression.

## References

1. Metzinger-Le Meuth V, Metzinger L. miR-223 and other miRNA's evaluation in chronic kidney disease: innovative biomarkers and therapeutic tools. *Noncoding RNA Res.* 2019;4:30–35. doi: 10.1016/j.ncrna.2019.01.002.
2. Rong D, Sun H, Li Z, Liu S, Dong C, Fu K, Tang W, Cao H. An emerging function of circRNA-miRNAs-mRNA axis in human diseases. *Oncotarget.* 2017;8:73271–73281. doi: 10.18632/oncotarget.19154.
3. Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature.* 2010;466:835–840. doi: 10.1038/nature09267.
4. Bartel, D.P. MicroRNAs: Target recognition and regulatory functions. *Cell* **2009**, *136*, 215–233.
5. Bhatt, K.; Mi, Q.S.; Dong, Z. MicroRNAs in kidneys: Biogenesis, regulation, and pathophysiological roles. *Am. J. Physiol.-Ren. Physiol.* **2011**, *300*, 602–610
6. Mitchell, P.S.; Parkin, R.K.; Kroh, E.M.; Fritz, B.R.; Wyman, S.K.; Pogosova-Agadjanyan, E.L.; Peterson, A.; Noteboom, J.; O'Briant, K.C.; Allen, A.; et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 10513–10518.
7. Liu, Y.; Usa, K.; Wang, F.; Liu, P.; Geurts, A.M.; Li, J.; Williams, A.M.; Regner, K.R.; Kong, Y.; Liu, H.; et al. MicroRNA-214-3p in the kidney contributes to the development of hypertension. *J. Am. Soc. Nephrol.* **2018**, *29*, 2518–2528. [[Google Scholar](#)] [[CrossRef](#)]
8. Fourdinier, O.; Schepers, E.; Metzinger-Le Meuth, V.; Glorieux, G.; Liabeuf, S.; Verbeke, F.; Vanholder, R.; Brigant, B.; Pletinck, A.; Diouf, M.; et al. Serum levels of miR-126 and miR-223 and outcomes in chronic kidney disease patients. *Sci. Rep.* **2019**, *9*, 1–12. [[Google Scholar](#)] [[CrossRef](#)] [[PubMed](#)]

9. Fujii, R.; Yamada, H.; Munetsuna, E.; Yamazaki, M.; Ohashi, K.; Ishikawa, H.; Maeda, K.; Hagiwara, C.; Ando, Y.; Hashimoto, S.; et al. Associations of Circulating MicroRNAs (miR-17, miR-21, and miR-150) and Chronic Kidney Disease in a Japanese Population. *J. Epidemiol.* **2019**, *30*, 177–182.
10. Waikar, S. S., Betensky, R. A. & Bonventre, J. V. Creatinine as the gold standard for kidney injury biomarker studies? *Nephrol Dial Transplant* **24**(11), 3263 (2009).
11. Kern, E. F. *et al.* Early urinary markers of diabetic kidney disease: a nested case-control study from the Diabetes Control and Complications Trial (DCCT). *Am J Kidney Dis* **55**(5), 824 (2014).
12. Nguyen, T. Q. *et al.* Urinary connective tissue growth factor excretion correlates with clinical markers of renal disease in a large population of type 1 diabetic patients with diabetic nephropathy. *Diabetes Care* **29**(1), 83 (2006).
13. Boes, E. *et al.* Apolipoprotein A-IV predicts progression of chronic kidney disease: the mild to moderate kidney disease study. *J Am Soc Nephrol* **17**(2), 528 (2006)
14. Nassirpour, R., Raj, D., Townsend, R. & Argyropoulos, C. MicroRNA biomarkers in clinical renal disease: from diabetic nephropathy renal transplantation and beyond. *Food Chem Toxicol* **98**(Pt A), 73 (2016).
15. Gilad, S. *et al.* Serum microRNAs are promising novel biomarkers. *PLoS One* **3**(9), e3148 (2008).
16. Mitchell, P. S. *et al.* Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* **105**(30), 10513 (2008).
17. Etheridge, A. *et al.* Extracellular microRNA: a new source of biomarkers. *Mutat Res* **717**(1-2), 85 (2011)
18. Kerr, K. F. *et al.* Evaluating biomarkers for prognostic enrichment of clinical trials. *Clin Trials* **14**(6), 629 (2014).
19. Neal, C. S. *et al.* Circulating microRNA expression is reduced in chronic kidney disease. *Nephrol Dial Transplant* **26**(11), 3794 (2011).
20. Gidlof, O. *et al.* Cardiospecific microRNA plasma levels correlate with troponin and cardiac function in patients with ST elevation myocardial infarction, are selectively dependent on renal elimination, and can be detected in urine samples. *Cardiology* **118**(4), 217 (2011).
21. Taibi, F., Metzinger-Le Meuth, V., Massy, Z. A. & Metzinger, L. miR-223: An inflammatory oncomiR enters the cardiovascular field. *Biochim Biophys Acta* **1842**(7), 1001 (2014)