

**Title: MIR-193a AS A FOCAL SEGMENTAL GLOMERULOSCLEROSIS BIOMARKER**

**Running Title : MIR-193a AS DIAGNOSTIC BIOMARKER**

**Authors:**

**\*Ibrahim Abdul-Majeed Altamemi**

**\*\*Ali Ridha**

**Affiliations:**

**\* PhD. Medical Microbiology.** assistant professor in department of Medical Microbiology/  
College of medicine/ University of Al-Qadisiyah **Email;** [ibrahim.altamemi@qu.edu.iq](mailto:ibrahim.altamemi@qu.edu.iq)

**\* \* B.Sc. medical laboratory technology at Al-sadder medical city /**

Corresponding author: **Ibrahim A. Altamemi**

Email of corresponding author: [ibrahim.altamemi@qu.edu.iq](mailto:ibrahim.altamemi@qu.edu.iq)

**Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/ have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

**Conflicts Of Interest:** There is no conflicts of interest.

**Author Contribution:**

**\*Ibrahim Altamemi:** Contributing to sample collection, writing the manuscript, statistical analysis, and publication.

**\*\*Ali Ridha:** Contributing to sample collection, writing the manuscript, and statistical analysis.

## **miRNA-193a As a Diagnostic Biomarker for focal segmental glomerulosclerosis**

### **Abstract:**

**Objectives:** biopsy is the best way to diagnose patients with focal segmental glomerulosclerosis. But it is considered an invasive method for diagnosis, therefore present study suggests to evaluate the significant importance of miRNA-193a as biomarkers for diagnosis of focal segmental glomerulosclerosis.

**Material and Methods:** A urine sample was collected from two groups. The first group was patients with focal segmental glomerulosclerosis (FSGS), the Second group was healthy volunteers. Urine of this sample used to RNA purification and cDNA application with stem-loop specific primer then miRNA-193a was quantitated by using RT-PCR.

**Result:** The level of miR-193a fold change was significantly highest in the FSGS group then the control group ( $P < 0.001$ ). A receiver operator characteristic (ROC) analysis; the cut off value was identified at miRNA-193a of  $>0.31$  fold change with a sensitivity of 100 % and a specificity of 50%,

**Conclusion:** miRNA-193a is high sensitivity and Specificity in this study which was bushed to using them as a biomarker for FSGS diagnosis.

**Key words:** miRNA-193, FSGS.

### **Introduction**

Focal segmental glomerulosclerosis: it is major cause of progressive renal disease and end stage renal disorder with progressive glomerular scarring and proteinuria (1) characterized by increased level of protein in urine (proteinuria) and defect in podocyte injury.(2).other studies have shown that podocytes are essential cell that cause development of Focal segmental glomerulosclerosis (3) (4). The patient with FSGS suffering from proteinuria ,hypoalbuminemia ,hypercholesterolemia and peripheral edema (5). Classification of FSGS is various and includes pathophysiologic, histologic, and genetic considerations (6). initially proposed that FSGS can be classified into primary (idiopathic) and secondary forms. The latter might be considered to involve familial/genetic forms, virus-associated forms, drug-induced forms, and forms mediated by adaptive structural-functional responses. clinical response may relate to the histologic variant, most notable the glucocorticoid responsiveness of the tip lesion and the aggressive of the collapsing variants ,more recently, pains to identify genetic diversity of FSGS in at risk population have acquired momentum with the most recent addition involving the

APOL1 genetic variant as a major causes of FSGS in individual of sub-sahran African descent with FSGS. when putting together the genetic susceptibility ,pathophysiologic . clinical history and effect of the therapy, we believe that it is useful to the group , FSGS can be classified into six clinical forms including two groups the first common forms (primary FSGS and adaptive FSGS) and three less common forms (genetic FSGS, viral mediated FSGS ,and medication associated FSGS). In 1957 the ARNOLD RICH was first described the focal segmental glomerulosclerosis ,he hypothesized that the development of glomerulosclerosis accounted for the progression to end stage renal disorder seen in a group of children with idiopathic nephrotic syndrome . however it was not until the 1970s that FSGS appear as separate clinic pathologic entity according a report by the International study of kidney disease in children (7). FSGS is a defining by present the proteinuria typically accompanied by hypoalbuminemia , hypercholesterolemia and peripheral edema .in children 10% - 30% of patients with proteinuria are detected on routine checkups and physical examination.

In adult may detection in military induction examination , obstetric checkups and physical examination . the incidence of nephrotic- range proteinuria at onset in children is 70% to 90% whereas only 50% to 70% of adult with FSGS present with nephrotic syndrome. but in secondary forms of FSGS associated with hyper-filtration such as remnant kidney and ORG, typically to present lower levels of proteinuria and many such patient with FSGS have sub nephrotic proteinuria and a normal serum albumin concentration (8, 9). MicroRNAs comprise a large family of 21–22-nucleotide-long RNAs that have emerged as key post-transcriptional regulators of gene expression in animals and plants. In animals, microRNAs are predicted to control the activity of ~50% of all protein-coding genes(10) .

Thus, according to such contraversary the aims of present study are : Investigate whether patients with focal segmental glomerulosclerosis (FSGS) have distinct miRNA193a that could lead to potential development of non-invasive diagnostic biomarkers of the disease. And finally determine which of them have higher sensitivity and specificity.

## **Material and method**

A case control study has been conducted based on mid-stream urine sample from 24 Iraq patients with FSGS which include (13 male and 11 female) with FSGS, who attended the consultant clinic for nephrology in AL-Diwaniyah teaching hospital in the period between 1January 2018 to 10 May 2018 under the supervision of nephrologist specialists were included in this study patients were diagnosed with FSGS according to histopathological report of kidney in addition to that the information about each case collected from patient as well as the test such as urea, serum creatinine , protein in urine and histopathological test performed in the hospital In addition to that

about 24 healthy volunteers were included as a control group. Urine sample were collected from 24 patient and their healthy controls and putting in tube and separated by centrifugation 1500 rpm for 5 minute. The urine has been collected in tube then stored at -20c for total RNA were extracted from urine samples by using (TRIzol<sup>®</sup> reagent kit. Bioneer. Korea) and done according to company instructions. The extracted RNA was treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to the method described by Promega company, USA instructions The Taq Man MicroRNA assays were used looped-primer RT-PCR, a new real-time quantification method, for detemine of mature miRNAs. Total RNA containing miRNA was the starting material in RT-PCR reaction which was performed in two steps.

For reproducible and accurate results in miRNA quantification, a normalization control was used in real-time PCR, where it was crucial to normalize the target miRNA amount by the use of an appropriate endogenous reference RNA. This method is called a relative quantification. Factors that may result in inaccurate quantification are corrected through this normalization. These factors include differences in the quantity of RNA input, the probability of degradation of RNA, the availability of inhibitors in the samples of RNAs, as well as the variation in sample handling. Furthermore, normalization makes it possible to compare different samples directly. In this experiment, RNU6-2 was used as a reference gene according to the manufacturer recommendations. (**TaqMan small RNA assays protocol, 2011**). The F and R primer for miRNA-193a is shown in table 1.

**Table 1: miRNA-193a F and R primer.**

Primer	Sequence	
hsa-miR-193a RT primer	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAG AGCCAACTCAACA	
hsa-miR-193a primer	F	GTTTGGTAGCTTATCAGACTGA
	R	GTGCAGGGTCCGAGGT
hsa-miR-193a probe	FAM- CCAGAGCCAACTCAACA-MGB	

## Results and Discussion

The mean age of patients with FSGS was  $28.38 \pm 13.70$  years, the mean age of the control group was  $30.38 \pm 12.78$  years. There was no significant difference in mean age among study and control groups enrolled in the present study ( $P=0.604$ ), which ensures age matching that is mandatory for such a study.

Regarding to gender distribution about 13 patients (54.2%) were male, and 11 patients (45.8%) were female, while the control group included 19 (79.2%) male and 5 (20.8%) female. There was no significant difference in mean age among the three groups regarding the distribution of patients according to gender ( $P=0.066$ ), which ensures gender match that is mandatory for such a study.

The median fold change and inter-quartile range of miRNA-193a in patients groups were 2.125 (5.86), and 0.375 (1.1) of the control group respectively.. Thus, the level of miRNA-1 fold change was significantly highest in the FSGS group then by control group ( $P<0.001$ ), as shown in figure (1).

Receiver operating characteristic curves (ROC) carried out. The area under the curve (AUC), were 0.826. The cut off value was identified at the miRNA-193a level of  $>0.31$  fold change with a sensitivity of 100 % and a specificity of 50%, as shown in table 2.

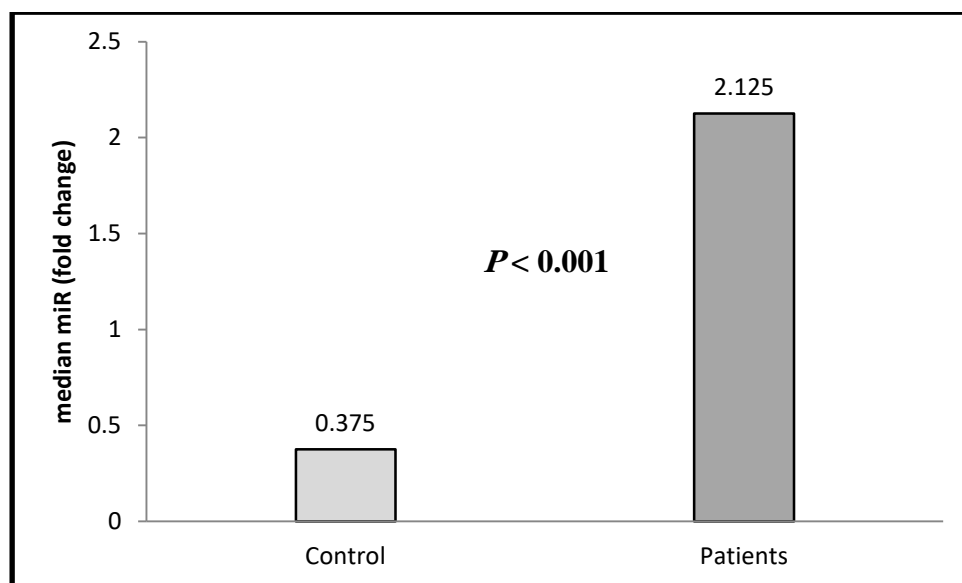
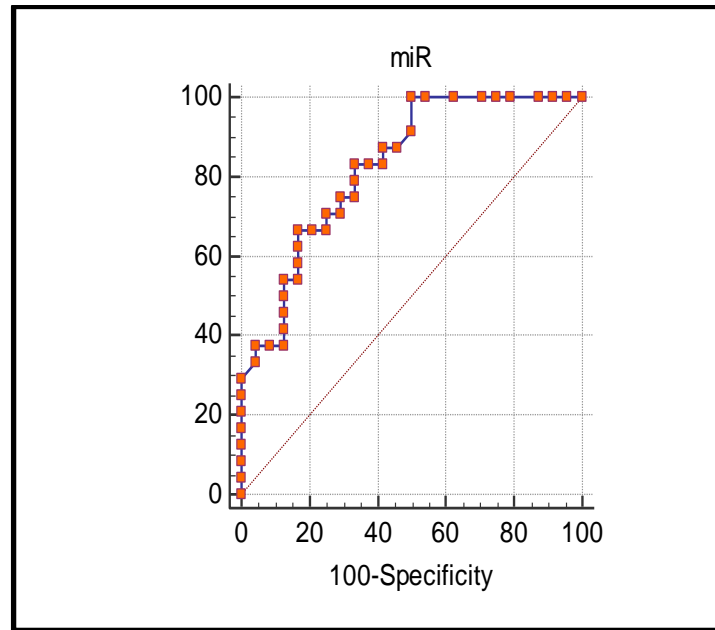


figure (1).median of miRNA 193a in study.



**Figure 2: Characteristics of ROC curve for miRNA-193a.**

**Table 2: ROC cutoff value of miRNA-193a that diagnosis of FSGS**

Cutoff value	>0.31 fold change
AUC (accuracy)	0.826
P	<0.001
Sensitivity	100
Specificity	50

### Discussion:

Circulating levels of miRNA-193a were significantly increased in patients with FSGS, this result came in agreement with study done by Menke et al. they revealed that mechanistic insight into the molecular pathogenesis of glomerular damage induced by dysregulation of a single miRNA. Inducible up-regulation of miR-193a in transgenic mice led to rapidly progressing FSGS and death from renal failure within 12 weeks. Mechanistically, miR-193a binds to and represses *WT1*, a gene that is essential for the development and maintenance of normal podocytes and glomeruli (11, 12).

The study were done by Karolina et al, found that miRNA is being increasingly found to have important regulatory roles in the development, physiology, and maintenance of adult-

kidney microstructure. Though expression profiles of miRNAs in various renal diseases have already been examined, further studies are needed for a thorough understanding of the roles of miRNA in renal pathophysiology. miRNAs form valuable tools for diagnosis of several kidney diseases. This review provides an overview on the crucial roles that miRNAs have in renal function and diseases. The involvement of miRNAs in various kidney diseases/pathogenesis(13).

The miRNA plays a key role in preventing protein synthesis through DNA work, and inhibits the translation process in an early step, possibly at the beginning of the translation, which eventually follows the mRNA decay.(14)

Through previous studies have shown that the miRNA affect the cellular metabolism and have a direct or indirect role in the impact on the physiology of disease for a range of diseases, the most important kidney disease through its impact on Nephron and then can be indicative of its presence in the diagnosis of diseases especially FSGS(15, 16).

In addition, the miRNA play an important role in the regulation of tubular and glomerular damage and proteinuria due to podocyte specific deletion of dicer(17).

The miRNA play important in regulate gene expression at posttranscriptional level and then play role in different cellular function and physiological activities(15) .

More research suggests the present miRNA 193a has a direct relationship to the early detection of FSGS in a patients through its association with oxygen deficiency (hypoxia )(18) . These results is agreement with study done by Zhang et al. that to evaluate the diagnostic values of urinary exosomal miR-193a for primary FSGS, ROC curves were generated to discriminate primary FSGS from MCD in children. the found an area under the ROC curve (AUC) of 0.85 (95% confidence interval [CI] 0.63–1.07)). A ROC analysis identified an optimal threshold of urinary exosomal miR-193a for the diagnosis of FSGS at 530, with a high sensitivity of 75% and a high specificity of 80%. This finding indicated that urinary exosomal miR-193a may be a good index for the differentiation between primary FSGS and MCD in children. levels of urinary exosomal miR-193a were significantly higher in children with primary FSGS than those in children with MCD. (19)the role of specific miRNAs in normal renal development and physiology, but also the initiation and the progression of the interstitial fibrosis that underlies progressive forms of chronic kidney disease. It follows, that miRNAs detected in either plasma o urine, the two

fluidic compartments directly affected by renal processing, may be mechanistically plausible, rational biomarkers for diverse forms of kidney diseases. In fact, miRNA associations found in observational human studies may offer a unique opportunity to “reverse translate” such findings into animal studies, which provide mechanistic insights into novel therapeutics that are tested in rigorous interventional clinical trials in humans.

miRNAs are endogenously expressed in the kidney and several have been found to be up- or downregulated in renal tissue in various kidney diseases. (13, 20) A recent elegant study showed that miR-193a is

Up-regulated significantly in podocytes in FSGS, where it directly targets the expression of WT1, a key transcription factor for podocyte differentiation and health. Surprisingly, extracellular miRNAs are abundant in blood and other biological fluids,(21) where they are shielded from nucleases by being packaged in lipid micro particles (such as in exosomes and micro vesicles) or by association with protein (such as Argonaute 2) and lipoprotein (such as high-density lipoprotein) complexes. The remarkable stability of

circulating miRNAs has made them valuable for use as novel biomarkers in multiple human diseases . other Study done by Arce et al. that they found identify a number of unique miRNA signatures that are associated with human kidney diseases, including FSGS.(20).

miRNAs have also been used as biomarkers—both in serum and urine—to assess FSGS disease activity. In one study, researchers found elevated plasma hsa-miR-125b-5p, hsa-miR-186-50 and hsa-miR-193a-3p in patients with FSGS with area under curve (AUC) of 0.88, 0.78, and 0.91,

respectively . Patients in remission had lower hsa-miR-125b-5p and hsa-miR-186-5p concentrations . These miRNA levels remained unchanged in patients that did not achieve remission.(22)

circulating miRNAs has made them valuable for use as novel biomarkers in multiple human diseases . other Study done by Arce et al. that they found identify a number of unique miRNA signatures that are associated with human kidney diseases, including FSGS.(20). miRNAs have also been used as biomarkers—both in serum and urine—to assess FSGS disease activity. In one study, researchers found elevated plasma hsa-miR-125b-5p, hsa-miR-186-50 and hsa-miR-193a-3p in patients with FSGS with area under curve (AUC) of 0.88, 0.78, and 0.91, respectively .



## **Conclusion :**

The characteristic of miRNA-193a and their high sensitivity and Specificity in this study pushed forward to use them as biomarker or supported for Another biomarker in FSGS diagnosis.

## **References.**

1. D'Agati VD, Kaskel FJ, Falk RJ. Focal segmental glomerulosclerosis. *New England Journal of Medicine*. 2011;365(25):2398-411.
2. Fogo AB. Causes and pathogenesis of focal segmental glomerulosclerosis. *Nature Reviews Nephrology*. 2014;11:76.
3. Kriz W. The pathogenesis of 'classic' focal segmental glomerulosclerosis-lessons from rat models. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 2003;18 Suppl 6:vi39-44.
4. Wharram BL, Goyal M, Wiggins JE, Sanden SK, Hussain S, Filipiak WE, et al. Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *Journal of the American Society of Nephrology : JASN*. 2005;16(10):2941-52.
5. Peired A, Angelotti ML, Ronconi E, la Marca G, Mazzinghi B, Sisti A, et al. Proteinuria impairs podocyte regeneration by sequestering retinoic acid. *Journal of the American Society of Nephrology : JASN*. 2013;24(11):1756-68.
6. D'Agati VD, Fogo AB, Bruijn JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. *American journal of kidney diseases : the official journal of the National Kidney Foundation*. 2004;43(2):368-82.
7. Churg J, Habib R, White RH. Pathology of the nephrotic syndrome in children: a report for the International Study of Kidney Disease in Children. *Lancet (London, England)*. 1970;760(1):1299-302.
8. Kambham N, Markowitz GS, Valeri AM, Lin J, D'Agati VD. Obesity-related glomerulopathy: an emerging epidemic. *Kidney Int*. 2001;59(4):1498-509.
9. Kopp JB, Smith MW, Nelson GW, Johnson RC, Freedman BI, Bowden DW, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat Genet*. 2008;40(10):1175-84.

10. Rajewsky N. microRNA target predictions in animals. *Nat Genet.* 2006;38 Suppl:S8-13.
11. Chau YY, Brownstein D, Mjoseng H, Lee WC, Buza-Vidas N, Nerlov C, et al. Acute multiple organ failure in adult mice deleted for the developmental regulator *Wt1*. *PLoS genetics.* 2011;7(12):e1002404.
12. Guo JK, Menke AL, Gubler MC, Clarke AR, Harrison D, Hammes A, et al. *WT1* is a key regulator of podocyte function: reduced expression levels cause crescentic glomerulonephritis and mesangial sclerosis. *Human molecular genetics.* 2002;11(6):651-9.
13. Chandrasekaran K, Karolina DS, Sepramaniam S, Armugam A, Wintour EM, Bertram JF, et al. Role of microRNAs in kidney homeostasis and disease. *Kidney Int.* 2012;81(7):617-27.
14. Li S, Liu L, Zhuang X, Yu Y, Liu X, Cui X, et al. MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in *Arabidopsis*. *Cell.* 2013;153(3):562-74.
15. Wei Q, Mi QS, Dong Z. The regulation and function of microRNAs in kidney diseases. *IUBMB life.* 2013;65(7):602-14.
16. Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, et al. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. *Cell.* 2008;132(5):875-86.
17. Duisters RF, Tijssen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, et al. miR-133 and miR-30 regulate connective tissue growth factor: implications for a role of microRNAs in myocardial matrix remodeling. *Circulation research.* 2009;104(2):170-8, 6p following 8.
18. Fogo AB. Causes and pathogenesis of focal segmental glomerulosclerosis. *Nature reviews Nephrology.* 2015;11(2):76-87.
19. Huang Z, Zhang Y, Zhou J, Zhang Y. Urinary Exosomal miR-193a Can Be a Potential Biomarker for the Diagnosis of Primary Focal Segmental Glomerulosclerosis in Children. 2017;2017:7298160.
20. Kato M, Arce L, Natarajan R. MicroRNAs and their role in progressive kidney diseases. *Clinical journal of the American Society of Nephrology : CJASN.* 2009;4(7):1255-66.
21. Creemers EE, Tijssen AJ, Pinto YM. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? *Circulation research.* 2012;110(3):483-95.

22. Zhang C, Zhang W, Chen HM, Liu C, Wu J, Shi S, et al. Plasma microRNA-186 and proteinuria in focal segmental glomerulosclerosis. *American journal of kidney diseases : the official journal of the National Kidney Foundation*. 2015;65(2):223-32.