J Renal Inj Prev. 2022; x(x): 23778.

http://journalrip.com doi: 10.34172/jrip.2022.23778

Journal of Renal Injury Prevention

Circulating and cellular levels of miR-193 and miR-217 in patients with common glomerular diseases

Sima Abediazar[1], Soroush Mostafavi[1,2], Mahsa Razavi[1,2], Seyed Sadroddin Rasi Hashemi[1], Seyyedeh Mina Hejazian[1,2], Roya Jabbari[1], Sepideh Zununi Vahed[1], Rovshan Khalilov[3,4], Hamid Noshad[1*], Mohammadreza Ardalan[1*]

[1]Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Department of Biophysics and Molecular Biology, Baku State University, Baku, Azerbaijan
[4]Joint Ukraine-Azerbaijan International Research and Education Center of Nanobiotechnology and Functional Nanosystems, Drohobych, Ukraine

ARTICLEINFO

Article Type:
Original

Article History:
Received: 16 July 2020
Accepted: 12 Sep. 2020
Published online: 24 Sep. 2020

Keywords:
Nephrotic syndrome,
Proteinuria,
Focal segmental glomerulosclerosis,
Membranous glomerulonephritis,
microRNAs

ABSTRACT

Introduction: The definitive diagnosis of the common types of glomerular disease including FSGS (focal segmental glomerulosclerosis) and MN (membranous nephropathy) is still performed by biopsy studies, which has high risks and complications. MicroRNAs (miRNAs) can open a new horizon for the treatment and diagnosis of glomerular diseases.
Objectives: In the present study, we focused on miR-217, miR-193-3p, and miR-124 expression in patients with FSGS and MN.
Patients and Methods: Sixty cases (30 FSGS and 30 MN) were included based on strict criteria. A group of healthy controls were also included. The relative expression of the microRNAs was evaluated in the plasma and peripheral blood mononuclear cells (PBMCs) by quantitative real-time PCR. The association between the expression levels of microRNAs and clinicopathological parameters were also assessed.
Results: There were significant differences in miR-193-3p levels between FSGS and MN group in plasma samples ($P = 0.036$). Furthermore, significantly decreased levels of miR-217 were observed in plasma samples of patients with NS ($P = 0.026$) and MN ($P = 0.036$) groups.
Conclusion: The studied miRNAs are dysregulated in clinical samples of patients with nephrotic syndrome and they may be involved in the pathogenesis of FSGS and MN. More research is needed for understanding the relationship between these microRNAs and the pathogenesis of FSGS and MGN.

Original

Implication for health policy/practice/research/medical education:
Dysregulated levels of microRNAs were observed in clinical samples of adult patients with nephrotic syndrome and they may be involved in the pathogenesis of focal segmental glomerulosclerosis and membranous nephropathy.

Please cite this paper as: Abediazar S, Mostafavi S, Razavi M, Rasi Hashemi SS, Hejazian SM, Jabbari R, Zununi Vahed S, Khalilov R, Noshad H, Ardalan M. Circulating and cellular levels of miR-193 and miR-217 in patients with common glomerular diseases. J Renal Inj Prev. 2021; x(x): 23778. doi: 10.34172/jrip.2022.23778.

Introduction

Nephrotic syndrome (NS) is defined by proteinuria, peripheral oedema, hypercholesterolaemia, and hypoalbuminaemia (1). Increased permeability of glomerular to large molecules is the underlying pathological process in NS with different etiologies. Membranous nephropathy (MN) and focal segmental glomerulosclerosis (FSGS) are the common types of NS in adults that can progress to end-stage renal disease (ESRD) in many patients (2,3). FSGS usually manifests itself in the form of NS (4), less common manifestations of this disease include hypertension, hematuria, and elevated serum creatinine (5). FSGS is grouped into two primary and secondary forms (5). The idiopathic (primary) type, with no associated systemic cause, is more abundant (5) and is probably due to dysfunction in podocytes (6). Secondary FSGS is occurred due to gene mutations, injuries following infection and medications, obesity, vesicoureteral reflux and inappropriate response to the loss of kidney mass (7). The most important mechanism of injury in this disease is

***Corresponding authors**: Prof. Mohammadreza Ardalan, Email; ardalan34@yahoo.com, ardalanm@ tbzmed.ac.ir and Hamid Noshad, Email: hamidnoshad1@yahoo.com

podocyte apoptosis.

Although a rare disease, MN is another cause of NS in adults that has an autoimmune nature characterized by the deposition of immune complexes in renal glomeruli. The mainstream of patients identify with a nephrotic range proteinuria and some patients may also present hypertension and hematuria (8). The disease has been termed primary (idiopathic) and secondary with different causes such as systemic autoimmune disease, various infections, and malignancy (9). Most idiopathic cases of MN are triggered by the production of autoantibodies against PLA2R1 (phospholipase A2 receptor 1) and THSD7A (thrombospondin type-1 domain-containing 7A antigens on podocytes (9, 10).

At present, the diagnosis of the several types of NS is based on biopsy result that is intrinsically an invasive procedure (11). Early diagnosis and treatment of these diseases are important because of the negative impact on the patient's life quality (4). In the absence of treatment, the likelihood of progression to renal failure is 80% for 10 years (12).

MicroRNAs (miRNAs) are non-coding RNAs that controlling gene expression negatively. They have important roles in many biological pathways such as cell apoptosis, proliferation, metabolism, and oncogenesis (13). There has been a possible link between the dysregulation of miRNAs and pathogenesis of kidney diseases (5, 14).

Objectives

Considering an extreme need for a non-invasive and more sensitive biomarker for early detection of the glomerular diseases and roles of dysregulated miRNA in their pathophysiology, we evaluated the expression levels of miR-193-3p, miR-124, and miR-217 in the blood sample of cases with primary FSGS and MN using quantitative real-time PCR (qPCR) and compared the results with healthy control.

Patients and Methods

Study subjects

This cross-sectional study was performed in Tabriz Imam Reza hospital during 17 months. All of the involved patients (n = 60) were diagnosed as primary NS (FSGS and MN) through routine clinical data and renal biopsies. The clinical information of the patients consists of personal characteristics (age, gender and ethnicity), histopathological results, the results of the most recent blood tests were collected. Healthy individuals (n = 24) were also sampled from volunteers who matched with patients. Inclusion criteria were patients with NS with an age range of 20-60. The exclusion criteria for cases were diabetes, serologic positivity for variety of severe viral and bacterial infections, any malignancy, obstruction in renal system, inflammatory diseases, overlap syndrome, any other glomerulopathy, severe organ failure, autoimmune disorder and those with ESRD under dialysis. Moreover, patients with secondary causes of NS such as lupus nephritis, diabetes, amyloidosis, viral infection, and drug-related NS were not included. By providing written informed consent, subjects agreed to participate in this study.

RNA extraction from plasma and peripheral blood mononuclear cells (PBMCs)

Blood samples of all subjects were collected in EDTA-coated vacutainer tubes and plasma and PBMC cells were isolated within 2 hours after sampling. For plasma separation, 2 cc of blood samples were centrifuged twice at 1000 ×g for 10 minutes at 4°C. Finally supernatant (plasma) was aliquot in volumes of 300-500 µL and stored at -80°C. For PBMC extraction, 4 mL of blood was diluted with 4 mL of phosphate buffer solution (PBS) and gently transferred into 4 cc Ficoll-Paque. The solution was centrifuged at 800 ×g for 20 minutes at RT (room temperature). PBMCs layer between Ficoll and plasma was washed at 500 ×g for 10 minutes. Supernatant removed and pellet was washed with 1 mL PBS again and centrifuged at 500 ×g for 10 minutes. Finally, the PBMC cells were completely resolved after removal of the supernatant in 1 cc Trizol (RiboEx™, Geneall) and kept in micro-tubes at -80°C for subsequent use. For each 250 µL plasma sample, 750 µL of Trizol (RiboExTM LS, Geneall) was added and vortexed. Then, RNA extraction was performed for all samples according to the instructions. Finally, after drying RNA precipitation, 20 µL of DEPC water was added into each sample and solved by incubating at 65°C for 5 minutes. The optical absorption rates of A260⁄A230 and A260⁄A280 were accurately calculated with 1 µL RNA extracted via Nanodrop (NanoDrop™ One^{C} Thermo Scientific, USA) to determine the concentration, quality and quantity of RNAs.

cDNA synthesis and quantitative real-time PCR (qPCR)

The cDNA was synthesized from the extracted RNAs of plasma and PBMC samples separately in a volume of 15 µL. In summary, 5 µL RNA extracted from plasma and 1 µg RNA from PBMCs, 3 µL of diluted mixed primers (consist of miR-124, miR-193-3p, miR-217, Snord47, U6 and universal reverse (UR) (Table 1), 0.8 µL RT (RevertAid Reverse Transcriptase, Thermo Fisher), 3 µL RT buffer, 0.375 µL Ribolock (RevertAid Reverse Transcriptase, Thermo Fisher), 1.5 µL dNTP and 1.325 µL DEPC were incubated at 65°C for 5 minutes, 42°C for one hour and 72°C for 10 minutes. The cDNA samples were stored at -80°C for later use.

Ethical approval

The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran certified this study (IR.TBZMED.REC.1396.912). The protocol of the study was explained to all patients and healthy individuals and written informed consent was obtained from them. This research

Table 1. Sequences of designated primers in the study

Primers	Type	Sequence (5'-3')
miR-193a-3p	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTGGG
	F	AACTGGCCTACAAAGTCCCAG
	R	GTGCAGGGTCCGAGGT
miR-217	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCCAAT
	F	GCTACTGCATCAGGAACTGATTGG
	R	GTGCAGGGTCCGAGGT
miR-124-3p	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGCATTC
	F	ACTAAGGCACGCGGTGAATG
	R	GTGCAGGGTCCGAGGT
SNORD-47	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAACCTCA
	F	GCGATATCACTGTAAAACCGTTCC
	R	GTGCAGGGTCCGAGGT
U6	RT	GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGCATACGACAAAAATATGG
	F	AAGGATGACACGCAAATTC
	R	GAGCAGGGTCCGAGGT

RT: stem-loop primers for reverse transcription. F: forward. R: reverse.

was funded by the Kidney Research of Tabriz University of Medical Sciences, Tabriz, Iran. This article was extracted from the M.D, theses of Soroush Mostafavi (Thesis #60478) and Mahsa Razavi (Thesis #60742).

Data analysis

Variables were represented by numbers and percentages. In the normal distribution values, the mean ± SD and for values with non-normal distribution, the median (minimum to maximum) were used. Mann-Whitney U and *t* tests were used to compare quantitative variables and chi-squared was used for qualitative variables. One-way ANOVA test or Kruskal-Wallis was used for comparison of variables between more than two groups for normally and non-normally distributed variables, respectively. Correlation between variables was also determined by Spearman's correlation.

Results

Sixty cases with primary NS were included and divided into two groups (MN and FSGS) based on clinical and histopathology data. No significant difference was found in age [46.6 ± 13 vs. 44.5 ± 12.8, $P = 0.40$], GFR (mL/min/1.73 m^2) [80 (18-137) vs. 79 (10-130), $P = 0.32$], and serum creatinine (mg/dL) [2.21 ± 0.55 vs. 1.2 ± 0.5, $P = 0.17$] in patients with MN compared with FSGS group. Most of the cases in MN and FSGS groups had proteinuria [3013 (85-15000) vs. 1360 (97-15900), $P = 0.26$] despite receiving related therapies.

Plasma level of miR-217 in cases with NS was lower than healthy controls that was statistically significant ($P = 0.026$). However, levels of miR-217 in PBMC samples of patients with NS compared to controls were reduced ($P = 0.739$). In patients with MN, plasma levels of miR-217 were down-regulated compared with healthy controls that were statistically significant ($P = 0.035$; Figure 1A) and its PBMC concentration also decreased when compared to the control group ($P = 0.747$; Figure 1B). In FSGS patients, serum concentration of miR-217 was lower than healthy control that was not significant ($P = 0.085$; Figure 1A); likewise, its PBMC concentration was low ($P = 0.286$; Figure 1B). Patients with MN had a lower serum concentration of miR-217 than those with FSGS that was not significant ($P = 0.085$; Figure 1A). Patients with FSGS had a lower PBMC concentration of miR-217 than those with MN that was not significant (P= 0.127; Figure 1B).

Plasma levels of miR-193-3p were lower ($P = 0.062$) while its cellular levels in PBMC samples of all patients with NS were higher than healthy control ($P = 0.364$). In patients with MN, plasma levels of miR-193-3p significantly decreased when compared to healthy controls ($P = 0.015$). On the other hand, miR-193-3p expression levels were different between MN and FSGS patients in plasma samples ($P = 0.036$; Figure 2A). In PBMCs, an increased level of miR-193-3p was observed in MN ($P = 0.280$) and FSGS ($P = 0.820$) groups compared to healthy controls; however, they were not statistically significant (Figure 2B). Moreover, no significant differences were observed in miR-193 levels between subtypes of NS ($P = 0.58$). There were no significant differences in miR-124 levels between the studied groups.

Discussion

Circulating levels of miR-217 in all NS and MN patients were significantly diminished, however no significant differences were observed in PBMCs of NS, MN, or FSGS patients. Likewise, cell-free levels of miR-193-3p significantly decreased in plasma samples of MN patients

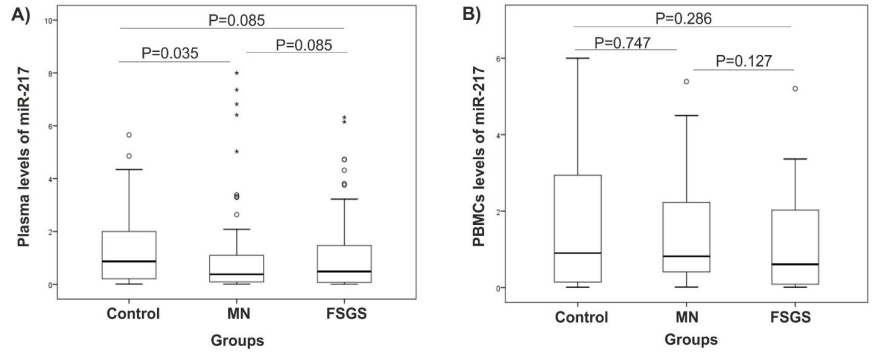

Figure 1. MicroRNA-217 expression level in plasma and peripheral blood cell samples. Comparison of microRNA-217 expression level in (A) plasma and (B) PBMCs samples of patients with nephrotic syndrome with MN and FSGS subclasses and healthy individuals. Fold change was calculated by $2^{-\Delta\Delta Ct}$ formula in the groups. FSGS: Focal Segmental Glomerulosclerosis, MN: Membranous glomerulonephritis, PBMC: Peripheral blood mononuclear cell,

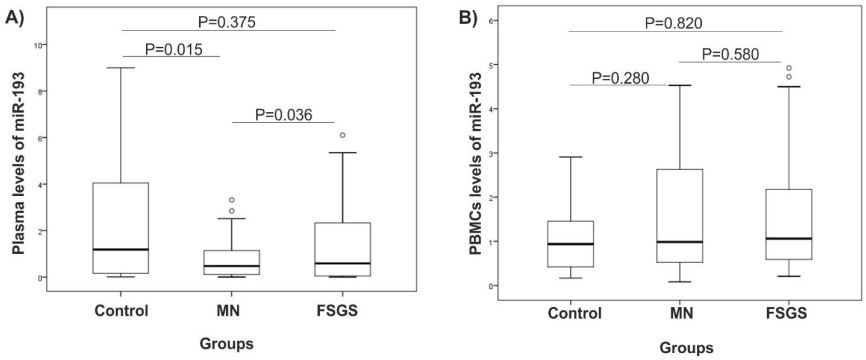

Figure 2. MicroRNA-193-3p expression level in plasma and peripheral blood cell samples. Comparison of microRNA-193-3p expression level in (A) plasma and (B) PBMCs samples of patients with nephrotic syndrome with MN and FSGS subclasses and healthy individuals. Fold change was calculated by $2^{-\Delta\Delta Ct}$ formula in the groups. PBMC: Peripheral blood mononuclear cell, FSGS: Focal Segmental Glomerulosclerosis, MN: Membranous glomerulonephritis

and there were significant differences in miR-193-3p levels between MN and FSGS groups. Nevertheless, in PBMCs samples of the studied groups no significant differences were observed in miR-217, and miR-193-3p levels.

Li et al demonstrated that miR-217 expression level was significantly decreased in MN patients compared to different subclasses of NS and had a high discrimination value to be used as a diagnosis marker for MN patients. They also showed that miR-217 is associated with process of apoptosis in the human podocyte cells (11). Jin et al showed that down-regulation in lncRNA X-inactive specific transcript by miR-217/TLR4 pathway could decrease podocytes apoptosis in MN patients (15). In a study by Szeto et al, it was revealed that alteration in urinary expression of miR-217 was associated with reduced tubulointerstitial fibrosis (16). In this study, dysregulated levels of miR-217 were seen between the studied groups.

In a study among 364 patients with idiopathic MN, a significant increase was observed in urinary miR-193a levels that was associated with the severity of idiopathic MN (17). Gebeshuber et al suggested that over-expression of miR-193a can initiate a series of molecular events that increase podocyte apoptosis by down-regulation of WT1in FSGS (18). However, it is reported that the role of WT1 in podocyte apoptosis is unrelated to miR-193a expression (19). Additionally, Altamemi and Ridha found that miR-193a level in urine samples of FSGS patients was higher than control group significantly and they concluded that this microRNA could be used as a highly sensitive and specific biomarker (20). Likewise, in the present study, a statistically significant dysregulation was observed in circulating levels of miR-193 in both FSGS and MN groups. miR-193a also plays a role in human parietal epithelial cell (hPEC) differentiation. The increase in miR-193a stimulates cells differentiation to the PEC phenotype and its decrease leads to differentiation to the podocyte phenotype (21). These studies imply that dysregulation levels of miR-193 in clinical specimens is associated with pathophysiology of NS with different etiology.

The role of miR-124 has not been studied in NS. Li and colleagues have shown that miR-124 is associated with podocytic adhesive capacity damage and may play a role in the pathogenesis of diabetic nephropathy in rats (22). It is also known to be an important factor in the poor response to glucocorticoid therapy in acute lymphoblastic leukemia (23).

Conclusion

In summary, we concluded that dysregulated levels of miR-193-3p and miR-217 in clinical samples may be associated with the pathophysiology of NS with different etiology. It is critical to research more about their roles since they may serve as a therapeutic and diagnostic candidate in the future.

Limitations of the study

Small sample size was the limitation of this study. Levels of miRNAs are changed during the course of disease and treatment of NS in different clinical samples. It is important to find more reliable clinical sample to evaluate the diagnostic and prognostic values of miRNAs in nephrotic syndrome.

Acknowledgments

The authors wish to express their gratitude toward the Nephrology Ward of Imam-Reza hospital for their honest assistance in sampling. They also want to express special gratitude to Dr. Milad Bastami and Dr. Ziba Nariman-Saleh-Fam for designing the primers.

Authors' contribution

SZV and MA designed the study. SMH and SM did sampling and conducted molecular methods. SZV analyzed data. SM and MR prepared the first draft of the article. SZV, HN, SA, and MA revised the manuscript. All authors read and signed the final paper.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical considerations

The authors completely have observed the ethical issues including data fabrication, falsification, plagiarism, double publication misconduct, or submission and redundancy.

Funding/Support

This work was financially supported by the Kidney Research Center at Tabriz University of Medical Sciences, Tabriz, Iran (Grants # 60742 and # 60478).

References

1. Hull RP, Goldsmith DJ. Nephrotic syndrome in adults. BMJ. 2008;336:1185-9.doi:10.1136/bmj.39576.709711.80
2. He F, Peng F, Xia X, Zhao C, Luo Q, Guan W, et al. MiR-135a promotes renal fibrosis in diabetic nephropathy by regulating TRPC1. Diabetologia. 2014;57:1726-36. doi: 10.1007/s00125-014-3282-0
3. Fan Y, Chen H, Huang Z, Zheng H, Zhou J. Emerging role of miRNAs in renal fibrosis. RNA Biol. 2020;17(1): 1-12. doi: 10.1080/15476286.2019.1667215
4. Sohi GK. A Review of Glomerular Diseases: Focal Segmental Glomerulosclerosis (FSGS) and Minimal Change Disease (MCD). Sciential-McMaster Undergraduate Science J. 2019;(2):9-17.
5. Nguyen S, Jen KY, Focal and Segmental Glomerulosclerosis (FSGS). in: M.N. Haddad, E. Winnicki, S. Nguyen (Eds.), Adolescents with Chronic Kidney Disease: From Diagnosis to End-Stage Disease, Springer International Publishing, Cham, 2019, pp. 129-151.
6. Fogo AB. Causes and pathogenesis of focal segmental glomerulosclerosis. Nat Rev Nephrol. 2015;11:76. doi: 10.1038/nrneph.2014.216
7. Kiffel J, Rahimzada Y, Trachtman H. Focal segmental glomerulosclerosis and chronic kidney disease in pediatric patients. Adv Chronic Kidney Dis. 2011;18:332-338. doi: 10.1053/j.ackd.2011.03.005
8. Jennette JC, Silva FG, D'Agati VD. Heptinstalls Pathology of the Kidney. 7th ed. Wolters Kluwer Health; 2014.
9. Cai Q, Hendricks AR. Membranous nephropathy: A ten-year journey of discoveries. Semin Diagn Pathol. 2020;37:116-120. doi: 10.1053/j.semdp.2020.01.001.
10. Wasserstein AG. Membranous glomerulonephritis. J Am Soc Nephrol. 1997;8:664-674.
11. Li J, Liu B, Xue H, Zhou QQ, Peng L. miR-217 is a useful diagnostic biomarker and regulates human podocyte cells apoptosis via targeting TNFSF11 in membranous nephropathy. Biomed Res Int. 2017;2017:2168767. doi: 10.1155/2017/2168767.
12. Lafayette RA. Increasing options for first-line therapy in primary FSGS? Kidney Int Rep. 2019;4:8-10. doi: 10.1016/j.ekir.2018.10.016
13. Petrillo F, Iervolino A, Zacchia M, Simeoni A, Masella C, Capolongo G, et al. MicroRNAs in renal diseases: a potential novel therapeutic target. Kidney Dis. 2017;3:111-119. doi: 10.1159/000481730
14. Yu J, Yu C, Feng B, Zhan X, Luo N, Yu X, et al. Intrarenal microRNA signature related to the fibrosis process in chronic kidney disease: identification and functional validation of key miRNAs. BMC Nephrol. 2019;20:336. doi: 10.1186/s12882-019-1512-x.
15. Jin LW, Pan M, Ye HY, Zheng Y, Chen Y, Huang WW, et al. Down-regulation of the long non-coding RNA XIST ameliorates podocyte apoptosis in membranous nephropathy via the miR-217-TLR4 pathway. Exp Physiol. 2019;104:220-30. doi: 10.1113/ep087190.
16. Szeto C-C, Ching-Ha KB, Ka-Bik L, Mac-Moune LF, Cheung-Lung CP, Gang W, et al. Micro-RNA expression in the urinary sediment of patients with chronic kidney diseases. Dis Markers. 2012;33:137-44. doi: 10.1155/2012/842764.
17. Zhang W, Ren Y, Li J. Application of miR-193a/WT1/PODXL axis to estimate risk and prognosis of idiopathic membranous nephropathy. Ren Fail. 2019;41:704-717. doi: 10.1080/0886022X.2019.1642210.
18. Gebeshuber CA, Kornauth C, Dong L, Sierig R, Seibler J, Reiss M, et al. Focal segmental glomerulosclerosis is induced by microRNA-193a and its downregulation of WT1. Nat Med. 2013;19:481-7. doi: 10.1038/nm.3142.
19. Zapata-Benavides P, Arellano-Rodriguez M, Bollain YGJJ, Franco-Molina MA, Rangel-Ochoa GA, Avalos-Diaz E, et al. Cytoplasmic Localization of WT1 and Decrease of miRNA-16-1 in Nephrotic Syndrome. Biomed Res Int.

2017;2017:9531074. doi:10.1155/2017/9531074
20. Altamemi IA, Ridha A. Micro-RNA-193a as a Focal Segmental Glomerulosclerosis Biomarker. J Pharm Sci Res. 2019;11:882-885.
21. Kietzmann L, Guhr SS, Meyer TN, Ni L, Sachs M, Panzer U, et al. MicroRNA-193a regulates the transdifferentiation of human parietal epithelial cells toward a podocyte phenotype. J Am Soc Nephrol. 2015;26:1389-401. doi: 10.1681/ASN.2014020190.
22. Li D, Lu Z, Jia J, Zheng Z, Lin S. MiR-124 is related to podocytic adhesive capacity damage in STZ-induced uninephrectomized diabetic rats. Kidney Blood Press Res. 2013;37:422-31. doi: 10.1159/000355721.
23. Liang Y-N, Tang Y-L, Ke Z-Y, Chen Y-Q, Luo X-Q, Zhang H, et al. MiR-124 contributes to glucocorticoid resistance in acute lymphoblastic leukemia by promoting proliferation, inhibiting apoptosis and targeting the glucocorticoid receptor. J Steroid Biochem Mol Biol. 2017;172:62-8. doi: 10.1016/j.jsbmb.2017.05.014.