



Elevated levels of plasma microRNA-192 in patients with lupus nephritis

Jalal Etemadi¹, Mohammad Reza Jafari Nakhjavani², Roza Motavalli³, Fariba Mahmoodpoor¹, Sima Abediazar^{1*}

¹Kidney Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

²Rheumatology, Connective Tissue Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

³School of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

*Correspondence to

Prof. Sima Abediazar,

Email:

abediazars@tbzmed.ac.ir

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Abstract

Introduction: Lupus nephritis (LN) is the most commonly occurring complications of systemic lupus erythematosus that leads to end-stage renal disease (ESRD). Besides genetic background and environmental factors, epigenetic factors especially microRNAs (miRs) are involved in the attainment of the disease.

Objectives: The aim of this study was to evaluate the plasma levels of miR-192 and miR-200b in patients with recently diagnosed as LN.

Patients and Methods: In this study, 26 patients with LN and 26 healthy individuals were included. The plasma levels of the microRNAs were evaluated and their correlation with disease activity and pathological findings along with their ability to distinguish patients with LN were assessed.

Results: Plasma levels of miR-192 were significantly increased in the cases compared to the controls ($P < 0.001$). Circulating miR-192 was not significantly correlated with clinical parameters. The receiver operating characteristic (ROC) curve analysis indicated that circulating plasma miR-192 could discriminate most of the patients with LN from controls with an area under the curve (AUC) of 0.95 [95% CI 0.90-1.00, < 0.001] with 88% sensitivity and 99% specificity.

Conclusion: The results suggested that miR-192 may take part in the pathogenesis of LN. Further studies are needed to confirm the role of circulating miR-192 as a biomarker of LN.

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Introduction

Lupus nephritis (LN) is the most commonly occurring complications of systemic lupus erythematosus, a chronic inflammatory autoimmune disease in which tolerance to self-antigens is lost, that leads to end-stage renal disease (ESRD) (1,2). LN is considered as one of the main problems of the healthcare system. It is estimated that about 40% of patients with lupus experience LN during the disease (3). Conventional important markers that are evaluated mostly in these patients are proteinuria, creatinine ratio, creatinine clearance, anti-double strand DNA, and complement (C3 and C4) levels (2). On the other hand, despite renal biopsy being a gold standard for degree classifying in these patients, it suffers from being invasive and consequently unsuitable for serial monitoring. For above-mentioned reasons, the novel biomarkers are indispensable and valuable in the realm of LN. The combination of traditional and

Key point

In a study on 26 patients with LN and 26 healthy individuals, we did find no significant association between miR-192 and clinical data. However, miR-192 could discriminate most of LN patients from controls with high sensitivity and specificity. This observation suggests that plasma level of miR-192 may have the potential to be used as a biomarker of LN.

novel biomarkers may ease the outcomes of patients with early diagnosis and appropriate therapy.

Despite the unknown etiology of this disease, studies have reported various factors involved in the disease, including genetic background and environmental factors. Recently published articles are also reported that epigenetic factors especially microRNAs (miRs) are involved in the attainment of the disease. The role of microRNAs, small non-coding RNAs, in kidneys opens a new era for better diagnosis and disease management in

renal diseases (4). Up to now, miRNAs have been considered as biomarkers for evaluating kidney function in different kidney pathological statuses from glomerulonephritis (5), renal transplantation (6-9), and beyond. Genetic changes, hormonal effects, environmental factors, or even the pro-inflammatory environment itself can cause dysregulation of the microRNAs expression. The different expression of miRNAs in kidneys during pathological processes may lead to the dysregulation of a wide range of targeted genes and development of kidney diseases.

An array of upregulated and downregulated miRNAs is observed among the cases with LN (10-14). Different expression profiles of microRNAs in LN patients with different ethnic groups indicate the importance of microRNAs as valuable biomarkers of renal injury in LN (15).

Objectives

Since previous studies displayed that miR-192 (16) along with miR-200 (17, 18) family can hinder the expression of important macromolecules involved in epithelial-mesenchymal transition (EMT) and lead to renal fibrosis. The aim of this study was to quantify miR-192 and miR-200b expression levels in plasma samples of patients with recently diagnosed LN that did not receive any LN-related therapy.

Patients and Methods

Subjects

We studied 26 consecutive SLE patients with biopsy proven nephritis. All patients were collected from September 2015 to December 2016. Cases meet SLE diagnostic criteria according to the American College of Rheumatology (ACR). The disease activity index and chronicity index of cases determined according to National Institutes of Health (NIH) system as we explained previously (15). Patients with active infection, previous malignancy, and diabetes mellitus were excluded. Moreover, healthy volunteer individual's samples were collected as controls (N = 26). All patients were free to participate and provided written informed consent to participate. Baseline serum anti-double strand (ds) DNA and anti-nucleic acid (ANA) antibody titers, complement levels (C3 and C4), serum creatinine, and amount of proteinuria in 24-hour urine sample were measured.

Quantification of miRNA expression

The Biofluid miRCURY™ RNA isolation kit was used for isolation of circulating RNA from plasma samples. The miRCURY LNA™ Universal RT cDNA Synthesis kit was used for reverse-transcription (RT) of the extracted RNAs based on the manufacturer recommended protocols. Expression of miR-192, miR-200b, and miR-191-5p was quantified by RT-quantitative polymerase chain reaction (RT-QPCR) based on the protocol of the SYBR® Green

master mix kits using the iCycler iQ system (Bio-Rad). All the commercially used kits were from Exiqon, Vedbaek, Denmark. The miR-191-5p was used as internal control.

Ethical approval

The protocol of the study was institutionally approved by the Clinical Research Ethics Committee of the Tabriz University of Medical Sciences, Tabriz, Iran (# IR.TBZMED.REC. 1395.494) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The protocol of the study was clarified to all participants and written informed consent was achieved from the patients.

Statistical analysis

The results were shown as the mean \pm SD and median (interquartile range) for normal and nonparametric data. The differences between the groups were analyzed for statistical significance using an unpaired t test or Mann-Whitney U test. The association between plasma levels of the studied miRNAs and clinical parameters was analyzed by Spearman's rank order correlation. In order to test the diagnostic performance of miR-192, receiver operating characteristic (ROC) analysis and the area under the curve (AUC) with 95% CI were applied. All statistical analyses were performed using IBM SPSS software version 17.0. A P value <0.05 was considered statistically significant.

Results

Clinical and demographic characteristics of the patients are presented in Table 1. The relative expression of circulatory miRNA-192 in plasma samples of participants in the LN group was compared to healthy individuals. Significantly, increased levels of miRNA-192 were observed in the LN group compared to controls (median 10 fold, $P < 0.001$) (Figure 1A). miR-200b was not detected in some plasma samples of LN group (Ct > 40), therefore, its result did not analyze.

The relationship between clinical parameters and fold change of miRNA-192 was explored. The expression level of the circulatory miRNA-192 was not significantly correlated with the patients' age ($r = 0.27$, $P = 0.17$), C3 ($r = -0.19$, $P = 0.34$), C4 ($r = -0.21$, $p = 0.31$), anti-double-strand DNA ($r = 0.08$, $P = 0.68$), ANA ($r = -0.03$, $P = 0.87$), serum creatinine ($r = 0.15$, $P = 0.44$), 24 hours' urine proteinuria ($r = 0.21$, $P = 0.29$), chronicity index ($r = 0.23$, $P = 0.24$), disease stage ($r = -0.14$, $P = 0.50$), activity index ($r = -0.13$, $P = 0.51$), and erythrocyte sedimentation rate ($r = -0.09$, $P = 0.65$). The ROC analysis was performed to evaluate whether the circulating miRNA-192 level (defined as fold change values) can discriminate the LN from controls. The predictive power of the circulating miRNA-192 level for LN made an AUC of 0.95 [95% CI 0.90 to 1.00, <0.001] with 88% [0.70 - 0.97] sensitivity and

Table 1. Demographic and baseline clinical data

Characteristics	LN patients	Control group
No. of cases	26	26
No. of male	6	9
No. of female	20	17
Age, mean \pm SD (y)	32.61 \pm 9	30.00 \pm 7.22
C ₃ (mg/dL)	27.00 \pm 9	89.94 \pm 17.34
C ₄ (mg/dL)	13.27 \pm 6	45.83 \pm 16.18
ANA	7.77 \pm 3	0.60 \pm 0.20
Anti-dsDNA	61.00 \pm 25	14.11 \pm 4.24
Creatinine, (mg/dL)	1.46 \pm 0.3	0.91 \pm 0.10
Proteinuria (mg/24 h)	2107 \pm 1094	94.66 \pm 15.55
ESR	33.61 \pm 12	12.66 \pm 3.67
Chronicity index	6.4 \pm 3.1	-
Activity index	10.0 \pm 3.4	-
Stages (3/4/5)	9/11/6	-

ANA: anti-nucleic acid, anti-dsDNA: anti-double strand DNA, ESR: erythrocyte sedimentation rate.

The quantity data are expressed as mean \pm SD.

99% [0.81-1.00] specificity (Figure 1B).

Discussion

It is well-known that TGF- β /Smad3 signaling stimulates renal fibrosis and miR-192 and miR-200 family may be its serious downstream mediator (19). miR-192 has functional significance in kidney biology and the preservation of cell function in the nephron (20). Moreover, miR-192 has a role in cell cycle control (21). miR-192 is highly expressed in kidney and presents pleiotropic roles in renal homeostasis and in the fibrotic kidney (20). miR-192 and miR-200b expression levels were increased by TGF- β treatment in mesangial cells (MCs) and kidney of diabetes experimental models and lead to accumulation of collagen in MCs through targeting E-box repressors (Zeb1/2) and modification of other renal miRNAs (16, 22). However,

miR-192 shows pro- and/or anti-fibrotic characteristics based on cell phenotype (20).

The functional role of miR-192 in human kidney disease seems to be subtler than originally reported. In human kidney, miR-192 level is reported to be altered in acute kidney injury (23), IgA nephropathy (24), hypertensive nephrosclerosis (25), and renal allograft fibrosis (26, 27). Although the published literature also suggests a particular role for miR-192 in pathogenesis and progression of diabetic nephropathy, the accurate function of miR-192 in diabetic nephropathy is controversial. Krupa et al concluded that in response to TGF- β , miR-192 expression is reduced in proximal tubular epithelial cells and this loss of expression is associated with tubulointerstitial fibrosis in biopsy samples of patients with diabetic nephropathy (28). Likewise, decreased levels of urine miR-192 were observed in diabetic nephropathy than other nephrotic syndrome causes (membranous nephropathy, minimal change nephropathy or focal glomerulosclerosis (29). However, in glomeruli of diabetic mice models, an increase in miR-192 expression could down-regulate E-box repressors and control the TGF- β -induced Col1a2 expression (16).

In a study, Wang and colleagues showed that serum and urinary levels of miR-192 and miR-200b were lower than the control group in patients with active LN. However, we observed an increase in circulating miR-192 but a decrease in miR-200b of patients with LN that may present kidney injury or fibrosis. The diminished levels of miR-200b in some cases were so minor that it could not be recognized by qPCR. Wang et al found no significant correlation between serum and urinary miRNA levels. Serum levels of miR-192 and miR-200b but not that of urine were correlated with glomerular filtration rate. However, they were not associated with any other clinical parameters (30).

Conclusion

In the present study, no significant association between

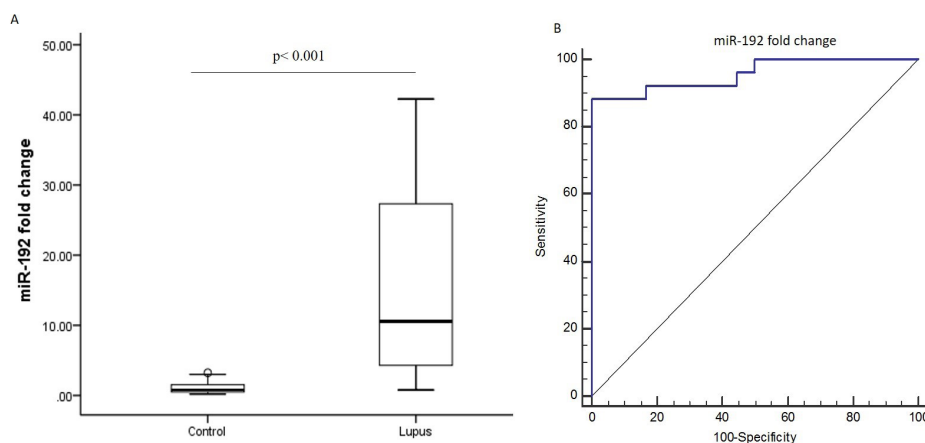


Figure 1. Significance of miRNA-192 in lupus nephritis group when compare to controls. (A) Level of circulating miR-192 in plasma samples of patients with lupus nephritis. miR-191 was used as normalizing endogenous control. (B) ROC curve analysis of plasma miR-192 based on q-PCR data.

miR-192 and clinical data was detected. However, miR-192 could discriminate most of LN patients from controls with high sensitivity and specificity. This observation suggests that plasma level of miR-192 may have the potential to be used as a biomarker of LN.

Study limitations

The measurements of the alteration in miRNA expression levels can be applied for early diagnosis of the disease, as well as the evaluation of the response to treatment and monitoring of the patients. Due to the clinical significance of this issue and the lack of definitive findings in medical references, further studies in this field are necessary for better decision making.

Conflicts of interest

The author declares no competing interests.

Authors' contribution

All authors engaged in the design of the research and acquisition of information and contributed equally to the manuscript.

Ethical considerations

Ethical matters such as (plagiarism, misconduct, data fabrication, falsification, and double publication or submission) have been thoroughly controlling by all authors.

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