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Original Article

Clinical significance of miR-200a in systemic lupus erythematosus and renal damage in children

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Abstract

The purpose was to analyze the clinical significance of miR-200a in children with initially diagnosed SLE and renal damage. Children with initially diagnosed SLE (n=100) and healthy children (n=50) undergoing physical examinations during the same period were recruited. Disease activity of SLE children was determined based on SLEDAI (systemic lupus erythematosus disease activity index), and they were divided into SLEDAI≤9 group and SLEDAI>9 group, respectively. Moreover, SLE children were divided into LN and non-LN groups based on the occurrence of lupus nephritis. Differential level of miR-200a between groups was detected by qRT-PCR. Spearman correlation test was conducted to analyze the influence of miR-200a on SLEDAI and other laboratory indexes of SLE children. Its diagnostic potential in SLE and LN was assessed through depicting ROC curves. MiR-200a level was remarkably lower in SLE children than that of healthy children. Lower level of miR-200a was detected in SLE children with high SLEDAI or accompanied LN. MiR-200a level was negatively correlated to SLEDA

I (r=-0.425), ESR (r=-0.284), CRP (r=-0.338), BUN (r=-0.263) and Scr (r=-0.345), while it was positively correlated to C3 (r=0.631), C4 (r=0.524) and ALB (r=0.394) in SLE children. The AUC of miR-200a in diagnosing SLE was 0.8379 (cut-off value=2.225, sensitivity=70%, specificity=70%). Besides, the AUC of miR-200a in diagnosing LN was 0.7619 (cut-off value=2.005, sensitivity=80%, specificity=76%). MiR-200a level has a certain correlation to the disease activity of children with initially diagnosed SLE, which can be utilized as an adjuvant indicator in evaluating SLE severity. Meanwhile, miR-200a has predictive value for SLE-induced renal damage.

Keywords: SLE, LN, miR-200a, Diagnosis

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease, which can involve multiple systems and organs. The main pathological change is vasculitis mediated by the immune complex. SLE is characterized by severe interferences in the functions and activities of innate and adaptive immune cells. Disease manifestations, pathological processes, and clinical outcomes vary significantly among individuals, ethnicities, and age groups. About 10-20% of SLE cases initiate in childhood or puberty, which mainly affects the population in 12-16 years [1]. Compared with adult patients, SLE children have more atypical early manifestations, more dangerous progression, more rapid involvement of organs and worse prognosis [2].

Lupus nephritis (LN) is an important complication of SLE, with diverse clinical manifestations, including asymptomatic hematuria and/or proteinuria, nephrotic syndrome and acute progressive nephritis with renal dysfunction. About 40%-70% of SLE children have clinical manifestations of LN, and the incidence of LN in SLE children is 10-30% higher than in SLE adults. It is reported that 90% of SLE patients suffer from renal damage as renal biopsy results suggest. Seriously, 5-20% of LN patients will aggravate uremia within 10 years [3]. Recently, the incidence of childhood SLE is on the rise, as well as that of LN [4].

MiRNAs are a type of endogenous, non-coding, single-stranded RNAs, and they have about 25 nucleotides in length [5]. They guide mRNA degradation or post-transcriptionally suppress protein translation by binding 3'UTR of target genes, thereby participating in life activities [6]. Due to the stability, disease specificity, and availability, plasma miRNAs have gradually been discovered as disease biomarkers [7,8]. Plasma miRNAs are capable of diagnosing tumors, determining therapeutic efficacy, and monitoring tumor recurrence and metastasis [9-11]. Their vital regulations in autoimmune diseases have been recognized as well [12,13]. MiR-200a is differentially expressed in tumor samples, which is closely linked to malignant phenotypes of tumor cells and clinical prognosis [14,15]. This study aims to explore the clinical significance of miR-200a in childhood SLE and LN.

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2. Materilas and Methods

2.1. Subjects

This study was approved by the ethics committee of Zhanjiang Central People's Hospital. Signed written informed consent were obtained from the patients and/or guardians. Children with initially diagnosed SLE (n=100) in our hospital were recruited. Inclusion criteria: (1) Age < 18 years; (2) They were diagnosed as SLE based on the standard released by the Systemic Lupus International Collaborating [16]; (3) Clinical data were complete; (4) Other diseases were excluded, including drug-induced lupus, rheumatoid arthritis, blood system diseases, mixed connective tissue diseases, etc. Based on the diagnosis of SLE, lupus nephritis (LN) with any of the following manifestations of renal involvement could be diagnosed: (1) Urinary protein test met any of the following: Positive urine protein qualitative examination three times in one week; Or 24-hour urine protein >150 mg; Or urine protein/urinary creatinine > 0.2 mg/mg; Or higher microalbuminuria than the normal three times a week; (2) > 5 erythrocytes per high power field of vision in centrifugal urine; (3) Abnormal function of glomerulus and/or renal tubules; (4) Abnormal findings in renal biopsy that was consistent with pathological changes of LN. During the same period, fifty healthy children undergoing physical examinations were recruited as a control group.

2.2. Acquisition of clinical data

The following data of each subject were recorded. (1) Routine blood test data: WBC, HGB, PLT, NE, LY, MPV and RDW; (2) Baseline characteristics: Age, sex and clinical manifestations; (3) Laboratory indexes: ALB, BUN, Scr, C3, C4, CRP, ESR, ds-DNA and SLEDAI.

2.3. SLEDAI scoring

The disease activity of SLE children was determined using SLEDAI-2000. According to the symptoms and examinations within 10 days, SLEDAI was assessed with the involvement of 24 clinical indexes on 9 organs and systems. Its total score was 105 grades (<4: no disease activity; 5-9: mild stable disease; 10-14: moderate disease activity; ≥15: active disease) [17].

2.4. qRT-PCR

Serum miRNAs were isolated and reversely transcribed to cDNAs. A qRT-PCR mixture involving 1.0 μl of cDNA, 0.8 μl of primers, 10 μl of 2 × miRNA qPCR Mix and 8.2 μl of ddH_2O was prepared. It was subjected to qRT-PCR at 95°C for 10 min, followed by 45 cycles at 95°C for 10 s, 60°C for 20 s and 72°C for 12 s. U6 (forward: 5'-CTCGCTTCGGCAGCACATTTT-3' and reverse: 5'-AACGCTTCACGAATTTGCGT-3') was the internal reference. MiR-200a sequences were: Forward: 5'-TAACACTGTCTGGTAACGATGT-3' and reverse: 5'-ATCGTTACCAGACAGTGTTATT-3'.

2.5. Statistical analysis

Statistic Package for Social Science (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Normally distributed quantitative data were expressed as mean ± standard deviation, and differences between groups were compared by the *t*-test. Enumeration data were expressed as a percentage (%) and compared by the χ^2 test. Spearman correlation test was conducted to analyze the influence of miR-200a on SLEDAI and other laboratory indexes of SLE children. The diagnostic potential of miR-200a was assessed by depicting ROC curves. $P<0.05$ was considered as statistically significant.

3. Results

3.1. Comparison between SLE children and healthy subjects

No significant differences in age and sex rate were detected between SLE children and healthy subjects ($P>0.05$). Lower levels of WBC, HGB, PLT and LY, and higher levels of RDW and MPV were detected in SLE children than in healthy subjects. In addition, the serum level of miR-200a was lower in SLE children compared with that of healthy subjects (Table 1). MiR-200a may be involved in the progression of SLE.

3.2. Correlation between miR-200a and laboratory indexes relevant to SLE activity

The recruited 100 SLE children were divided into SLEDAI≤9 group (n=41) and SLEDAI>9 group (n=59). Higher levels of ESR and CRP, as well as higher rate of positive anti-dsDNA, were detected in SLEDAI>9 group in comparison to the other group. Besides, C3, C4 and miR-200a levels were lower in SLEDAI>9 group (Table 2). It is indicated that miR-200a could affect the disease activity of SLE.

3.3. Correlation between miR-200a and laboratory indexes relevant to SLE-induced LN

Recruited 100 SLE children were divided into LN group (n=68) and non-LN group (n=32). Higher levels of

Table 1. Comparison of clinical data between SLE group and control group.

Variable	SLE group (n=100)	Control (n=50)	t/χ^2	*P*
Age (years old)	10.4±2.1	10.6±2.8	-0.490	0.625
Male/Female	21/79	8/42	0.534	0.518
WBC ($\times10^9$/L)	4.1±1.15	6.2±1.34	-9.969	<0.001
HGB (g/L)	90±12.1	128±17.3	-15.630	<0.001
PLT ($\times10^9$/L)	130±25.3	247±31.7	-24.489	<0.001
NE ($\times10^9$/L)	2.4±0.75	2.51±0.81	-0.824	0.411
LY ($\times10^9$/L)	1.5±0.09	2.3±0.21	-32.644	<0.001
RDW (%)	15.8±2.4	12.9±2.0	7.358	<0.001
MPV (fl)	8.7±1.02	8.5±0.92	1.169	0.244
miR-200a	1.31±0.56	2.41±0.97	-8.796	<0.001

Table 2. Comparison of laboratory indicators between high and low SLEDAI groups.

Variable	SLEDAI≤9 (n=41)	SLEDAI>9 (n=59)	t/χ^2	*P*
C3 (g/L)	0.35±0.07	0.31±0.05	3.335	0.001
C4 (g/L)	0.05±0.03	0.03±0.01	4.763	<0.001
ESR (mm/h)	32±8.22	47±11.53	-7.157	<0.001
CRP (mg/L)	1.14±0.88	2.31±0.93	-6.324	<0.001
Anti-ds-DNA antibody (Positive/Negative)	18/23	39/20	4.864	0.040
miR-200a	2.72±1.04	2.14±0.85	3.060	0.003

Table 3. Comparison of laboratory indexes between LN group and non-LN group.

Variable	LN group (n=68)	non-LN group (n=32)	t	*P*
ALB (g/L)	30.13±7.42	34.64±8.03	-2.762	0.007
BUN (mmol/L)	5.2±1.42	4.5±1.17	2.426	0.017
Scr (μmmol/L)	53.15±19.74	44.82±16.39	2.073	0.041
SLEDAI	15±2.15	10±1.06	12.439	<0.001
miR-200a	2.14±0.86	2.77±1.13	-3.081	0.003

Table 4. Correlation analysis of miR-200a and various indicators.

Variable	r	*P*
SLEDAI	-0.425	0.002
ESR (mm/h)	-0.284	0.018
CRP (mg/L)	-0.338	0.007
C3 (g/L)	0.631	<0.001
C4 (g/L)	0.524	<0.001
BUN (mmol/L)	-0.263	0.006
Scr (μmmol/L)	-0.345	0.021
ALB (g/L)	0.394	0.028

BUN, Scr and SLEDAI, as well as lower levels of ALB and miR-200a were detected in LN group in comparison to non-LN group (Table 3). It is suggested that low serum level of miR-200a may trigger the incidence of LN in SLE children.

3.4. Correlation between miR-200a and laboratory indexes relevant to SLE activity

Spearman correlation test was conducted to analyze the influence of miR-200a on SLEDAI and other laboratory indexes of SLE children. MiR-200a level was negatively correlated to SLEDAI (r=-0.425), ESR (r=-0.284), CRP (r=-0.338), BUN (r=-0.263) and Scr (r=-0.345), while it was positively correlated to C3 (r=0.631), C4 (r=0.524) and ALB (r=0.394) in SLE children (Table 4).

3.5. Diagnostic potential of miR-200a in SLE and LN

To ascertain the prognostic potential of miR-200a in SLE and renal damage, ROC curves were depicted. The AUC of miR-200a in diagnosing SLE was 0.8379 (cut-off value=2.225, sensitivity=70%, specificity=70%) (Figure 1A). Besides, the AUC of miR-200a in diagnosing LN was 0.7619 (cut-off value=2.005, sensitivity=80%, specificity=76%) (Figure 1B).

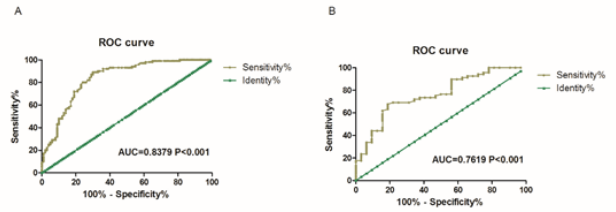

Fig. 1. Sensitivity and specificity of miR-200a in predicting SLE and LN. (A) The AUC of miR-200a in diagnosing SLE was 0.8379 (cut-off value=2.225, sensitivity=70%, specificity=70%); (B) The AUC of miR-200a in diagnosing LN was 0.7619 (cut-off value=2.005, sensitivity=80%, specificity=76%).

4. Discussion

Childhood SLE is a chronic autoimmune disease characterized by vascular inflammation and connective tissue inflammation. Positive expressions of specific antinuclear antibodies and anti-dsDNA can be detected in SLE patients [18]. The incidence of childhood SLE varies in different races, ranging from 10/100,000-20/100,000, and it covers 15-50% of SLE cases. The 10-year survival of SLE is nearly 90%. However, the life quality of SLE children is poor [19]. About 67-82% of SLE children suffer from renal damage, mainly manifested as proteinuria and hematuria [20,21].

During the active phase of SLE, a large number of immune complexes are deposited in tissues and organs, which activate the complement system to eliminate them through complements-induced classical and bypass pathways [22,23]. As a result, serum C3 and C4 are abundantly consumed. CRP is an acute phase reaction protein and it can activate the complement system. CRP level in the remission phase of SLE decreases significantly compared to the active phase, and it is positively correlated with

SLEDAI [22]. Recent evidences have proven the close relation between red blood cell distribution width and autoimmune diseases. RDW is an independent risk factor for autoimmune hepatitis (AIH)-induced cirrhosis, which is a promising indicator for revealing the progression of AIH [24]. Tecer D at el. [25] suggested that RDW is able to reflect inflammatory state of rheumatoid arthritis, and it is linked to the disease activity and pain degree. Platelet activation is an inflammatory marker. Platelet activation in SLE patients may be related to immune complex deposition, antiphospholipid antibodies, and infectious factors such as viruses, and it is an important cause of the pathogenesis of SLE [24]. In clinical practice, relative levels of antinuclear antibodies and anti-dsDNA are detected to reflect the disease activity of SLE [26]. Consistently, our findings uncovered that routine blood test data (WBC, HGB, PLT, NE, LY, MPV and RDW) and laboratory indexes (ALB, BUN, Scr, C3, C4, CRP, ESR and anti-dsDNA) were closely linked to the onset of SLE.

The production of proinflammatory factors, cell death and antigen presentation during the progression of SLE are all affected by miRNAs [27-31]. Sheedy et al. [32] discovered 7 differentially expressed miRNAs between SLE patients and healthy controls. Our findings showed that serum level of miR-200a was remarkably downregulated in SLE children. Its level was negatively correlated to SLEDAI, ESR, CRP, BUN and Scr, while it was positively correlated to C3, C4 and ALB in SLE children. In addition, ROC curves demonstrated the diagnostic potential of miR-200a in SLE and LN.

Taken together, this study evaluated the potential interaction between miR-200a and SLE and renal damage. It is concluded that miR-200a level was positively correlated to SLEDAI, which could be an inflammatory indicator for assessing the disease activity of SLE children. Moreover, miR-200a could predict the onset of SLE and the following renal damage.

5. Conclusion

MiR-200a level has a certain correlation to the disease activity of children with initially diagnosed SLE, which can be utilized as an adjuvant indicator in evaluating SLE. Meanwhile, miR-200a has predictive value for SLE-induced renal damage.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This study was approved by the ethics committee of Zhanjiang Central People's Hospital.

Informed Consent

Signed written informed consent were obtained from the patients and/or guardians.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

HZ and MZ designed the study and performed the experiments, XZ collected the data, QL analyzed the data, HZ and MZ prepared the manuscript. All authors read and approved the final manuscript.

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