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Original Research Article

MiR-196a and miR-122 as potential markers in cholestatic liver disease induced by drugs

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Abstract

Purpose: *To determine the role of miR-196a and miR-122 in cholestatic liver disease (CSLD) induced by drugs.*
Methods: *Sixty (60) children treated in Cangzhou Infectious Disease Hospital for drug-induced CSLD from January 2017 to January 2020 were recruited as the CSLD group, and 60 healthy people in the same period were enrolled as the control group. Polymerase chain reaction (PCR) was applied to evaluate the expression of serum miR-196a and miR-122, while enzyme-linked immunosorbent assay (ELISA) was used to determine the serum levels of nuclear factor erythroid 2-related factor 2 (Nrf–2) and heme oxygenase-1 (HO-1). Diagnostic efficacy of miR-196a and miR-122 was assessed using receiver operating characteristic (ROC) curve.*
Results: *Compared to the control group, miR-196a and miR-122 in CSLD group were highly-expressed ($p < 0.05$). The serum expression of miR-196a in patients with mild, moderate and severe CSLD was noticeably higher than for control group ($p < 0.05$); the expression level of moderate and severe CSLD was lower than that of mild CSLD ($p < 0.05$). The serum miR-122 expression of patients with mild, moderate and severe CSLD was also higher compared to control group ($p < 0.05$). Expressions of miR-196a and miR-122 were positively correlated with Nrf-2 and HO-1.*
Conclusion: *The expression of miR-196a and miR-122 in the serum of patients with drug-induced CSLD significantly increases, but decreases with the severity of CSLD. Thus, these molecules can potentially as biomarkers for the condition and prognosis of patients with CSLD.*

Keywords: *MiR-196a, MiR-122, Cholestatic liver disease, Diagnostic value*

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INTRODUCTION

Cholestatic liver disease (CSLD) is defined as the accumulation of bile in hepatic tissue following intrahepatic or extrahepatic biliary obstruction [1-3]. The major symptoms include fatigue, jaundice and dark urine, etc. [4,5]. Due to the complex pathogenesis of CSLD, the clinical efficacy of CSLD remains unsatisfactory [6]. It is documented that drug-induced liver injury (DILI) contributes to nearly half of CSLD [7]. Therefore, the relevant pathogenesis of drug-induced CSLD

development and the biomarkers for diagnosis and treatment should be addressed in a timely fashion. To date, it has been largely reported that the abnormal regulation of microRNA (miRNA) is strongly associated with the occurrence and development of multiple diseases, and it participates in the regulation of bile acid signals and the occurrence of cholestatic liver fibrosis [8].

Additionally, it has been noted that miR-196a and miR-122 are specifically expressed in liver tissue, and miR-196a is involved in the occurrence and development of liver cancer [9], miR-122 affects steatosis and plasma cholesterol levels, yet the expression of these two parameters in liver diseases are rarely reported. In consideration of these findings, it remains to be explored whether these two indicators can be used as biomarkers for early diagnosis of drug-induced CSLD. In this regard, the authors attempted to determine the value of miR-196a and miR-122 in the early diagnosis of drug-induced CSLD by detecting their expression.

METHODS

General information

The protocol was approved by Medical Science Research Ethics Committee of Jinan City People's Hospital (approval no. 2016-23(034) and followed international guidelines for human studies [10]. Drug-induced CSLD children who were treated in Cangzhou Infectious Disease Hospital from January 2017 to January 2020 were enrolled in this study. Inclusion criteria were (1) patients who met the clinical diagnostic criteria for drug-induced CSLD [11]; (2) patients who were diagnosed as CSLD by imaging or endoscopy; (3) patients who agreed to participate in this study. Exclusion criteria were (1) patients with malignant tumors; (2) patients with extrahepatic biliary atresia and (3) patients with bile duct dilation. Finally, 60 patients were included in the CSLD group. Sixty (60) healthy people who took a physical examination in our hospital in the same period were selected as the control group. The baseline data for the two groups were identical ($p > 0.05$, Table 1).

Sample collection

5 mL of fasting venous blood of drug-induced CSLD children and healthy people after overnight fasting was obtained by venipuncture. All samples were placed in ethylene diamine tetraacetic acid anticoagulation tubes and labeled. The sample was centrifuged at 1500 r/min for 10 min, supernatant was collected and placed in another sterile anticoagulation tube, and stored at -80 ºC for use. The serum levels of Nrf-2 and HO-1 were detected by Elisa kits.

Table 1: Baseline data (n = 60)

Group	Male	Female	Mean age (mean ± SD, month)
CSLD group	36	24	3.39±1.08
Control group	37	23	3.36±1.02
χ^2/t	0.035		0.156
P-value	0.852		0.876

Determination of serum miRNA

(1) RNA extraction. TRIzol reagent was used to extract 1 μL total RNA in the serum, and the concentration and purity of the RNA were detected by ultraviolet spectrophotometer. The instruments and consumables used in the extraction process were subjected to high temperature and water immersion to eliminate RNase. (2) miRNA reverse transcription reaction. miR-196a, miR-122 reverse transcription reaction reagents were purchased from Shanghai Haoran Biological Technology Co., Ltd. Reverse transcription reaction was performed in strict accordance with instructions. The primer sequences are shown in Table 2. (3) PCR reaction. Amplification was performed using real-time quantitative PCR instrument (Shanghai Bio-Rad Laboratories Co., Ltd.). It was processed at 95°C for 15min, 95°C for 15 sec, and 60 °C for 1min, totaling 40 cycles, and 72 °C extension for 5 min. The internal reference was U6RNA (forward primer: 5'-CTCGCTTCGGCAGCACA-3', reverse primer: 5'-AACGCTTCACGAATTT-3'). The above measurements were repeated 3 times to obtain average. The relative expression levels of miR-196a and miR-122 were calculated using the formula 2-△△CT.

Table 2: Primer sequences for miR-196a and miR-122

Item	Location	Sequence
miR-196a	Upstream Primer	5' -CGTCAGAAGGAATGATGCACAG-3'
	Downstream Primer	5'-ACCTGCGTAGGTAGGTTTCATGT-3'
miR-122	Upstream Primer	5'-ATTGCGGTGGAGTGTGTCATGG-3'
	Downstream Primer	5'-AACCAGTGCAGCGTCCGAGG-3'

Statistical analysis

SPSS 22.0 was applied for statistical analysis. Quantitative data were reported as x±s, and the significance was assessed using t test; qualitative data were expressed as cases or percentage, and the significance was assessed using chi-square test. ROC curve was employed to evaluate the diagnostic efficacy of miR-196a and miR-122; Pearson correlation coefficient was adopted to analyze the correlation. Significance was set at $p < 0.05$.

RESULTS

Expression of miR-196a and miR-122 in serum of children with drug-induced CSLD

Compared to control group, the expressions of miR-196a and miR-122 in drug-induced CSLD group remarkably increased ($p < 0.05$), as shown in Table 3.

Table 3: Comparison of expression of miR-196 and miR-122 (mean ± SD, n = 60)

Group	miR-196a	miR-122
CSLD	6.69±1.15	7.94±2.41
Control	1.24±0.36	1.56±0.43
T	35.031	20.192
P-value	＜0.001	＜0.001

Expression of miR-196a in children with varying severity of drug-induced CSLD

When compared to the control group, the serum expressions of miR-196a in patients with mild, moderate and severe drug-induced CSLD was noticeably higher ($p < 0.05$); the expression of moderate and severe drug-induced CSLD was lower than that of mild CSLD ($p < 0.05$); the expression of severe CSLD was lower than that of moderate CSLD, but the difference was not significant ($p > 0.05$). See Table 4.

Expression of miR-122 in children with different severity of drug-induced CSLD

The serum expressions of miR-122 in patients with mild, moderate and severe CSLD was found to be significantly higher comparing against the control group ($P < 0.05$); the expression of moderate and severe CSLD was lower than that of mild CSLD, whereas no remarkable difference was found ($p > 0.05$); the expression of severe CSLD lower than that of moderate CSLD, but this was not significant ($p > 0.05$), as shown in Table 5.

Table 4: Comparison of expression of miR-196a varying degrees of severity of CSLD (mean ± SD)

Group	n	MiR-196a	t	*P*-value
Control	60	1.24±0.36		
Mild CSLD	24	7.45±1.74*	26.492	<0.001
Moderate CSLD	19	6.32±1.51$^{*\#}$	24.273	<0.001
Severe CSLD	17	6.03±1.34$^{*\#\&}$	25.031	<0.001

$^{*}P < 0.05$ compared to control group; $\#P < 0.05$ compared to mild CSLD group; $^{\&}p < 0.05$ compared to severe CSLD

Table 5: Comparison of expression of miR-122 at different degrees of severity of CSLD (mean ± SD)

Group	N	MiR-122	t	*P*-value
Control	60	1.56±0.43		
Mild CSLD	24	8.53±2.14*	24.241	<0.001
Moderate CSLD	19	7.65±1.76$^{*\#}$	24.863	<0.001
Severe CSLD	17	7.43±1.57$^{*\#\&}$	25.084	<0.001

Note: $^{*}P < 0.05$ compared to control group; $\#P < 0.05$ compared to mild CSLD group; $^{\&}p < 0.05$ compared to severe CSLD

Correlation analysis of miR-196a and miR-122 with Nrf-2 and HO-1

Pearson correlation analysis showed that the expression of miR-196a and miR-122 were positively related to Nrf-2 and HO-1 ($p < 0.05$), as shown in Table 6

Table 6: The correlation analysis of miR-196a and miR-122 with Nrf-2 and HO-1

Item	Nrf-2		HO-2	
	r-value	*P-value*	*r-value*	*P-value*
MiR-196a	0.638	<0.001	0.471	0.011
MiR-122	0.516	0.005	0.386	0.023

MiR-196a and miR-122 expression in diagnosis of drug-induced CSLD

The AUC of miR-196a and miR-122 was 0.756 and 0.686, respectively, with higher sensitivity and specificity (Table 7 and Figure 1).

DISCUSSION

Drug-induced CSLD can result in a series of disorders including hepatocyte transporter gene expression or function inhibition, bile duct cell damage, vanishing bile duct syndrome (VBDS), biliary cirrhosis caused by allergic or hypersensitivity, and bile duct skeleton micro-

Table 7: ROC analysis of miR-196a and miR-122 expressions in diagnosis of CSLD

Parameter	AUC	Sensitivity (%)	Specificity (%)	*P*-value	95%CL	
					Lower limit	*Upper limit*
MiR-196a	0.756	86.47	80.24	0.023	0.637	0.875
MiR-122	0.686	83.16	79.62	0.034	0.548	0.824

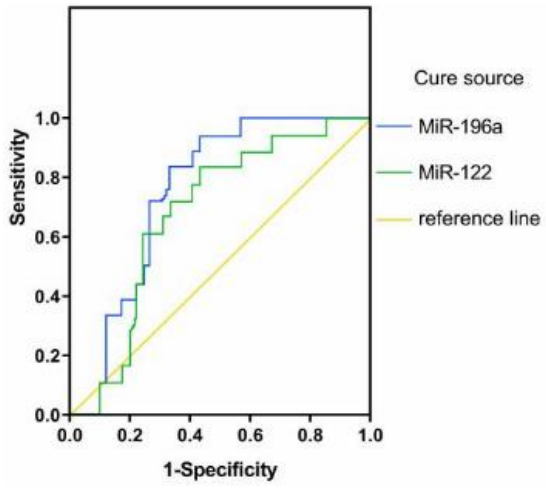

Figure 1: Diagnosis of CSLD by the expression levels of miR-196a and miR-122

tubules damage or cell dysfunction affects microtubule contraction [12,13]. The pathogenesis of the disease is relatively complicated, wherein various factors that cause liver damage may contribute to cholestasis [14,15], and persistent cholestasis promotes the progression of liver disease [16]. Along with the soaring incidence of drug-induced CSLD and the rising awareness of the pathophysiology, considerable attention has been attached to clinical research in this field [17].

However, a robust outcome of drug-induced CSLD hasn't been yielded due to its obscure pathogenesis and various stages [18,19]. Therefore, it is crucially important to explore the relevant pathogenesis of drug-induced CSLD and seek the biomarkers for diagnosis and treatment of drug-induced CSLD.

As is known, miRNA is a non-coding RNA with a length of about 20-25 nucleotides. At present, over 500 miRNAs have been identified, the total number is expected to exceed 1,000, and about 30% of them can regulate human genes. It is highly conservative, acts similar biological functions in different organisms. In addition, it has been confirmed to be associated with numerous diseases, and is closely related to liver cell regeneration, proliferation, apoptosis, immune response, inflammatory response, and liver cancer [20]. It is also known that miRNA also participate in regulating the function of hepatic stellate cells and affect the progress of liver fibrosis [21].

Currently, reports on miRNA in CSLD are scanty. A previous study found that H19/miR-148a/USP4 can promote liver fibrosis through the TGF-β signal enhancement of hepatic stellate cells and hepatocytes [22]. It was pointed out by a prior study that inhibiting the expression of miR-122 can reduce the degree of fatty liver and plasma cholesterol levels [23]. However, the expression and significance of miR-196a and miR-122 in drug-induced CSLD are so far rarely reported at home and abroad. Previous studies have proved that Nrf–2 and HO-1, important factors in resisting oxidative stress, are closely related to the hepatocyte damage. The authors found that these two parameters were positively related to serum levels of Nrf-2 and HO-1, suggesting miR-196a and miR-122 could regulate Nrf2/HO-1 pathway to induce the liver cell injury.

Importantly, both miR-196a and miR-122 were revealed to be highly expressed in drug-induced CSLD patients in the present study; miR-196a and miR-122 in the serum decreased with the severity of CSLD; the ROC area of miR-196a was 0.756, the diagnostic sensitivity was 86.47%, and the specificity was 80.24%; the AUC of miR-122 was 0.686, the diagnostic sensitivity was 83.16%, and the specificity was 79.62%. Taken together, miR-196a and miR-122 are speculated to be sensitive predictors of the severity of drug-induced CSLD and the dynamic changes.

CONCLUSION

The findings of this study demonstrate the diagnostic value of miR-196a and miR-122 in CSLD induced by drugs. Exploration of their potential mechanisms of action involving Nrf-2 and HO-1, suggest that they may be of diagnostic value for drug-induced CSLD patients.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Zefeng Kang, Yongsheng Yang, Qianqian Chen and Yekai Lu conducted the experiments and collected the data; Zefeng Kang, Ran Han, Wei Lu, and Mingfeng Zhang designed the experiments and wrote the paper. Zhiyong Ma, Guiqin Huang, and Huixuan Xu analyzed the data. All authors reviewed and approved the final version of the manuscript for publication.

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