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Serum microRNA-181a Expression Level in Patients with Acute Liver Failure and Its Correlation with Prognosis

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Objective: This paper examined miR-181a expression in the serum of patients with acute liver failure (ALF) and investigated the impact of its expression in the prognosis of ALF patients.

Methods: A total of 112 ALF patients (ALF group) and 100 healthy controls during the same period (control group) were recruited as study subjects, and ALF patients were separated into the survival group and the death group. Serum ALT, AST, SCr, TBil, PTA, and International Normalized Ratio (INR) indices as well as serum miR-181a expression were assessed by using a fully automated biochemistry analyzer and RT-qPCR. Patients in the ALF group were evaluated using the Model for End-Stage Liver Disease (MELD) score. Correlation between serum miR-181a expression and MELD scores of ALF patients was processed by Pearson correlation analysis, and the diagnostic value of miR-181a level for the occurrence of ALF was estimated by ROC curve analysis. Multivariate logistic regression analysis was executed to assess the factors influencing the occurrence of death in ALF patients.

Results: ALF patients had higher levels of ALT, AST, TBiL, SCr, INR and miR-181a and lower PTA levels in comparison to healthy controls. Serum miR-181a expression level in ALF patients revealed a significant positive correlation with MELD score. Multivariate logistic regression analysis unveiled that TBil, INR, SCr, and miR-181a were the independent risk factors for the occurrence of death in ALF patients, and that PTA was an independent protective factor for the prognosis of ALF patients. miR-181a exhibited a favorable diagnostic value in ALF and its prognosis.

Conclusion: miR-181a expression is upregulated in the serum of ALF patients, and it can be utilized as an indicator for ALF diagnostic and prognostic assessment.

Keywords: acute liver failure, microRNA-181a, prognosis, influencing factors, diagnostic value, model for end-stage liver disease, multivariate logistic regression analysis, Pearson correlation analysis

Introduction

Acute liver failure (ALF) is known as a rare, acute, and potentially reversible disease that contributes to severe liver damage and quick clinical deterioration in those patients without prior liver disease.[1] The ALF management relies on strengthening collaborative clinical nursing and support. Correctly understanding and treating common complications of this disease is crucial for optimizing outcomes.[2] In the past few decades, the survival rate of ALF patients has steadily elevated, and the significant improvement in survival rate is due to the combined impact of improved medical practices and the utilization of emergency liver transplantation.[3] However, ALF is an intensive care unit emergency that needs tight monitoring, extensive examination to determine the cause and frequent support of respiratory, hemodynamic, and renal function, as well as targeted therapies based on the cause.[4] Therefore, ALF is a treatable disease that demands a very early diagnosis and adequate treatment.

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The histopathological results of liver biopsy or liver transplantation may aid in identifying potential causes or offer important directions for subsequent clinical, laboratory, along with radiological research.[5] As biomarkers may indicate potential pathophysiological processes in ALF, some candidate biomarkers have been supplemented to the prognostic model to improve its performance.[6] However, commonly used serum biomarkers for ALF, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are not liver specific and have a restricted prognostic value.[7] microRNAs (miRs) have been broadly investigated to mediate gene expression post-transcriptionally and multiple biological and cellular processes.[8] Owing to its extraordinary stability, less complicated chemical structure, as well as lack of post-processing modifications, miRs are considered the "best" serum biomarkers.[9] miRs, for example, miR-146a-5p, miR-26a-5p, and miR-191-5p, have been demonstrated to be dysregulated in the liver, which are described to function in the pathophysiology of liver diseases.[10] A unique liver miR signature suggests a potential new hallmark that can forecast the outcomes of both acute and chronic liver disease in selecting liver transplantation.[11] Among these, miR-181a is an immunoregulatory miR,[12] and it has highest expression in the thymus, highlighting its potency in T lymphocyte maturation, sensitivity as well as selection.[13] Evidence has shown that miR-181a is upregulated in non-alcoholic fatty liver disease (NAFLD) patients, demonstrating that it may exert function in lipid metabolism and insulin resistance of the liver.[14] In this paper, we examined miR-181a expression in the serum of ALF patients and investigated the impact of its expression in the prognosis of ALF patients.

Materials and Methods

Ethics Statement

The study was under the approval of the Ethic Committee of Qingdao Sixth People's Hospital. Written informed consent was acquired form all subjects. This study complied with the Declaration of Helsinki.

Basic Information

One hundred and twelve patients with ALF admitted to Qingdao Sixth People's Hospital from May 2021 to May 2023 were selected as the ALF group. Inclusion criteria: all patients met the diagnostic criteria related to ALF in the Guidelines for Diagnosis and Treatment of Liver Failure (2018 edition) published by the Chinese Journal of Clinical Infectious Diseases; those with complete clinical data. Exclusion criteria: those combined with coronary heart disease, hypertension, diabetes mellitus and other chronic diseases; those combined with immune dysfunction or hematological system diseases; those combined with severe cognitive dysfunction or psychiatric disorders and cannot cooperate with this study; those combined with malignant tumors; pregnant and lactating women. Another 100 healthy controls were selected as the control group.

Methods

Sample Collection

In the ALF group, 5 mL × 2 tubes of venous blood were drawn in a sterile blood collection tube in the fasting state in the early morning of the day after admission; the blood was allowed to stand at ambient temperature for 30 min and then centrifuged at 3000 r/min for 10 min. Subsequently, the upper layer of the blood serum was dispensed into another clean centrifugal tube, which was stored at −80°C for use. In the control group, venous blood was drawn on the day of physical examination and centrifuged to obtain serum.

Biochemical Indicator Testing

A biochemical analyzer was employed to test serum ALT, AST, serum creatinine (SCr), total bilirubin (TBil), prothrombin activity (PTA), International Normalized Ratio (INR) and other indices.

miR-181a Expression Level Measurement

Total RNA in serum was isolated by using Trizol reagent, and RNA concentration was tested by UV spectrophotometry. RNA was reverse transcribed to cDNA by utilizing a reverse transcription kit (TaKaRa, Japan), and analyzed by reverse transcription quantitative polymerase chain reaction (RT-qPCR) using SYBR RT-qPCR kit. The primers were designed and synthesized by Invitrogen (USA). miR-181a upstream primer: 5′-AACATTCAACGCTGTCGGTGAGT-3′, downstream

primer: universal primer; U6 was used as an internal reference, upstream primer: 5′-CTCGCTTCGGCAGCACA-3′, downstream primer: 5′-AACGCTTCACGAATTTGCGT-3′. The relative expression level of miR-181a was calculated using the $2^{-\Delta\Delta CT}$ method.

Model of End-Stage Liver Disease (MELD) Score

The MELD score was performed based on the TBiL and SCr concentrations and the INR of the prothrombinogen time of the study subjects, and was calculated using the following formula: MELD = 9.57 × ln[SCr (mg/dL)] + 3.78 × ln[TBiL (mg/dL)] + 11.20 × ln(INR) + 6.40 × etiology (0: alcoholic or cholestatic; 1: other causes).

Follow-Up

The patients in the ALF group were followed up regularly, which was mainly accomplished through outpatient review/telephone tracking, and the follow-up period was up to August 2023. The survival status of the patients was recorded, and then these patients were separated into the survival group (n = 69) and the death group (n = 43).

Statistical methods

Data were analyzed by SPSS 25.0 (IBM SPSS Statistics, Chicago, IL, USA) and GraphPad Prism8.0 (GraphPad software, Inc., La Jolla, CA, USA). Measurement data was depicted as mean ± standard deviation ($\bar{x} \pm s$), and *t*-test was implemented for comparison between groups. Numeration data were expressed as n or n (%) and comparisons were carried out by the χ^2 test. Diagnostic value of miR-181a for ALF and its prognosis was analyzed using ROC curves, and independent risk factors impacting death in ALF patients were analyzed by multivariate logistic regression analysis. $P < 0.05$ was considered significant.

Results

Comparison of Each Index of the Patients in the Control Group and the ALF Group

ALF patients had higher levels of ALT, AST, TBiL, SCr, INR and miR-181a and lower PTA levels in comparison to healthy controls ($P < 0.05$), but the differences showed no significance when comparing the age and gender of patients in the two groups ($P > 0.05$) (Table 1).

Table 1 Comparison of Each Index of the Patients in the Control Group and the ALF Group

Indicator	Control Group (n = 100)	ALF Group (n = 112)	t/χ^2	P
Age (years)	49.85 ± 9.26	51.22 ± 11.15	0.969	0.334
Gender (male/female)	64/36	71/41	0.008	0.927
ALT (U/L)	35.41 ± 7.20	645.54 ± 124.12	49.070	<0.001
AST (U/L)	26.15 ± 9.35	171.90 ± 40.21	35.390	<0.001
TBil (μmol/L)	13.82 ± 2.76	159.97 ± 68.26	21.390	<0.001
PTA (%)	74.53 ± 6.47	33.66 ± 7.04	43.840	<0.001
INR	1.05 ± 0.27	2.16 ± 0.89	12.020	<0.001
SCr (μmol/L)	50.45 ± 15.47	79.94 ± 21.17	11.460	<0.001
MELD score	/	25.24 ± 6.52	/	/
miR-181a	0.77 ± 0.25	1.34 ± 0.61	8.716	<0.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBiL, total bilirubin; PTA, prothrombin activity; INR, international normalized ratio; SCr, serum creatinine; MELD, model of end-stage liver disease; miR, microRNA.

Correlation Analysis Between Serum miR-181a and MELD Score in ALF Patients

Serum miR-181a expression level in ALF patients revealed a significant positive correlation with MELD score (r = 0.542, $P < 0.001$; Figure 1).

Comparison of Each Index of ALF Patients in the Survival Group and the Death Group

Comparisons in terms of the age and gender of ALF patients in the survival and death groups showed no difference ($P > 0.05$); higher levels of ALT, AST, TBil, SCr and INR, MELD score and miR-181a expression and lower PTA levels were observed in ALF patients of the death group versus the survival group ($P < 0.05$; Table 2).

Analysis of the Factors Affecting Death in ALF Patients

With the patients' prognosis as the dependent variable (survival group = 0; death group = 1) and the data (ALT, AST, TBil, SCr, INR, MELD score, PTA and miR-181a expression level) that differed between ALF patients in the survival group and the death group as the independent variables, the results of multivariate logistic regression analysis unveiled that TBil, INR, SCr, and miR-181a were the independent risk factors for the occurrence of death in ALF patients, and that PTA was an independent protective factor for the prognosis of ALF patients (Table 3).

Predictive Value of miR-181a in ALF and Its Prognosis

After the ROC curve analysis, the area under the curve (AUC) of miR-181a for the diagnosis of ALF was 0.807 (95% CI: 0.747–0.868), with a sensitivity of 0.625, a specificity of 0.920, and a Youden index of 0.545 ($P < 0.001$); and the AUC for the prognostic diagnosis of miR-181a for ALF was 0.716 (95% CI: 0.617–0.815), with a sensitivity of 0.750, a specificity of 0.594, and a Youden index of 0.344 ($P < 0.001$) (Figure 2).

Discussion

ALF is a rare disease that involves the quick development and worsening of liver dysfunction, which is featured with coagulopathy and encephalopathy, as well as high mortality.[15] At present, there is no treatment plan to reverse or prevent liver cell necrosis, so the current focus of treatment is to support failed organs and prevent life-threatening complications, waiting for spontaneous liver recovery or transplantation.[16] Doctors must quickly diagnose these patients while estimating other diseases and complications.[17] In this paper, we examined miR-181a expression in the serum of ALF patients and investigated the impact of its expression in the prognosis of ALF patients.

The discovery of acellular miRs in diverse body fluids have suggested that miRs may serve as extracellular signaling molecules and may serve as appropriate biomarkers to support the diagnosis and monitoring of therapeutic responses in various diseases.[18] Cumulative evidence has revealed that miRs are implicated in the process of both liver injury and

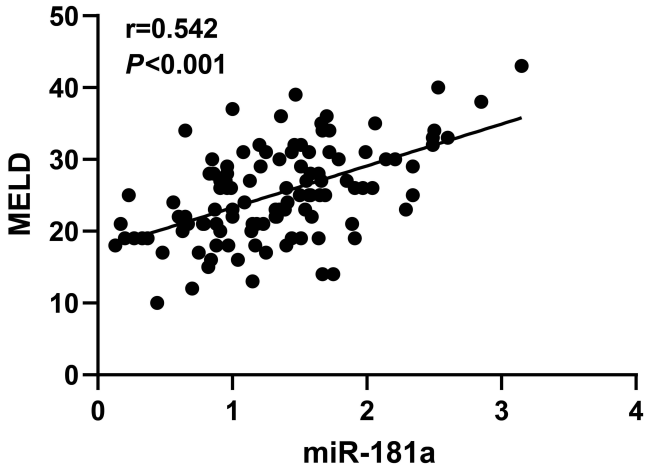

Figure 1 Correlation analysis between serum miR-181a expression level and MELD score. MELD: model for end-stage liver disease; miR: microRNA.

Table 2 Comparison of Each Index of ALF Patients in the Survival Group and the Death Group

Indicator	Survival Group (n = 64)	Death Group (n = 48)	t/χ^2	P
Age (years)	50.34 ± 10.12	52.40 ± 12.41	0.963	0.338
Gender (male/female)	42/22	29/19	0.321	0.571
ALT (U/L)	574.31 ± 95.67	740.52 ± 89.40	9.356	<0.001
AST (U/L)	152.34 ± 32.19	197.99 ± 34.84	7.169	<0.001
TBil (μmol/L)	139.27 ± 60.42	187.56 ± 68.91	3.940	<0.001
PTA (%)	36.36 ± 6.67	30.05 ± 5.84	5.222	<0.001
INR	1.79 ± 0.61	2.65 ± 0.96	5.788	<0.001
SCr (μmol/L)	69.67 ± 16.98	93.64 ± 18.35	7.141	<0.001
MELD score	22.75 ± 5.57	28.56 ± 6.25	5.185	<0.001
miR-181a	1.14 ± 0.46	1.60 ± 0.68	4.271	<0.001

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBiL, total bilirubin; PTA, prothrombin activity; INR, international normalized ratio; SCr, serum creatinine; MELD, model of end-stage liver disease; miR, microRNA.

Table 3 Analysis of the Factors Affecting Death in ALF Patients

Indicator	*β*	*SE*	Wald	*P*	OR	95% CI
TBil	0.013	0.005	6.020	0.014	1.014	1.003–1.025
PTA	−0.169	0.057	8.665	0.003	0.844	0.754–0.945
INR	1.552	0.461	11.338	0.001	4.722	1.913–11.655
SCr	0.072	0.021	11.682	0.001	1.075	1.031–1.120
miR-181a	1.509	0.670	5.080	0.024	4.524	1.218–16.806

Abbreviations: TBiL, total bilirubin; PTA, prothrombin activity; INR, international normalized ratio; SCr, serum creatinine; miR, microRNA.

liver failure.[19,20] In particularity, some miRs have been demonstrated to correlate with the prognosis of ALF.[21] For example, expression levels of miR-122-3p, miR-146a-5p, and miR-328-3p have a positive relation with the liver inflammation severity in acute-on-chronic liver failure (ACLF) patients.[22] In our study, we observed that ALF patients had higher levels of miR-181a in comparison to healthy controls. In addition, miR-181a were the independent risk factors for the occurrence of death in ALF patients, and that miR-181a exhibited a favorable diagnostic value in ALF and its prognosis. miR-181a has been previously reported to modulate the development of the lymphatic system, embryonic organs, and blood cell lines, along with the maturation of T lymphocytes and the differentiation of megakaryocytes.[23] In vivo experiments have disclosed that constitutive mitomiR-181a-5p expression contributes to glucose metabolism reprogramming, thereby enhancing liver cancer tumor growth and early lung metastasis.[24] Notably, in the liver tissue and serum of GT4-HCV-infected patients, miR-181a expression is negatively correlated with its expression in liver cancer. Besides, miR-181a is upregulated in liver tissues of liver cancer and is normally expressed in patient serum.[25] Another study has unveiled that miR-181a expression is increased in NAFLD patients, high-fat die and ob/ob mice, as well as non-esterified fatty acids-treated hepatocytes in contrast to the corresponding controls.[26] These studies have all demonstrated the role of miR-181a in liver disease, but there is still limited research on its role in ALF.

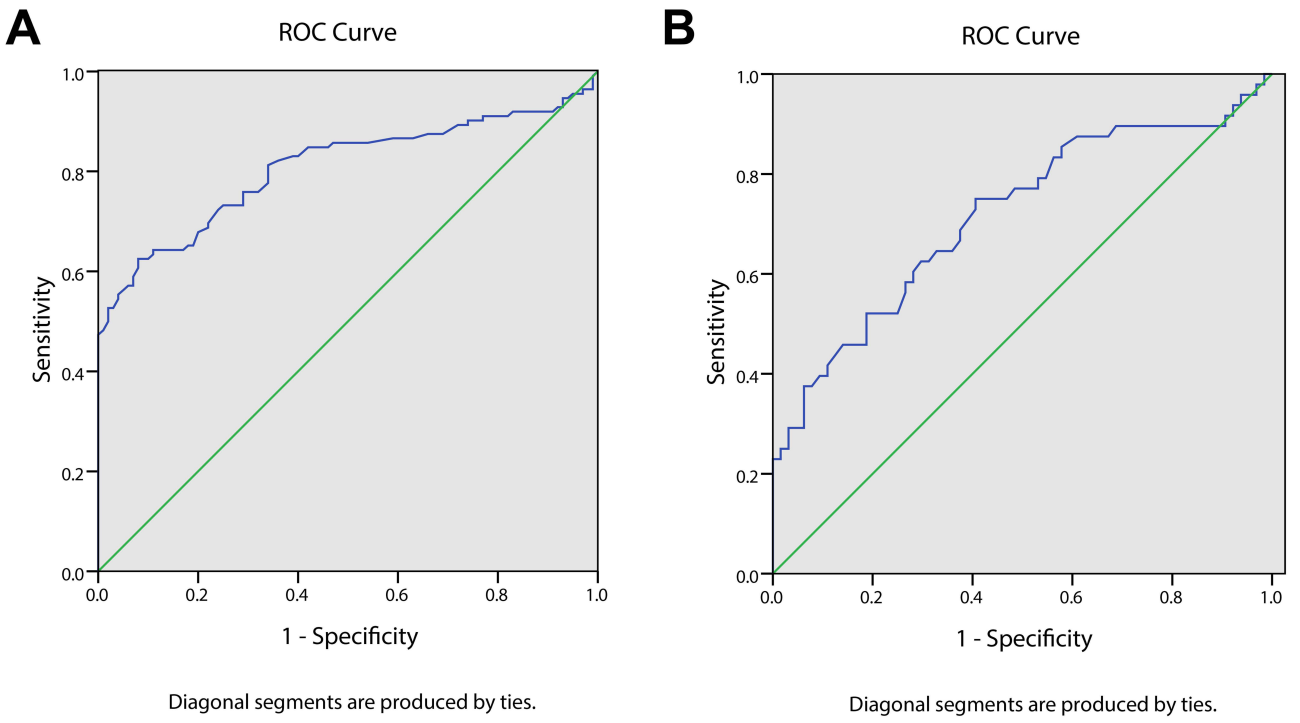

Figure 2 Diagnostic ROC curve of miR-181a for ALF and its prognosis. (**A**) ROC curve of miR-181a for the diagnosis of ALF. (**B**) ROC curve of miR-181a for prognostic diagnosis of ALF.

In the meantime, ALF patients had higher levels of ALT, AST, TBiL, SCr, and INR and lower PTA levels in comparison to healthy controls. We also found that TBil, INR, and SCr were the independent risk factors for the occurrence of death in ALF patients, and that PTA was an independent protective factor for the prognosis of ALF patients. Albumin, ALT, AST, and choline esterase are upregulated in liver cells or synthesized by liver cells, and their levels or activities can reflect the liver function state in a direct way.[27] Several indicators such as age, Tbil, prothrombin, INR, alpha-fetoprotein, and hepatic encephalopathy have prognostic power in evaluating liver failure.[28] INR measurement in post-hepatectomy liver failure patients can identify and stratify patients objectively who may be eligible for enhanced rehabilitation plans and those who deserve close examination in high dependency areas.[29] Recently, MELD score has been applied for forecasting the mortality of end-stage liver disease, which has been broadly utilized to evaluate the prognosis of HBV-associated ACLF.[30] Similar to our findings, elevated serum ALT and AST levels have been observed in ALF rat liver tissues.[31] Another research has revealed that the baseline ALT, AST, INR, and Tbil serum levels are independent risk parameters for liver failure.[32] However, due to limited conditions, we have not conducted in vivo and in vitro experiments to validate the related mechanism of miR-181a in ALF for the time being. Further studies are needed to convince our results.

Conclusion

miR-181a expression is upregulated in the serum of ALF patients, and it can be utilized as an indicator for ALF diagnosis and prognostic assessment. Importantly, miR regulation in the liver indicates a new therapeutic regimen in the treatment equipment of future hepatologists.

Data Sharing Statement

The experimental data used to support the findings of this study are available from the corresponding author upon request.

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Disclosure

The authors declared that they have no conflicts of interest regarding this work.

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