

Original Research

Clinical Significance of miR-339-5p in Early Diagnosis and Predicting Pregnancy Outcome of Chinese Patients with Liver Injury in Pregnancy

Ying Wang^{1,†}, Lichao Yuan^{1,†}, Falong Hu¹, Huilan Yu¹, Qiaofang Yang¹, Xiujuan Zheng^{1,*}¹Department of Obstetrics, Jinhua Municipal Central Hospital, 321000 Jinhua, Zhejiang, China*Correspondence: zhengxiujuandr@163.com (Xiujuan Zheng)

†These authors contributed equally.

Academic Editor: Michael H. Dahan

Submitted: 17 January 2024 Revised: 6 March 2024 Accepted: 15 March 2024 Published: 19 July 2024

Abstract

Background: Liver injury in pregnancy significantly impacts the physical and mental health of pregnant women, and finding a potential therapeutic target is crucial for early prediction and improving adverse pregnancy outcomes. This study aims to examine the relationship between miR-339-5p expression and early diagnosis and pregnancy outcomes in patients experiencing liver injury in pregnancy. **Methods:** A retrospective study of 63 patients with liver injury in pregnancy. The expression of miR-339-5p in plasma of patients with liver health and liver injury in pregnancy was detected using quantitative real-time polymerase chain reaction (qRT-PCR). The value of miR-339-5p in the diagnosis and pregnancy outcomes of patients with liver injury in pregnancy was evaluated by receiver operating characteristic (ROC) and Cox regression analysis. **Results:** The alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bile acids (TBA), total bilirubin (TBIL) levels and miR-339-5p expression of patients in the intrahepatic cholestasis of pregnancy (ICP), hemolysis, elevated liver enzymes, and low platelet count (HELLP) and acute fatty liver of pregnancy (AFLP) groups, respectively, were statistically significant compared with those in the healthy control (HC) group ($p < 0.05$). MiR-339-5p expression was significantly lower in patients with liver injury in pregnancy compared to healthy individuals. This difference could be used to distinguish between healthy individuals and those with liver injury in pregnancy (area under the curve (AUC) = 0.897, 95% confidence interval (95% CI) = 0.843–0.951). In addition, ALT ($r = -0.686$), AST ($r = -0.699$) and TBA ($r = -0.706$) were highly negatively correlated with miR-339-5p expression, respectively. MiR-339-5p can be used as a biomarker of liver injury in pregnancy to predict adverse pregnancy outcomes. **Conclusions:** MiR-339-5p could potentially be used as a potential molecular marker for early diagnosis of liver injury in pregnancy and the prediction of adverse pregnancy outcomes.

Keywords: miR-339-5p; liver injury in pregnancy; ALT; AST; TBA; early diagnosis; pregnancy outcome

1. Introduction

Liver injury in pregnancy is a common maternal comorbidity that significantly impacts the physical and mental health of expecting mothers. Normal pregnancy increases the liver's burden due to elevated carbohydrate metabolism and increased physiological activity of estrogen. This can lead to changes in liver function and potentially cause liver disease and injury [1,2]. Liver disease in pregnancy includes both pregnancy and non-pregnancy induced liver diseases, of which those existing during pregnancy are relatively rare, as pregnant women are usually young and healthy [3]. Liver diseases specific to pregnancy include intrahepatic cholestasis of pregnancy (ICP), hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome, and acute fatty liver of pregnancy (AFLP), which can lead to serious liver injury in pregnant women and endanger the life and health of pregnant women and the fetus. Therefore, early diagnosis is important, and the choice of reasonable treatment and whether to terminate pregnancy are decisive [4]. Early diagnosis is crucial, and the choice of rational treatment and whether to terminate the pregnancy is decisive [5,6]. Currently, liver disease is typically diag-

nosed through pathological examination, but this method is invasive and should not be performed unless needed. Presently, there are no well-established techniques for early diagnosis of liver injury in pregnancy. There is therefore an urgent need for a non-invasive, accurate and rapid method to assist physicians in the early diagnosis of liver injury and to predict the prognosis of pregnant women and infants, which is important in the choice of rational treatment and whether or not to terminate a pregnancy.

Micro RNAs (miRNAs) are non-coding RNAs implicated in the initiation and progression of various human diseases, such as cancer [7], diabetes [8], cardiovascular [9], and pregnancy complications [10]. miRNAs have been widely used in the study of a variety of pregnancy complications [11]. For example, exosomal miRNA is a potential therapeutic target for gestational diabetes mellitus, affecting patients' prognostic outcomes during pregnancy by inhibiting phosphoinositide 3-kinase/protein kinase B, wingless/integrated and mammalian target of rapamycin signaling pathways [12]. Overexpressed in the placenta, miRNA-141 can regulate the biological activity of cancer cells by targeting *PLAG1* [13]. Similarly, highly expressed



miRNA-424 causes placental vascular underdevelopment leading to hypoxia by targeting oxygen gene expression [14]. The liver is crucial in regulating blood glucose homeostasis. MiR-339-5p targets glucose-6-phosphatase, which is involved in glycogenolysis [15]. As a significant component of the regulatory network of chronic/acute liver failure, miR-339-5p is implicated in hepatocellular carcinoma development [16]. miR-339-5p was found to be involved in hepatocellular carcinoma development by Wang *et al.* [17]. Therefore, it is hypothesized that aberrant expression of miR-339-5p during pregnancy could indirectly indicate liver conditions. This study investigates the potential of miR-339-5p as a biomarker of liver injury in pregnancy.

The objective of this study was to identify a novel biomarker to enhance the early diagnosis of liver injury in pregnancy and to improve pregnancy outcomes.

2. Materials and Methods

2.1 Study Subjects

This study is a retrospective study. The experimental group consisted of 63 Chinese patients with liver injury in pregnancy who gave birth in Jinhua Maternal and Child Health Care Hospital from 2018 to 2022. The diagnostic criteria for liver injury in pregnancy are as follows:

- (1) the pregnant woman identified an intrauterine single pregnancy;
- (2) normal liver function before pregnancy, abnormal liver function indexes during pregnancy;
- (3) laboratory results indicate serum alanine aminotransferase (ALT) >40 IU/L or aspartate aminotransferase (AST) >40 IU/L or total bile acids (TBA) >10 $\mu\text{mol/L}$ or Total Bilirubin (TBIL) >17.1 $\mu\text{mol/L}$.

Exclusion Criteria:

- (1) patients with serious underlying diseases, organ dysfunction and malignant tumors;
- (2) patients with other diseases during pregnancy, such as thyroid disease, diabetes, cardiovascular disease, etc;
- (3) patients with abnormal cognitive and mental state and communication difficulties;
- (4) patients who are poorly compliant and do not cooperate with regular maternity check-up [18].

Another 57 pregnant women with normal liver function, who gave birth at Jinhua Maternal and Child Health Care Hospital during the same period, were selected as the healthy control group (HC). Inclusion criteria are as follows:

- (1) pregnant women in the control group should have normal liver function indexes;
- (2) no complications during pregnancy and be in good health;
- (3) there was no statistically significant difference between the two groups in terms of gender, age, and number of deliveries ($p > 0.05$), and they were comparable.

Clinical information including age, gestational age (time of diagnostic enrollment), gravida, parity gestational,

body mass index ($\text{BMI} = \text{weight (kg)}/\text{height squared (m}^2\text{)}$), ALT, AST, TBIL and TBA were collected from all participating subjects. The experiment was approved by the Academic Ethics Committee of Jinhua Maternal and Child Health Care Hospital (ethics number 201726) and adhered strictly to ethical guidelines. Participates were fully informed about the purpose of the study and signed an informed consent form.

2.2 Followup of Adverse Pregnancy Outcomes

The study population needs to be followed up until delivery. Data on the gestational week of delivery, newborn weight, incidence of fetal growth restriction (FGR), and pregnancy outcomes were collected from participants. Adverse pregnancy outcomes included maternal outcome: eclampsia, heart failure, acute kidney injury, cerebrovascular accident, etc., and fetal outcome: preterm labor (delivery at more than 28 weeks of gestation but less than 37 weeks of gestation), intrauterine distress (acute or chronic hypoxia in utero with fetal heart rate >160 or <120 beats/min), stillbirth (in utero death at 20 weeks of gestation), premature rupture of membranes (rupture of membranes prior to delivery, and premature rupture of membranes), etc.

2.3 Sample Collection and miR-339-5p Detection

Venous blood was collected from the subjects while fasting, using an EDTA-K2 anticoagulation vacuum sampler (YA1291, Solarbio, Beijing, China), centrifuged at 3000 rpm for 15 min. The supernatant, obtain after separation, served as the serum specimen and was stored at -80°C .

The expression level of miR-339-5p was detected by fluorescence quantitative polymerase chain reaction (qPCR). RNA was extracted by Trizol method (Invitrogen, Carlsbad, CA, USA). Reverse transcription and amplification were performed carried out with the PrimeScript RT Enzyme Mix I kit (TaKaRa, Tokyo, Japan) and SYBR Green qPCR Super Mix (Invitrogen, Carlsbad, CA, USA) kit, respectively. U6 was used as an internal standard for miR-339-5p, and the relative expression was calculated using the $2^{-\Delta\Delta\text{Ct}}$ method.

2.4 Statistical Analysis

The ability of miR-339-5p to differential patients with liver injury in pregnancy was evaluated by receiver operating characteristic (ROC) analysis. The role of miR-339-5p in predicting delivery outcomes was assessed through Kaplan-Meier and multivariate Cox regression analysis. In addition, Spearman correlation analysis was used to estimate the correlation between miR-339-5p and clinicopathological characteristics of patients. The experimental data were represented by mean $\pm$ standard deviation (SD), and the paired measurement data were analyzed by a *t*-test. $p < 0.05$ indicates significant difference, $p < 0.01$ indicates a significant difference.

Table 1. Basic clinical features of study subjects.

Parameters	HC	ICP	HELLP syndrome	AFLP
	n = 57	n = 39	n = 14	n = 10
Age (years)	28.6 ± 4.6	28.7 ± 4.5	28.1 ± 3.8	26.4 ± 3.1
Gravidity (time)	1.5 ± 0.6	1.5 ± 0.6	1.4 ± 0.5	1.4 ± 0.7
Parity (time)	1.4 ± 0.6	1.4 ± 0.5	1.4 ± 0.5	1.4 ± 0.7
BMI (kg/m ²)	23.3 ± 1.7	23.3 ± 2.2	22.9 ± 2.0	23.0 ± 2.2
ALT (IU/L)	21.8 ± 3.5	170.6 ± 45.0 ^a	145.4 ± 46.6 ^b	154.5 ± 41.4 ^c
AST (IU/L)	18.8 ± 2.2	121.9 ± 22.9 ^a	120.1 ± 25.7 ^b	115.7 ± 22.9 ^c
TBA (umol/L)	2.5 ± 0.5	30.6 ± 11.8 ^a	29.5 ± 9.8 ^b	28.3 ± 9.1 ^c
TBIL (umol/L)	7.4 ± 2.1	50.2 ± 14.1 ^a	48.1 ± 8.5 ^b	44.2 ± 10.9 ^c
newborn weight (g)	3460.9 ± 374.2	3327.8 ± 448.0	3423.4 ± 389.0	3381.8 ± 434.3
Gestational week of delivery (weeks)	38.3 ± 1.2	37.7 ± 1.9	39.6 ± 1.6	37.8 ± 1.9
FGR (%)	2 (3%)	3 (8%)	1 (8%)	2 (20%)

^a: ICP patients compared to HC, $p < 0.05$. ^b: HELLP patients syndrome patients compared to HC, $p < 0.05$. ^c: AFLP patients compared to HC, $p < 0.05$. HC, healthy control group; ICP, Intrahepatic cholestasis of pregnancy; HELLP, hemolysis, elevated liver enzymes, and low platelet count; AFLP, acute fatty liver of pregnancy; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBA, total bile acids; TBIL, total bilirubin; FGR, fetal growth restriction.

3. Results

3.1 Basic Characteristics of Study Subjects

The patients with liver injury in pregnancy included three pregnancy-specific liver diseases, including 39 with ICP, 14 with HELLP syndrome, and 10 with AFLP. Among the enrolled study subjects, there was no significant difference between the HC and patients with ICP, HELLP syndrome and AFLP, in terms of age, gestational week of delivery, gravidity, parity, BMI, newborn weight, and FGR ($p > 0.05$, Table 1). Patients in the ICP, HELLP syndrome and AFLP groups showed significant differences in ALT, AST, TBA and TBIL levels compared to the HC group ($p < 0.05$), respectively, whereas the differences between the three diseases were not significant.

3.2 Expression of miR-339-5p in the Patients with Liver Injury in Pregnancy

As shown in Fig. 1a, the difference in miR-339-5p expression among ICP, HELLP syndrome and AFLP patients was not statistically significant ($p > 0.05$), but all of them differed significantly from miR-339-5p expression in the HC group ($p < 0.0001$). Therefore, the three pregnancy-specific diseases were combined as liver injury in pregnancy to investigate the clinical role of miR-339-5p in healthy population and patients with liver injury in pregnancy. The expression of miR-339-5p in serum of HC group and liver injury in pregnancy group was detected by quantitative real-time polymerase chain reaction (qRT-PCR). Results as shown in Fig. 1b, compared with the HC group, the expression of miR-339-5p in the liver injury in pregnancy group was significantly decreased ($p < 0.0001$). To further evaluate the diagnostic potential of miR-339-5p evaluate the diagnostic potential of for liver injury in pregnancy, a ROC was plotted. As shown in Fig. 1c, the

area under the curve (AUC) was 0.897, with a sensitivity of 80.95 and a specificity of 85.96%. These results indicate that miR-339-5p can assist in the diagnosis of pregnant patients with liver function injury.

According to the average expression level of miR-339-5p in pregnant patients with liver function, the patients were divided into two groups: low expression (34 cases) and high expression (29 cases). It was found that the levels of ALT ($p < 0.0001$, Fig. 2a), AST ($p = 0.0002$, Fig. 2b) and TBA ($p = 0.0001$, Fig. 2c) in the high expression group of miR-339-5p were significantly lower than those in the low expression group of miR-339-5p, while the expression level of TBIL was not significant in the two groups ($p = 0.0753$, Fig. 2d). Further analysis showed that ALT ($r = -0.686$, Fig. 3a), AST ($r = -0.699$, Fig. 3b) and TBA ($r = -0.706$, Fig. 3c) were highly negatively correlated with the expression of miR-339-5p, respectively. However, there was a low correlation between TBIL level and miR-339-5p expression ($r = -0.403$, Fig. 3d).

3.3 Prognostic Value of miR-339-5p in the Patients of Liver Injury in Pregnancy

As shown in Fig. 4, the incidence of adverse pregnancy outcomes between the two groups was compared. It was found that the group with low miR-339-5p expression had a higher incidence of adverse pregnancy outcomes at the same gestational age compared the group with high miR-339-5p expression (log-rank $p = 0.024$). In addition, miR-339-5p ($p = 0.038$, hazard ratio (HR) = 0.162), ALT ($p = 0.048$, HR = 3.223), AST ($p = 0.044$, HR = 5.880) and TBA ($p = 0.049$, HR = 6.656) can serve as predictors for poor prognosis in patients with liver injury in pregnancy (Table 2).

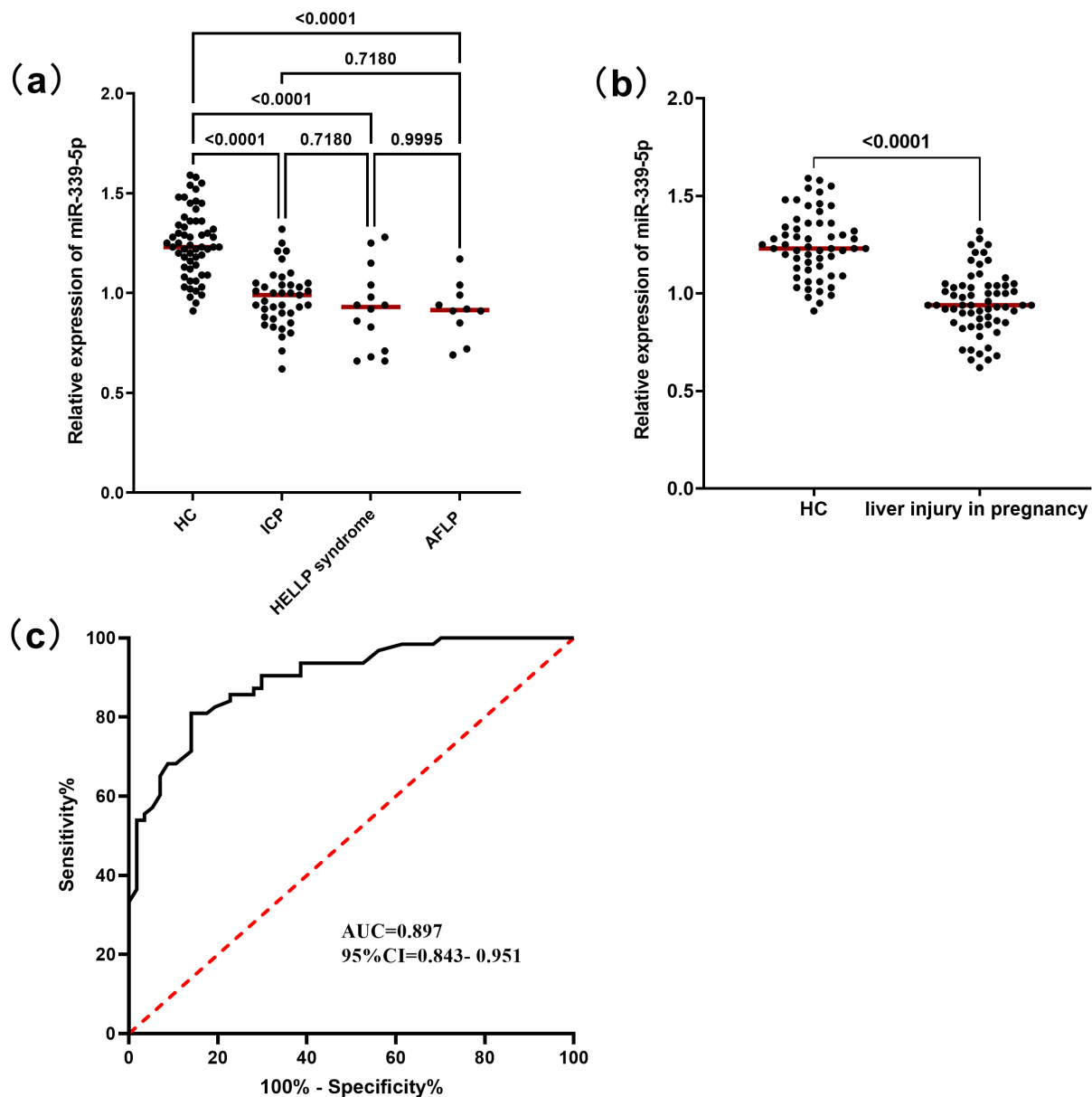


Fig. 1. Receiver operating characteristic (ROC) analysis to evaluate the significance of miR-339-5p in the diagnosis of hepatic injury in pregnancy. There was no significant difference in miR-339-5p expression among ICP, HELLP syndrome and AFLP patients (a). Compared with normal liver function during pregnancy, the expression of miR-339-5p in patients with liver injury in pregnancy is downregulated (b). miR-339-5p has high sensitivity and specificity in distinguishing between liver health and liver injury during pregnancy (c). AUC, area under the curve; 95% CI, 95% confidence interval.

4. Discussion

During pregnancy, hepatic blood flow balance is disrupted, which increases the load on the liver and consequently alters liver function. This situation can likely lead to liver injury, resulting in related pregnancy complications and adverse pregnancy outcomes [19,20]. MiRNAs are extensively involved in gene regulation related to immune response, placental development and angiogenesis during pregnancy, which are closely related to pregnancy-related complications [21]. For example, enrichment anal-

ysis of differentially expressed genes in ICP has shown that miRNAs mediate the janus kinase-signal transducer and activator of transcription 3 (JAK-STAT3) signaling pathway, a key pathway in ICP, and that miRNAs play an important role in ICP treatment [22].

Research has shown that miR-339-5p can target regenerative liver phosphatase-1 (PRL-1), which is associated with anaerobic mitochondrial metabolism in chronic liver disease [23,24]. Valletlunga *et al.* [25] found that miR-339-5p affects cortical neuronal over reactivity by targeting neuronatin and influencing calcium homeostasis. Gartz *et al.*

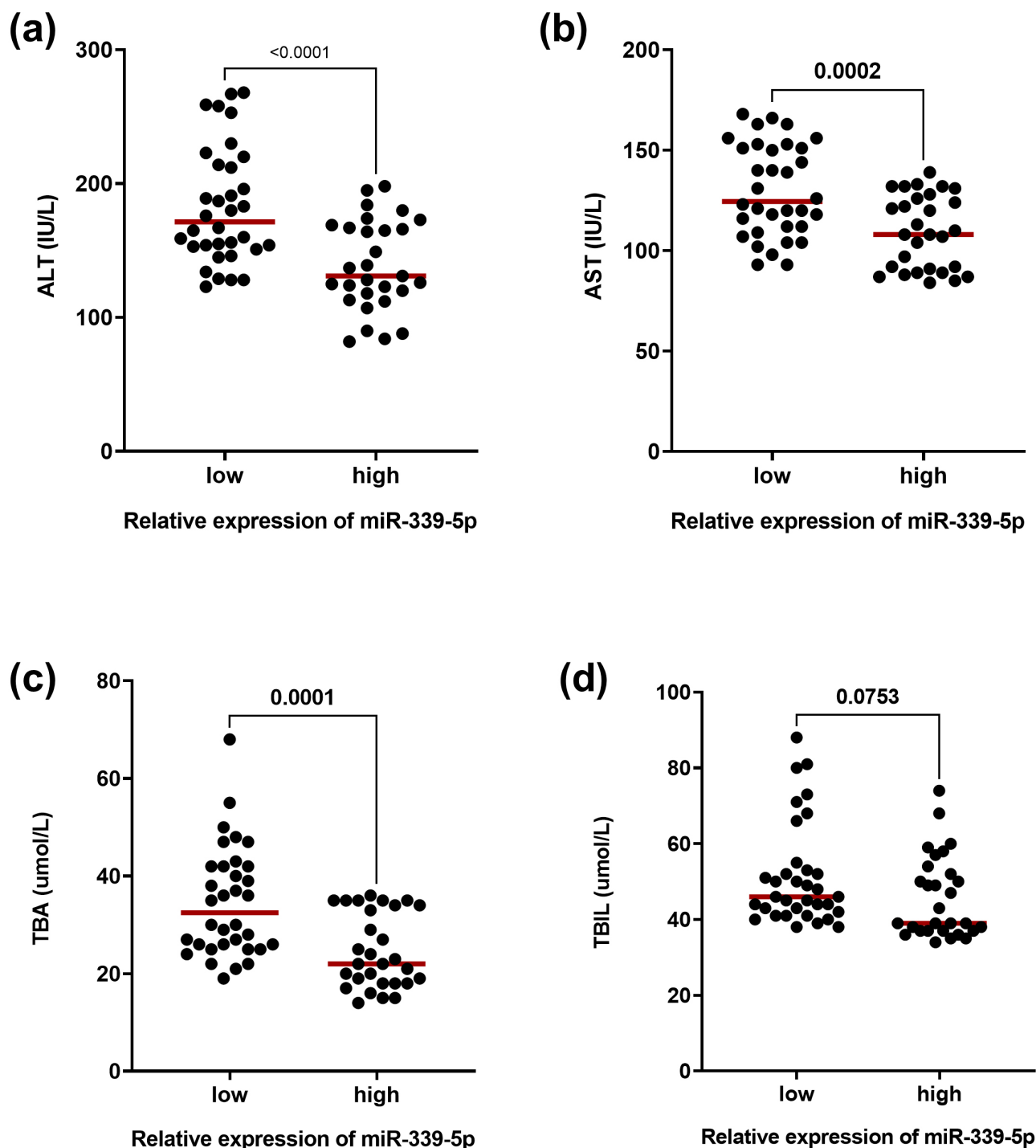


Fig. 2. Differences in the levels of liver injury indicators between miR-339-5p high and low expression groups. ALT levels were significantly lower in the miR-339-5p high expression group than in the low expression group (a). AST levels were significantly lower in the miR-339-5p high expression group than in the low expression group (b). TBA levels were significantly lower in the miR-339-5p high expression group than in the low expression group (c). TBIL levels were not significant in the miR-339-5p high and low expression groups (d).

[26] found that miR-339-5p was upregulated in Duchenne muscular dystrophy-derived cardiomyocytes and was involved in the regulation of stress response signaling pathways mouse double minute 2 (MDM2) and glycogen syn-

thase kinase 3 alpha (GSK3A), which is a potential target for the early diagnosis and treatment of Duchenne muscular dystrophy (DMD) cardiomyopathies. In this study, miR-339-5p expression was downregulated in patients with liver

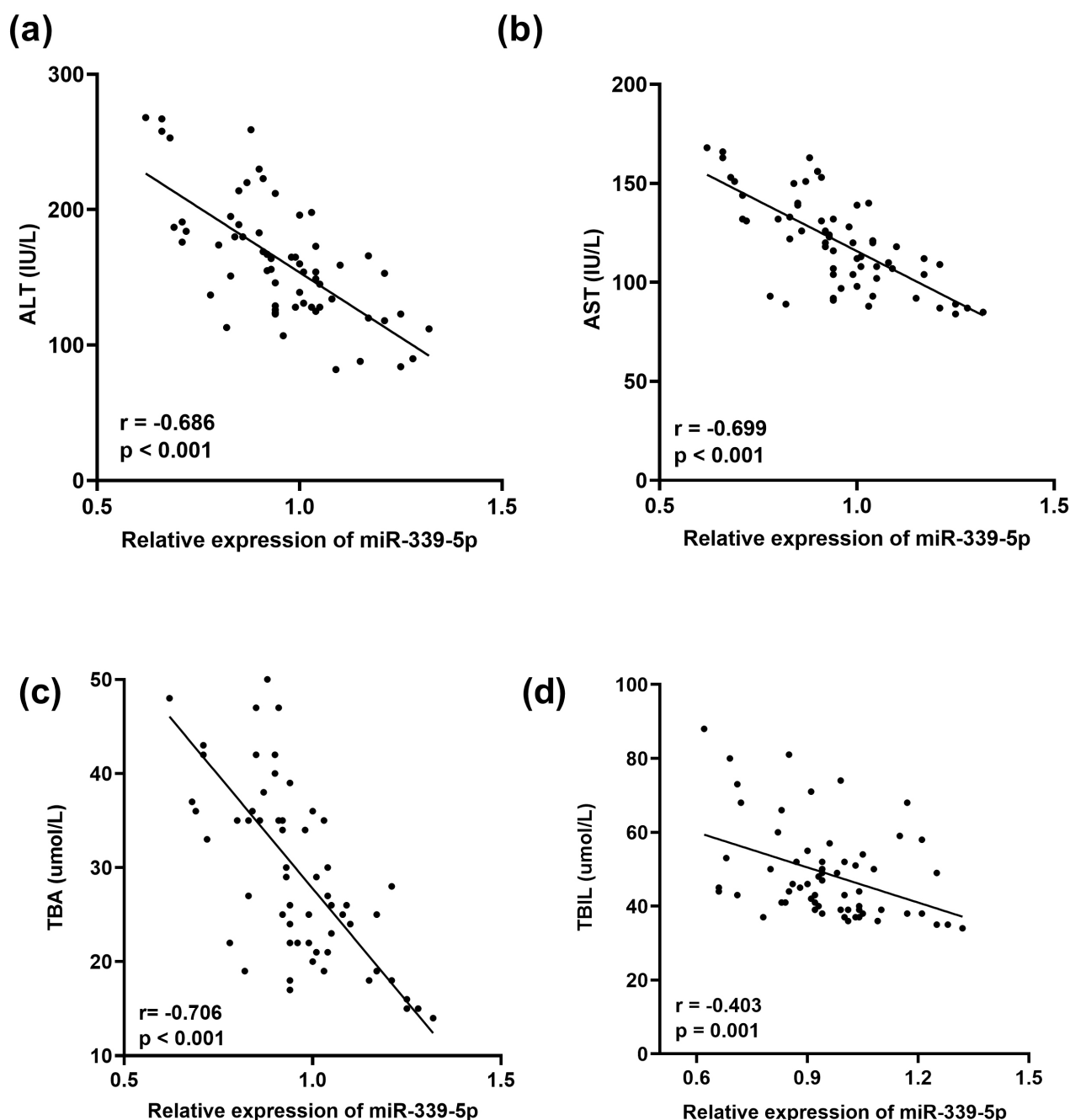


Fig. 3. Correlation between miR-339-5p expression and liver injury indexes. ALT levels were highly negatively correlated with miR-339-5p expression (a). AST levels were highly negatively correlated with miR-339-5p expression (b). TBA levels were highly negatively correlated with miR-339-5p expression (c). TBIL levels are lowly correlated with miR-339-5p expression (d). ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBA, total bile acids; TBIL, total bilirubin.

injury in pregnancy compared to pregnant women with normal liver function. The sensitivity and specificity in the early diagnosis of patients are more than 80%, suggesting that miR-339-5p could serve as an early marker for diagnosing liver injury in pregnancy. Moreover, patients at the same gestational age with higher miR-339-5p expression had a lower incidence of adverse pregnancy outcomes than those with low expression. The levels of ALT, AST, and TBA, which are indicators of liver health, were signifi-

cantly correlated with the occurrence of liver injury in pregnancy. Importantly, miR-339-5p expression was shown to be inversely connected with blood levels of ALT, AST, and TBA. These results indicate that miR-339-5p can be used as a predictor for early diagnosis of liver injury in pregnancy.

A study has shown that miR-200a-3p can predict adverse pregnancy outcomes in pregnant patients with hypertension [27]. The expression of miR-330-3p is up-regulated in gestational diabetes patients than non-diabetic

Table 2. Cox regression analysis evaluating the prognostic value of clinical features of patients.

Parameters	<i>p</i>	HR	95% CI
Age (years)	0.231	0.480	0.145–1.593
Gravidity (time)	0.461	0.238	0.005–10.843
Parity (time)	0.559	1.594	0.334–7.613
BMI (kg/m ²)	0.485	0.580	0.126–2.669
ALT (IU/L)	0.048	3.223	1.009–10.298
AST (IU/L)	0.044	5.880	1.052–32.868
TBA (umol/L)	0.049	6.656	1.011–43.815
TBIL (umol/L)	0.096	2.904	0.828–10.185
newborn weight (g)	0.509	0.666	0.200–2.221
Gestational week of delivery (weeks)	0.970	0.931	0.021–40.929
FGR (%)	0.317	2.443	0.425–14.044
miR-339-5p	0.038	0.162	0.029–0.906

BMI, body mass index = weight (kg)/height squared (m²); ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBA, total bile acids; TBIL, total bilirubin; FGR, fetal growth restriction; HR, hazard ratio; 95% CI, 95% confidence interval.

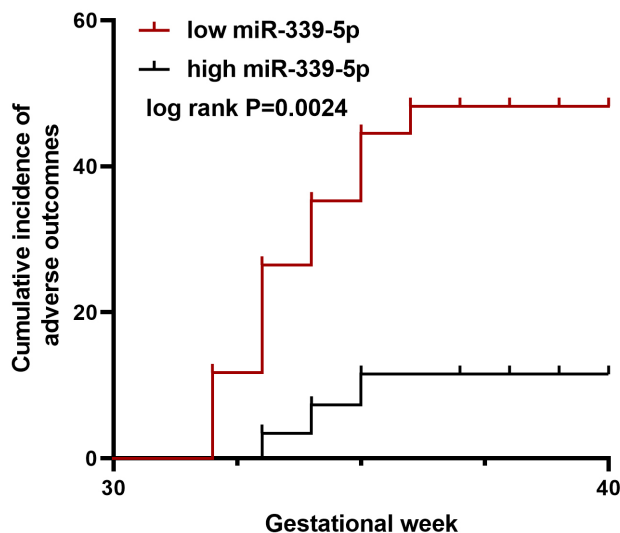


Fig. 4. Correlation between miR-339-5p levels and pregnancy outcome in patients with liver injury in pregnancy analyzed by the Kaplan-Meier method. The incidence of adverse pregnancy outcomes was higher in the group with lower miR-339-5p expression. log rank $p = 0.0024$.

pregnant women, and it significantly correlated with the risk of primary cesarean section [28]. MiR-519d expression is associated with adverse pregnancy outcomes such as pre-eclampsia, placental adhesion disorders, fetal death, or growth restriction [29]. Similarly, in this study, miR-339-5p is related to the occurrence of adverse pregnancy outcomes in patients, and the lower the expression of miR-339-5p in the serum of patients, the higher the probability of adverse pregnancy outcomes in pregnant women. These information suggest that miR-339-5p could serve as biomarkers for predicting pregnancy outcomes.

Studies have shown that miRNAs are involved in a wide range of biological processes, such as cell cycle control, apoptosis, and several developmental and physiological processes. miRNA can regulate the expression of redox sensors and change the key components of cellular antioxidants [30,31]. Therefore, exploring the targets and mechanisms of miRNA regulation will play a positive role in the diagnosis and treatment of clinically related diseases. Studies have shown that miR-339-5p is involved in the development of lung [32] and colorectal cancer [33] cells by targeting PRL-1. It was shown that upregulation of placental phosphatase of PRL-1 promotes liver regeneration in a rat model [34]. In a TAA-induced liver injury model, PRL-1 upregulation activated the antioxidant effects of liver and promoted liver regeneration [35]. Therefore, it is speculated that miR-339-5p targets PRL-1 and plays a role in regulating liver regeneration in the injured liver, which will be verified in cells and animals in the future. It has been noted pointed out that serum AST, ALT, TBA, and TBIL are considered important indicators of liver injury in pregnancy, and their abnormally elevated concentrations suggest pathological changes in pregnant women [36]. However, in this study, the expression level of miR-339-5p was found to have a low degree of correlation with TBIL, and whether this is related to the mechanism of action of miR-339-5p deserves further investigation. In addition, this study was designed to preliminarily investigate the role of miR-339-5p in the early diagnosis of liver injury in pregnancy and prediction of patient outcomes and did not include patients with other combined cases in pregnancy. However, some studies have pointed out that patients with hepatic impairment during pregnancy are prone to other comorbidities, such as diabetes mellitus (GDM): impaired hepatocyte function can affect hepatic glycogen synthesis, reduce glucose utilization and increase the risk of

combined GDM in pregnancy [37,38]. Therefore, in future studies, we will include more research subjects to deeply investigate the clinical significance of miR-339-5p in pregnancy and to lay more theoretical foundations for its clinical application.

5. Conclusions

In conclusion, low miR-339-5p expression can assist in the early diagnosis of liver injury in pregnancy and predicts adverse pregnancy outcomes.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by YW, LCY, FLH, HLY, QFY and XJZ. The first draft of the manuscript was written by YW, LCY and XJZ. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study protocol was approved by The Ethics Committee of Jinhua Maternal and Child Health Care Hospital (ethics number 201726) and followed the principles outlined in the Declaration of Helsinki. In addition, informed consent has been obtained from the participants involved.

Acknowledgment

We would like to express our gratitude to all those who helped us during the writing of this manuscript. Thanks to all the peer reviewers for their opinions and suggestions.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Gonzalez-Brown V, Frey HA. The Hepatobiliary System: An Overview of Normal Function and Diagnostic Testing in Pregnancy. *Clinical Obstetrics and Gynecology*. 2020; 63: 122–133.
- [2] Blume C, Pischke S, von Versen-Höynck F, Günter HH, Gross MM. Pregnancies in liver and kidney transplant recipients: a review of the current literature and recommendation. *Best Practice & Research. Clinical Obstetrics & Gynaecology*. 2014; 28: 1123–1136.
- [3] Katarey D, Westbrook RH. Pregnancy-specific liver diseases. *Best Practice & Research. Clinical Obstetrics & Gynaecology*. 2020; 68: 12–22.
- [4] Guarino M, Cossiga V, Morisco F. The interpretation of liver function tests in pregnancy. *Best Practice & Research. Clinical Gastroenterology*. 2020; 44–45: 101667.
- [5] Q Bartlett A, Vesco KK, Purnell JQ, Francisco M, Goddard E, Guan X, *et al.* Pregnancy and weaning regulate human maternal liver size and function. *Proceedings of the National Academy of Sciences of the United States of America*. 2021; 118: e2107269118.
- [6] Mufti AR, Reau N. Liver disease in pregnancy. *Clinics in Liver Disease*. 2012; 16: 247–269.
- [7] He B, Zhao Z, Cai Q, Zhang Y, Zhang P, Shi S, *et al.* miRNA-based biomarkers, therapies, and resistance in Cancer. *International Journal of Biological Sciences*. 2020; 16: 2628–2647.
- [8] He X, Kuang G, Wu Y, Ou C. Emerging roles of exosomal miRNAs in diabetes mellitus. *Clinical and Translational Medicine*. 2021; 11: e468.
- [9] Wojciechowska A, Braniewska A, Kozar-Kamińska K. MicroRNA in cardiovascular biology and disease. *Advances in Clinical and Experimental Medicine: Official Organ Wrocław Medical University*. 2017; 26: 865–874.
- [10] Braoudaki M, Lambrou GI. MicroRNAs in pediatric central nervous system embryonal neoplasms: the known unknown. *Journal of Hematology & Oncology*. 2015; 8: 6.
- [11] Mavreli D, Papantoniou N, Kolialexi A. miRNAs in pregnancy-related complications: an update. *Expert Review of Molecular Diagnostics*. 2018; 18: 587–589.
- [12] Mitra T, Gulati R, Uppal A, Kumari SR, Tripathy S, Ranjan P, *et al.* Prospecting of exosomal-miRNA signatures as prognostic marker for gestational diabetes mellitus and other adverse pregnancy outcomes. *Frontiers in Endocrinology*. 2023; 14: 1097337.
- [13] Tang Q, Wu W, Xu X, Huang L, Gao Q, Chen H, *et al.* miR-141 contributes to fetal growth restriction by regulating PLAG1 expression. *PLoS One*. 2013; 8: e58737.
- [14] Huang L, Shen Z, Xu Q, Huang X, Chen Q, Li D. Increased levels of microRNA-424 are associated with the pathogenesis of fetal growth restriction. *Placenta*. 2013; 34: 624–627.
- [15] Jia Y, Cong R, Li R, Yang X, Sun Q, Parvizi N, *et al.* Maternal low-protein diet induces gender-dependent changes in epigenetic regulation of the glucose-6-phosphatase gene in newborn piglet liver. *The Journal of Nutrition*. 2012; 142: 1659–1665.
- [16] Xu W, Yu M, Wu Y, Jie Y, Li X, Zeng X, *et al.* Plasma-Derived Exosomal SncRNA as a Promising Diagnostic Biomarker for Early Detection of HBV-Related Acute-on-Chronic Liver Failure. *Frontiers in Cellular and Infection Microbiology*. 2022; 12: 923300.
- [17] Wang YL, Chen CM, Wang XM, Wang L. Effects of miR-339-5p on invasion and prognosis of hepatocellular carcinoma. *Clinics and Research in Hepatology and Gastroenterology*. 2016; 40: 51–56.
- [18] Ch'ng CL, Morgan M, Hainsworth I, Kingham JGC. Prospective study of liver dysfunction in pregnancy in Southwest Wales. *Gut*. 2002; 51: 876–880.
- [19] García-Romero CS, Guzman C, Cervantes A, Cerbón M. Liver disease in pregnancy: Medical aspects and their implications for mother and child. *Annals of Hepatology*. 2019; 18: 553–562.
- [20] Westbrook RH, Dusheiko G, Williamson C. Pregnancy and liver disease. *Journal of Hepatology*. 2016; 64: 933–945.
- [21] Lycoudi A, Mavreli D, Mavrou A, Papantoniou N, Kolialexi A. miRNAs in pregnancy-related complications. *Expert Review of Molecular Diagnostics*. 2015; 15: 999–1010.
- [22] Fang Y, Fang D. Comprehensive analysis of placental gene-expression profiles and identification of EGFR-mediated au-

tophagy and ferroptosis suppression in intrahepatic cholestasis of pregnancy. *Gene*. 2022; 834: 146594.

- [23] Wang J, Jiang M, Xia S. miR-339-5p Increases Radiosensitivity of Lung Cancer Cells by Targeting Phosphatases of Regenerating Liver-1 (PRL-1). *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. 2018; 24: 8408–8416.
- [24] Kim JY, Kim SH, Seok J, Bae SH, Hwang SG, Kim GJ. Increased PRL-1 in BM-derived MSCs triggers anaerobic metabolism via mitochondria in a cholestatic rat model. *Molecular Therapy. Nucleic Acids*. 2023; 31: 512–524.
- [25] Vallelunga A, Iannitti T, Capece S, Somma G, Russillo MC, Foubert-Samier A, *et al.* Serum miR-96-5P and miR-339-5P Are Potential Biomarkers for Multiple System Atrophy and Parkinson's Disease. *Frontiers in Aging Neuroscience*. 2021; 13: 632891.
- [26] Gartz M, Beatka M, Prom MJ, Strande JL, Lawlor MW. Cardiomyocyte-produced miR-339-5p mediates pathology in Duchenne muscular dystrophy cardiomyopathy. *Human Molecular Genetics*. 2021; 30: 2347–2361.
- [27] He X, Ding D. High miR-200a-3p expression has high diagnostic values for hypertensive disorders complicating pregnancy and predicts adverse pregnancy outcomes. *BMC Pregnancy and Childbirth*. 2022; 22: 490.
- [28] Pfeiffer S, Sánchez-Lechuga B, Donovan P, Halang L, Prehn JHM, Campos-Caro A, *et al.* Circulating miR-330-3p in Late Pregnancy is Associated with Pregnancy Outcomes Among Lean Women with GDM. *Scientific Reports*. 2020; 10: 908.
- [29] Xie L, Sadovsky Y. The function of miR-519d in cell migration, invasion, and proliferation suggests a role in early placentation. *Placenta*. 2016; 48: 34–37.
- [30] Rani V, Sengar RS. Biogenesis and mechanisms of microRNA-mediated gene regulation. *Biotechnology and Bioengineering*. 2022; 119: 685–692.
- [31] Tüfekci KU, Meuwissen RL, Genç S. The role of microRNAs in biological processes. *Methods in Molecular Biology*. 2014; 1107: 15–31.
- [32] Li P, Liu H, Li Y, Wang Y, Zhao L, Wang H. miR-339-5p inhibits lung adenocarcinoma invasion and migration by directly targeting BCL6. *Oncology Letters*. 2018; 16: 5785–5790.
- [33] Zhou C, Liu G, Wang L, Lu Y, Yuan L, Zheng L, *et al.* MiR-339-5p regulates the growth, colony formation and metastasis of colorectal cancer cells by targeting PRL-1. *PLoS One*. 2013; 8: e63142.
- [34] Choi JH, Park S, Kim GD, Kim JY, Jun JH, Bae SH, *et al.* Increased Phosphatase of Regenerating Liver-1 by Placental Stem Cells Promotes Hepatic Regeneration in a Bile-Duct-Ligated Rat Model. *Cells*. 2021; 10: 2530.
- [35] Park HJ, Jun JH, Kim JY, Jang HJ, Lim JY, Bae SH, *et al.* Phosphatase of Regenerating Liver-1 (PRL-1)-Overexpressing Placenta-Derived Mesenchymal Stem Cells Enhance Antioxidant Effects via Peroxiredoxin 3 in TAA-Injured Rat Livers. *Antioxidants (Basel, Switzerland)*. 2022; 12: 46.
- [36] Gao Q, Ma Y, Qu X, Zheng X. Risk factors in patients with acute fatty liver of pregnancy: the role of abortion, total bilirubin and serum creatinine. *Archives of Gynecology and Obstetrics*. 2024; 310: 153–159.
- [37] Linder T, Eppel D, Kotzaeridi G, Rosicky I, Yerlikaya-Schatten G, Kiss H, *et al.* Fatty liver indices and their association with glucose metabolism in pregnancy - An observational cohort study. *Diabetes Research and Clinical Practice*. 2022; 189: 109942.
- [38] Wicklow B, Retnakaran R. Gestational Diabetes Mellitus and Its Implications across the Life Span. *Diabetes and Metabolism Journal*. 2023; 47: 333–344.