ORIGINAL RESEARCH

Diagnostic Potential of microRNA-122 for Stage Classification in Patients with HBV-Related Cirrhosis

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ABSTRACT

Objective • Previous studies have suggested that microRNA-122 has a relatively high diagnostic value for chronic viral hepatitis detection. In this study, we evaluated the diagnostic value of serum microRNA-122 in different stages of HBV-related cirrhosis, and serum microRNA-122 may serve as a potential biomarker for staging HBV related cirrhosis patients..

Methods • A total of 80 patients with HBV-related cirrhosis were included. Patients were characterized according to Child-Pugh score, laboratory parameters, and complications, and divided into compensated cirrhosis group and decompensated cirrhosis group. Wherein, the compensatory group for liver cirrhosis includes 21 patients, the compensatory group has 59 patients. Blood was collected from all patients, and RT-qPCR analyzed the expression levels of microRNA-122.

Results • Serum microRNA-122 was decreased, while Child-Pugh score, Meld score, Prothrombin time, total bilirubin, and Direct bilirubin were higher in a decompensated group compared to the compensated group (all $P < .05$). For further stage classification, the mean serum microRNA-122 level was higher in stage 1 (11.3±5.1, compensated cirrhosis) compared to stage 2~5 (8.5±4.2, 4.9±1.0, 4.7±1.6, 3.5±1.1, decompensated cirrhosis, all $P < .05$). The expression of serum microRNA-122 independent of Child-Pugh score and complications, including ascites, varices, HCC ($P > .05$). However it was affected by Meld score and Prothrombin time ($P < .05$). Moreover, ROC analysis indicated microRNA-122 could differentiate compensated HBV-related cirrhosis (0.97 of AUC, $P < .01$). Furthermore, it could differentiate patients in stage 1 (compensated cirrhosis without esophageal varices) from HBV-related cirrhosis (0.91 of AUC, $P < .01$), with a sensitivity of 77.8% and satisfactory specificity of 88.7%. The significance of the relationship between the decrease in serum microRNA-122 levels and the stage of liver cirrhosis will be beneficial.

Conclusion • Our results strongly support the diagnostic value of serum microRNA-122 as a potential biomarker of stage classification in patients with HBV-related cirrhosis, which could facilitate risk stratification and careful management. Provide new biomarkers for the diagnosis of patients with hepatitis B cirrhosis. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Hepatitis B virus (HBV) infection remains a major global public health issue. Chronic hepatitis B (CHB) is highly endemic in China. It has been estimated that the hepatitis B surface antigen (HBsAg) prevalence is about 5%~6% and that approx. 70 million persons suffer from chronic infection.[1] There remained considerable variation in estimates of HBsAg seroprevalence across China in 2021, from below 1.5% in North China to >3% in South-West China and >6% in Taiwan and Hong Kong. Children experienced the most pronounced decrease in prevalence, while a concurrent increase in HBsAg seroprevalence was observed in older persons (≥60 years).[2] Patients with CHB often develop serious complications, such as cirrhosis and hepatocellular carcinoma (HCC). Patients with decompensated cirrhosis have a very poor prognosis due to different complications and require careful management. Early diagnosis of liver cirrhosis can improve patient

prognosis. Thus, the search for non-invasive biomarkers for early diagnosis, prognosis, and assessment of HBV-related cirrhosis is of critical importance.

MicroRNAs (miRNAs) are small, non-coding RNAs that negatively regulate gene expression and are involved in various biological processes, including cell differentiation, proliferation, and apoptosis. Over the last two decades, a high number of microRNAs have been discovered. Considering their high stability in the blood, miRNAs have been suggested as novel biomarkers for detecting various diseases.[3]

MicroRNA-122, composing about 70% of the total miRNAs found in hepatocytes, is one of the most abundant miRNAs in the liver and plays a critical role in liver fibrosis by negatively regulating the proliferation and transactivation of HSCs.[4,5] microRNA-122 has a relatively high diagnostic value for chronic viral hepatitis detection.[6] Studies have shown that miR- 122 affects HBV replication in vitro and is significantly associated with HBV-related liver cirrhosis.[7] The anti-fibrotic disorders of miRNAs induced the occurrence of liver cirrhosis in patients with CHB [35], and the decreased expression of miR- 122 weakened the inhibitory effect of liver fibrosis, thereby aggravating the occurrence of liver cirrhosis.[8] It is expressed at high levels in the mature liver while is markedly downregulated in HCC; downregulation of microRNA-122 in HCC has been associated with poor prognosis and a higher risk of HCC metastasis.[4] In addition, Franck *et al.* discovered that low microRNA-122 was significantly associated with better prognosis in patients with advanced cirrhosis (Child-Pugh class B/C), advanced tumor stage (BCLC B/C/D), and normal alpha-fetoprotein (AFP).[9] Perhaps due to excessive attention to mir122 in liver malignancies, its role in patients with cirrhosis has been overlooked, so far, only a few studies have reported on the role of microRNA-122 in cirrhosis diagnosis and stage classification. Thus, in this study, we evaluated the diagnostic value of serum microRNA-122 in different stages of HBV-related cirrhosis, and it is related to the risk stratification of HBV-related cirrhosis patients.We can provide more accurate treatment plans based on the different stages of liver cirrhosis patients.

MATERIALS AND METHODS

Patients

Based on literature review, there are 80 Patients with HBV-related cirrhosis who admitted to the Department of Gastroenterology and Hepatology, Minhang Hospital, affiliated with Fudan University (Shanghai, China) between January 2020 and December 2021 were included in the study. HBV-related cirrhosis was defined as cirrhosis confirmed by clinical images and/or liver biopsy in those who suffered from hepatitis B or who had positive HBsAg for more than 6 months and positive HBsAg and/or HBV DNA. Participants who had co-infection with extrahepatic tumor disease or other causes for cirrhosis, such as Hepatitis C, alcoholic consumption, non-alcoholic fatty liver disease, and autoimmune liver diseases, were excluded.

The study was approved by the Ethics Committee for Clinical Research of MinHang Hospital (Number: 006-01K) and corresponded with the declaration of Helsinki. All patients provided written informed consent.

Description of the patients

Patients' characteristics are presented in Table 1. Eighty patients were separated into a compensated cirrhosis group and a decompensated cirrhosis group according to the diagnosis criteria of Chinese guidelines on the management of liver cirrhosis, which is more convenient for clinical staging of liver cirrhosis.[1] Different stages of cirrhosis were defined according to the diagnosis criteria of Chinese guidelines on the management of liver cirrhosis[1,10]: Stage 1: absence of ascites and esophageal varices; Stage 2: the presence of esophageal varices without bleeding and without ascites; Stage 3: the presence of ascites with or without esophageal varices; Stage 4: the presence of esophageal varices with bleeding, with or without ascites; Stage 5: the presence of sepsis, uncontrolled bleeding or refractory ascites, hepatorenal syndrome, hepatic encephalopathy and other multiple organ dysfunction. Include the first and second stages in the compensatory group for liver cirrhosis, and the third, fourth, and fifth stages in the decompensated group.

After blood sampling, all patients were characterized with respect to clinical and laboratory parameters. Based on literature review, we compared complications and other clinical characteristics to estimate the possible influence factors of microRNA-122 expression.

Extraction of total RNA

Blood was collected from all patients. A total RNA was extracted from serum using Nuclezol LS RNA Isolation Reagent (FP313-1), according to the manufacturer's instructions. The yield of RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and the integrity was evaluated using agarose gel electrophoresis stained with ethidium bromide.

Real-time quantitative RT-PCR

Quantification was performed with a two-step reaction process: reverse transcription (RT) and PCR. Each RT reaction consisted of 0.5 μg RNA, 5 μl of 2×TS miRNA Reaction Mix, and 0.5 μl of TransScrip miRNA RT Enzyme Mix, in a total volume of 10 μl. Reactions were performed in a GeneAmp® PCR System 9700 (Applied Biosystems, USA) for 60 min at 37°C, followed by heat inactivation of RT for 5s at 85°C. The 10 μl RT reaction mix was then diluted × 10 in nuclease-free water and held at -20°C.

Real-time PCR was performed using LightCycler® 480 II Real-time PCR Instrument (Roche, Swiss) with 10 μl PCR reaction mixture that included 1 μl of cDNA, 5 μl of 2×PerfectStartTM Green qPCR SuperMix, 0.2 μl of universal primer, 0.2 μl of microRNA-specific primer, and 3.6 μl of nuclease-free water. Reactions were incubated in a 384-well optical plate (Roche, Swiss) at 94°C for 30 s, followed by 45

cycles of 94°C for 5s, 60°C for 30 s. Each sample was run in triplicate. At the end of the PCR cycles, a melting curve analysis was performed to validate the specific generation of the expected PCR product. The microRNA-specific primer sequences were designed in the laboratory and synthesized by TsingKe Biotech based on the microRNA sequences obtained from the miRBase database (Release 20.0) as follows: TGGAGTGTGACAATGGTGTTTG.

The expression levels of microRNAs were normalized to (input the reference gene, e.g.: RNU6B, 5S rRNA) and were calculated using the 2-ΔΔCt method (Livak and Schmittgen, 2001).

Statistical analysis

Statistic Package for Social Science (SPSS) 23.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Continuous variables are presented as mean ± SD or median (interquartile range (IQR)) and were compared using a t-test or the Mann-Whitney U test. Categorical variables are presented as rates and were compared using the χ^2 test or the Fisher's exact test. Receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic performance of microRNA-122 by comparing different stages of HBV-related cirrhosis. A $P <$ .05 was considered to be statistically significant.

RESULTS

Demographic and clinical characteristics

Patients' characteristics are presented in Table 1. Child-Pugh score, Meld score, and Prothrombin time, as well as the level of total bilirubin and direct bilirubin were higher in the decompensated cirrhosis group (n=59) compared to the compensated cirrhosis group (n=21) (all $P <$ .05). In contrast, serum microRNA-122 was significantly decreased in patients with decompensated HBV-related cirrhosis as compared to those with compensated cirrhosis ($P <$ .05).

Serum microRNA-122 level in five different stages of HBV-related cirrhosis

We performed subgroup analyses based on the 5 stage classification due to the complications of cirrhosis according to the guidelines.[10] There were 9 patients with Stage 1, 12 with stage 2, 20 with stage 3, 24 with stage 4, and 15 with stage 5. As shown in Figure 1, the mean serum microRNA-122 level (11.3±5.1) was higher in stage 1 compared to stage 2 (8.5±4.2), stage 3 (4.9±1.0), stage 4 (4.7±1.6), and stage 5 (3.5±1.1) (all $P <$ .05). The results of the subgroup analysis supported the potential value of microRNA-122 as a diagnostic biomarker of stage classification for HBV-related cirrhosis, especially potential in early disease diagnosis.

Serum microRNA-122 level in correlation to Child-Pugh score and complications of HBV-related cirrhosis

RT-qPCR results indicated that serum microRNA-122 expression in HBV-related cirrhosis was independent of Child-Pugh score and complications of cirrhosis, including ascites, varices, and HCC ($P >$ .05) (Figure 2). This indicates

Table 1. Demographic characteristics of HBV-related cirrhosis patients

	Total (n=80)	compensated cirrhosis (n=21)	decompensated cirrhosis (n=59)	*P* value
Age, y (Mean±SD)	57.5±12.9	58.3±10.8	57.2±13.7	.744
Male, n (%)	60.0(75.0)	17.0(80.1)	43.0(72.9)	.470
microRNA-122 (Mean±SD)	5.8±3.5	9.6±4.7	4.5±1.4	.000
MELD score (Mean±SD)	14.8±5.9	12.1±4.9	15.8±5.9	.011
Child-Pugh score (Mean±SD)	7.2±2.5	6.0±2.0	7.7±2.6	.006
Hemoglobin, g/L (Mean±SD)	109.0±31.4	118.7±34.0	105.5±30.0	.100
Albumin, g/L (Mean±SD)	34.6±7.3	36.7±6.5	33.8±7.4	.118
Creatinine, μmol/L (Mean±SD)	73.6±25.4	68.9±14.6	75.3±28.2	.323
Prothrombin time, s (Mean±SD)	14.3±2.2	13.0±1.8	14.7±2.2	.002
ALT, U/L (M (P25, P75))	26.0(15.0,45.0)	28.0(15.0,38.0)	24.0(15.0,47.0)	.943
AST, U/L (M (P25, P75))	34.0(25.0,64.0)	32.0(25.0,38.0)	36.0(35.0,82.0)	.325
DBil, μmol/L (M (P25, P75))	12.3(8.3,18.7)	8.2(5.5,14.5)	14.2(9.3,20.5)	.007
TBil, μmol/L (M (P25, P75))	25.1(16.9,40.0)	19.8(12.0,27.7)	28.5(18.4,37.7)	.014
AFP, ng/ml (M (P25, P75))	3.2(1.7,16.4)	2.2(1.6,12.7)	3.3(1.7,40.0)	.426

Figure 1. Serum microRNA-122 level in five different stages of HBV-related cirrhosis. Stage 1: absence of ascites and esophageal varices (n=9); Stage2: the presence of esophageal varices without bleeding and without ascites (n=12); Stage 3: the presence of ascites with or without esophageal varices (n=20); Stage 4: the presence of esophageal varices with bleeding, with or without ascites (n=24); Stage 5: the presence of sepsis, uncontrolled bleeding or refractory ascites, hepatorenal syndrome, hepatic encephalopathy and other multiple organ disfunction (n=15).

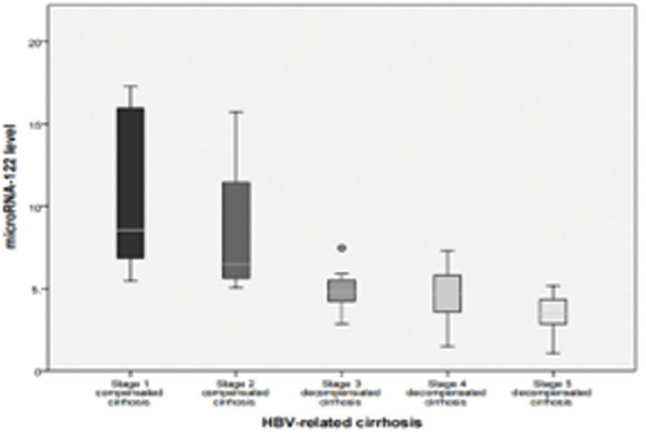


Figure 2. Serum microRNA-122 level in correlation to Child-Pugh score and complications of HBV-related cirrhosis. (A) Serum microRNA-122 level in relation to Child-Pugh score: class A (n=22), class B (n=18), and class C (n=11). (B) Serum microRNA-122 level in relation to HCC: with HCC (n=18), without HCC (n=62). (C) Serum microRNA-122 level in relation to ascites: with ascites (n=34), without ascites (n=46). (D) Serum microRNA-122 level in relation to gastroesophageal varices: with varices (n=47), without varices (n=33).

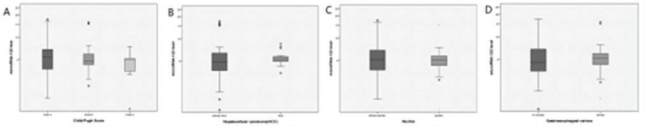


Table 2. Relationship of serum microRNA-122 with clinical parameters

Variables	microRNA-122	
	r	*P* value
Age	0.203	.071
MELD score	-0.238	.033
PT	-0.240	.032
ALT	-0.119	.293
AST	-0.127	.261
SCr	-0.081	.477
TBIL	-0.090	.429
DBIL	-0.097	.391
AFP	-0.064	.580
HBeAg	-0.135	.243

Figure 3. Serum microRNA-122 level in correlation to prothrombin time and Meld score (A) Serum microRNA-122 level in relation to Meld score. (B) Serum microRNA-122 level in relation to prothrombin time.

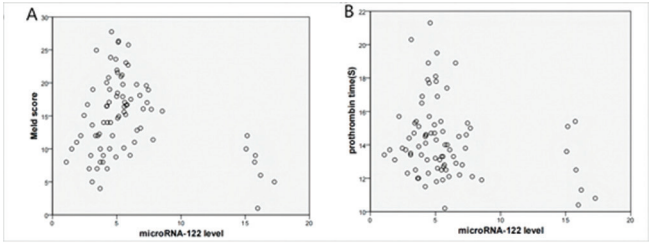
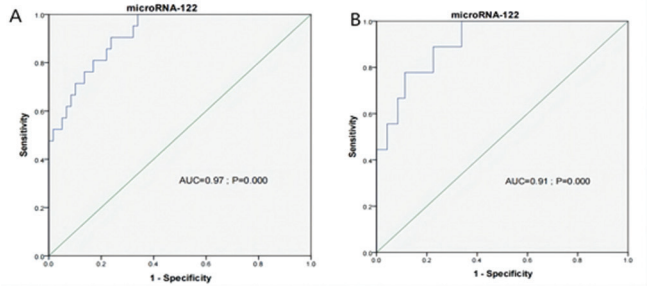


Table 3. ROC analysis of serum microRNA-122

Differentiate Stage	AUC (95%CI)	Best cutoff	Sensitivity	Specificity	*P* value
compensated stage	0.97(0.86~0.98)	5.44	90.5%	76.3%	.000
Stage 1 of compensated stage	0.91(0.83~0.99)	6.83	77.8%	88.7%	.000

Figure 4. ROC-analysis for differentiating between decompensated and compensated HBV-related cirrhosis. (A) compensated stage; (B) stage1(compensated cirrhosis): without esophageal varices.



that microRNA-122 could be a marker independent of traditional clinical assessments.

Relationships of Serum microRNA-122 with clinical parameters

Next, we analyzed the associations of serum miR122 levels with clinical parameters to estimate possible influencing factors that may impact serum microRNA-122 expression. The serum microRNA-122 expression in HBV-related cirrhosis was significantly associated with prothrombin time and Meld score ($P < .05$) (Table 2, Figure 3).This could imply a role of microRNA-122 in reflecting the liver's functional status in HBV-related cirrhosis.

Diagnostic value of serum microRNA-122 for compensated HBV-related cirrhosis

We conducted ROC analysis to evaluate the diagnostic performance of microRNA-122 alone for differentiating compensated HBV-related cirrhosis. The area under the curve (AUC) for serum microRNA-122 was 0.97 ($P < .01$). A reasonable cutoff value was 5.44, with a satisfied sensitivity of 90.5% and a specificity of 76.3%.

Moreover, we draw the ROC curve to further evaluate the diagnostic value for stage 1 of compensated HBV-related cirrhosis, defined as the absence of esophageal varices according to the guidelines[1], obtaining the AUC is 0.91 ($P < .01$). A reasonable cutoff value of 6.83 with sensitivity of 77.8% and a higher specificity of 88.7% were obtained (Table 3, Figure 4).

DISCUSSION

Chronic hepatitis B virus infection is the primary cause of severe liver disorders, including liver fibrosis, cirrhosis, and hepatocellular carcinoma. Cirrhosis is currently the 11th most common cause of death globally, resulting in approximately 2 million deaths every year.[11] The most common symptoms of cirrhosis are fatigue and loss of appetite. Scoring systems such as Child-Pugh score, model for end-stage liver disease (MELD), and MELD-Na score are widely used for assessing prognosis in cirrhosis patients but are not suitable for stage classification. Cirrhosis can be classified into compensated stage and decompensated stage.[1] Yet, differentiating decompensated from compensated cirrhosis patients remains challenging. In this study, we systematically characterized the diagnostic value of microRNA-122 in a different stage of HBV-related cirrhosis. Increase the diagnostic rate of early liver cirrhosis patients and improve their prognosis.

At present, histological evaluation is a "gold standard" for diagnosing and evaluating cirrhosis.[1] Yet, this method is invasive and not suitable for frequent monitoring or screening of cirrhosis. Moreover, endoscopic screening for a large proportion of patients without gastroesophageal varices may lead to unnecessary costs and risks. Thus, convenient and non-invasive biomarkers are urgently needed for risk stratification of HBV-related cirrhosis patients.Lipidomic, proteomic, and gut microbiome profiles, as well as microRNA signatures are promising techniques for fibrosis assessment in the future.[12]

As the most direct and reliable indicator of viral replication, HBV DNA can reflect the viral levels in vivo. Recent studies in HBV-infected patients revealed that microRNA-122 levels were negatively correlated with HBV-DNA,[13-15] but not with HBeAg.[14] Serum miR-122 was reported as an important predictor of HBsAg seroclearance in Japanese patients who did not receive antiviral therapy.[16] Meanwhile, several studies have reported that microRNA-122 is a serum biomarker for the severity of hepatocyte damage or the residual liver function in patients with cirrhosis and HCC.[9,17-19] Our study showed serum microRNA-122 expression is

negative correlation with prothrombin time and Meld score. The liver is an important site for synthesizing various coagulation factors. When liver function is incomplete, the consumption or synthesis of coagulation factors increases or decreases, leading to prolonged prothrombin time. The MELD score, as a traditional indicator for evaluating the prognosis of patients with various liver diseases, is widely used in clinical practice. The higher the MELD score, the worse the prognosis of patients. MELD score is more objective compared to indicators with stronger subjectivity, such as ascites and complications. Mounting evidence elucidated that miR-122 downregulation led to HSC activation and proliferation.[20,21] Therefore, we speculate that miR-122 may be associated with the severity of hepatocyte damage. Briefly, the lower the miR-122 expression, the severe the hepatocyte damage and the worse the prognosis.

In this study, serum microRNA-122 was apparently downregulated in the decompensated cirrhosis patients as compared to compensated stage patients. Moreover, serum microRNA-122 could effectively differentiate stage 1 (compensated cirrhosis) patients with or without esophageal varices, from patients with stages 2, 3, 4, and 5 (decompensated group), with high specificity and sensitivity. Moreover, we discovered that when serum microRNA-122 expression is higher than 6.83, endoscopic screening can be avoided. Otherwise, evaluation of the severity of portal hypertension, assessment, and management of complications should be conducted to HBV-related cirrhosis patients.

microRNA-122 is an anti-inflammatory and anti-tumorigenic effector in the liver.[12] Several studies have shown that the hepatic level of microRNA-122 is significantly decreased in the late stage of fibrosis and HCC.[22] MicroRNAs participat largely in the response to hypoxia by their regulation of the expression of critical genes involved in the apoptotic process, such as BCL2 or XIAP. Hypoxia plays a pivotal role in the natural history of cirrhosis as it stimulates angiogenesis, inhibits cell proliferation factors, and promotes fibrogenesis, contributing to progressive portal hypertension and hyperdynamic circulation.[23,24] An *in vivo* study indicated microRNA-122 as a key regulator of cholesterol and fatty acid metabolism, and its gene resulted in the development of liver tumors in mice.[25] The tumor-suppressive effect of microRNA-122 is believed to be mediated via the inhibition of a variety of tumorigenic genes.[4] Also, increasing evidence showed that microRNA-122 expression was up-regulated in patients with CHB and down-regulated in HBV-related HCC.[26-28] In the present study, we also observed that serum microRNA-122 level decreased in HBV-related HCC compared to HBV-related cirrhosis without HCC; however, only a non-significant trend was observed between the two groups ($P > .05$). Thus, our data do not support the use of microRNA-122 as a diagnostic biomarker for screening HCC in HBV-related cirrhosis. Perhaps it is related to the low number of HCC patients among the liver cirrhosis patients we included. Yet, the underlying mechanisms of HCC need to be further explored. Recent research discovered a novel liver-specific miR-122-G9a regulatory axis and suggested that miR-122 has a protective role in the liver from genotoxic carcinogens via targeting G9a.[29] Further comprehensive studies of the mechanism involved in microRNA-122 expression in HBV-related cirrhosis and HBV-related HCC should be performed.

ALT and Child-Pugh scores can reflect liver function impairment and the severity of cirrhosis, but the influence of ALT on the degree of liver cell damage, and Child-Pugh score is influenced by human factors. Our study showed that in HBV-related cirrhosis was independent of Child-Pugh score and complications of cirrhosis, as well as the hepatocyte damage markers, including ALT, HBeAg, and HBV-DNA. The relationship between microRNA-122 and the severity of hepatocyte damage might be affected by different treatments. The weakened relationship may also be related to the different antiviral treatments or various complications that coexisted in HBV-related cirrhosis patients.

This study has a few limitations. First, this cross-sectional study only showed the correlation between microRNA-122 and the stage classification of HBV-related cirrhosis, it unable to observe changes in variables, while it did not provide direct causal evidenceUnable to observe changes in variables. Second, the number of samples was relatively small. The scope of application of the conclusions obtained is smaller. Larger studies with prospective study designs are needed to further confirm these findings.in HBV-related cirrhosis was independent of Child-Pugh score and complications of cirrhosis, as well as the hepatocyte damage markers including ALT, HBeAg, and HBV-DNA, or to understand the long-term implications of using microRNA-122 as a biomarker. The relationship between microRNA-122 and the severity of hepatocyte damage might be affected by different treatments. However, the diagnostic value of serum microRNA-122 in the classifying stage with HBV-related cirrhosis is limited. In the future, we will expand the sample size to further confirm the diagnostic value of microRNA-122 in patients with hepatitis B cirrhosis. We propose to establish a non-invasive diagnosis model for cirrhosis. The model includes indicators of liver function and liver fibrosis, aiming to improve the diagnostic efficiency of non-invasive liver cirrhosis models, especially in the early stages of liver cirrhosis.

CONCLUSIONS

Identifying the HBV-related cirrhosis stage is crucial for the prognosis and management of the patient. Our data suggest that serum microRNA-122 could differentiate HBV-related cirrhosis patients in a compensated stage without esophageal varices, with high diagnostic specificity and sensitivity. Expected to become a biological marker for early diagnosis of liver cirrhosis. MicroRNA-122 is of great significance as a biomarker for distinguishing the compensatory and decompensated phases of HBV related cirrhosis. When serum microRNA-122 expression is higher than 6.83, endoscopic screening could be avoided, so as to

reduce the pain and medical expenses of patients. In the future, further research will be conducted on microRNA-122 for assessing the severity, complications, and management of portal hypertension in patients with HBV related cirrhosis, in order to recommend non-invasive diagnostic models for cirrhosis, improve the early diagnosis rate of cirrhosis, and improve the quality of life and survival time of patients.

ETHICAL COMPLIANCE

The study was approved by the Ethics Committee for Clinical Research of Minhang Hospital (Number: 006-01K) and corresponded with the declaration of Helsinki. All patients provided written informed consent.

FUNDING

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DECLARATIONS OF INTEREST

None.

DATA AVAILABILITY

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

AUTHOR CONTRIBUTIONS

Methodology, Writing - original draft, Visualization, Formal analysis: Qingqing Fang, Jin Liu, Ying Chen; Methodology, Investigation; Wei Chen, Feng Li; Conceptualization, Methodology, Data curation, Investigation, Formal analysis, Supervision: Shiyao Chen, Ying Chen. All authors read and approved the final manuscript. Qingqing Fang and Jin Liu contributed equally to this work.

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