European Review for Medical and Pharmacological Sciences 2020; 24: 6245-6251

Serum miR-17 levels in patients with hepatitis B virus induced liver fibrosis

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Abstract. – OBJECTIVE: To investigate the clinical significance of detecting serum level of miR-17 in patients with hepatitis B virus (HBV) induced liver fibrosis.

PATIENTS AND METHODS: A total of 200 HBV patients undergoing liver biopsy in Henan Provincial People's Hospital from June 2016 to December 2018, and 200 healthy subjects during the same period were included. Serum miR-17 level was detected by qRT-PCR. Multivariate Logistic regression analysis was conducted to evaluate independent risk factors of liver fibrosis in HBV patients. Meanwhile, ROC curves were used to assess the value of miR-17 in determining liver fibrosis severity of HBV patients.

RESULTS: 200 HBV patients were classified into 4 groups based on the severity of liver fibrosis, including 52 cases in S0-1, 47 cases in S2, 53 cases in S3, and 48 cases in S4. Serum level of miR-17 was lower in healthy subjects than that in HBV patients. In addition, the serum level of miR-17 was negatively correlated with liver fibrosis severity. The relative levels of aspartate aminotransferase (AST), HBV-DNA, albumin (ALB), platelets (PLT), aspartate aminotransferase-to-platelet ratio index (APRI) and miR-17 were the independent factors of advanced liver fibrosis in HBV patients. Serum level of miR-17 exerted a predictive potential in diagnosis of the severity of HBV-induced liver fibrosis.

CONCLUSIONS: Serum miR-17 level is highly expressed in HBV patients, and negatively correlated with liver fibrosis severity, which could be utilized as a non-invasive hallmark assessing liver fibrosis severity in HBV patients.

Key Words:
MiR-17, HBV, Liver fibrosis, Hallmark.

Introduction

Hepatitis B virus (HBV) infection is popular in the world. It is reported that 2 billion people in the world are infected with HBV, of whom 35 million are chronic carriers[1]. HBV infection has become the leading public health problem, and it is the tenth leading cause of death[2]. About 786,000 deaths per year are resulted from HBV infection. In addition, acute or chronic HBV infection is the major reason for chronic hepatitis, cirrhosis, and liver cancer. Approximately half of liver cancer deaths in 2010 can be attributed to HBV infection. From 1990-2010, mortality of liver cancer and cirrhosis has increased by 62% and 29%, respectively[2]. HBV infection has become a sever public health problem that we need to solve urgently.

MiRNAs are single-stranded, non-coding RNAs involved in biological activities by degrading target mRNAs or inhibiting their translation[3]. Finch et al[4] identified a great number of miRNAs that participated in liver development, degeneration, and diseases. Expression levels of these liver-associated miRNAs are closely related to viral hepatitis and other liver diseases.

MiR-17 is located on human chromosome 13q31.3[5], and it displays a carcinogenic role during tumor development[6]. MiR-17 is upregulated in brain cancer[7], hepatocellular carcinoma[8] and colorectal cancer species[9]. As a novel regulator of CYP7A1 signaling in liver lipid metabolism, miR-17 is a potential therapeutic target in fatty liver[10]. The primary purpose of this study was to investigate the serum miR-17 expression level in patients with hepatitis B induced liver fibrosis and the correlation between serum miR-17 levels with liver fibrosis severity.

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Patients and Methods

Patients

A total of 200 patients with HBV-induced liver fibrosis undergoing liver biopsy in Henan Provincial People's Hospital from June 2016 to December 2018 were included, involving 103 males and 97 females. Their age was 32-70 years and average BMI was 22.63 ±3.79. All patients with HBV-induced liver fibrosis were confirmed by pathological examination. Inclusion criteria were: (1) patients were diagnosed as chronic HBV infection; (2) HBsAg positive lasted for over 6 months; (3) total bilirubin (TBil) level was normal and alanine aminotrasferase (ALT) level < 400 U/L; (4) antiviral drugs were not received within one year before liver biopsy. Exclusion criteria were: (1) patients with abnormal liver function caused by other hepatophilic virus infection, alcoholic liver disease, fatty liver of various types, autoimmune liver disease or other factors; (2) patients with serious heart, lung and kidney diseases, rheumatism and immune system diseases, metabolic diseases (i.e., diabetes and hyperthyroidism), HIV infection and Wilson's disease.

A total of 50 healthy subjects undergoing health examinations during the same period were included as controls, involving 25 males and 25 females. Their age was 33-68 years and average BMI was 22.17 ±3.06. No significant differences in age, sex and BMI were identified between HBV patients and healthy subjects (p>0.05). This study was performed after approval of Hospital Ethic Committee and informed consent from patients.

Serological Examination

Venous blood was collected in each subject under the fasting state in the morning for analyzing alanine aminotrasferase (ALT), aspartate aminotrasferase (AST), HBV-DNA, HBV markers, platelets (PLT), albumin (ALB), glutamyl transferase (GGT), etc. Fibrosis-4 (FIB-4) index[11] and aspartate aminotransferase-to-platelet ratio index (APRI)[12] were calculated based on ALT, AST and PLT. Meanwhile, the serum levels of liver fibrosis markers, including propeptide of type III procollagen (PIIINP), type IV collagen (IV-C), hyaluronic acid (HA) and laminin (LN), were detected. In addition, 3 ml of venous blood of each subject was centrifuged at 3,500 r/min for 5 min, and the serum was collected and stored at -80°C.

Liver Biopsy and Pathological Diagnosis

Under the guidance of color Doppler ultrasound, a 16-G puncture needle was used for obtaining liver tissues (1.5-2.0 cm). The tissues were then fixed, paraffin embedded, sliced, and stained by hematoxylin and eosin (HE) and Masson (Boster, Wuhan, China). Three pathologists were independently responsible for pathological examinations. Liver fibrosis severity was classified into S0-1 (mild fibrosis), S2 (pronounced fibrosis), S3 (advanced fibrosis) and S4 (cirrhosis).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Serum miRNAs were extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany), which were reversely transcribed using the TaqMan microRNA reverse Transcription Kit (Thermo Fisher, Waltham, MA, USA). QRT-PCR was performed to amplify miR-17, with U6 as the internal reference. Primer sequences were listed as follows. MiR-17, F: 5'-TGCAAAGTGCTTACAGTGCAG-3', R: 5'-GTGCAGGGTCCGAGGTATTC-3'; U6, F: 5′-GCTTCGGCAGCACATATACTAAAAT-3′, R: 5′-CGCTTCACGAATTTGCGTGTCAT-3′.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Data were expressed as mean ± SD (standard deviation). Differences between two groups were analyzed by using the Student's t-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). Multivariate Logistic regression analysis was conducted to evaluate independent risk factors of liver fibrosis in HBV patients. Receiver operating characteristic (ROC) curves were depicted to assess the diagnostic potential of miR-17. p<0.05 indicated the significant difference.

Results

Baseline Characteristics of HBV Patients with Different Severities of Liver Fibrosis

200 HBV patients were classified into four groups according to liver fibrosis severity, including 52 cases in S0-1, 47 cases in S2, 53 cases in S3 and 48 cases in S4. No significant differences were found in age, sex and BMI between HBV patients and healthy subjects (p>0.05). By analyzing baseline characteristics of HBV patients with

Table I. Baseline characteristics of patients with different severities of HBV induced liver fibrosis.

Variable	S0-1 (n=52)	S2 (n=47)	S3 (n=53)	S4 (n=48)	F	p
Age	40.24±7.52	41.33±7.92	40.67±7.02	41.29±7.57	0.953	0.416
Sex (male/female)	24/28	26/21	29/24	24/24	1.132	0.769
BMI (kg/m2)	22.43 ± 3.11	22.53 ± 3.51	22.65±3.57	23.13 ± 3.78	1.023	0.542
HBeAg (+/-)	25/27	27/20	25/28	25/23	1.293	0.731
ALT (U/L)	57.66±20.57	62.81±32.69	85.69±39.15	82.09±30.68	11.173	<0.001
AST (U/L)	46.59±20.77	52.61±32.10	66.02±39.24	61.38±35.06	3.535	0.016
HBV-DNA (Log copies/mL)	6.48±1.98	5.57±1.43	5.31±1.32	5.25±1.19	6.544	<0.001
ALB (g/L)	42.97±14.71	41.98±13.18	38.9±12.67	35.99±11.87	2.749	0.044
GGT (U/L)	28.49±4.87	41.76±6.28	53.91±6.98	62.76±7.98	39.11	<0.001
PLT (109/L)	179.22±38.94	152.87±33.87	131.77±28.96	102.77±21.91	5.183	0.002
APRI index	1.28±0.43	1.88±0.49	2.65±0.53	3.37±0.87	3.654	0.001
FIB-4 index	0.61±0.41	1.14±0.58	1.32±0.87	1.76±0.98	26.893	<0.001
PIIINP (ng/mL)	17.07±3.95	16.51±4.11	25.05±4.35	34.47±6.94	17.34	<0.001
IV-C (ng/mL)	74.33±5.99	98.07±5.87	135.76±6.94	178.77±7.38	9.678	<0.001
HA (ng/mL)	32.75±10.75	72.86±11.35	117.57±11.95	189.34±13.24	24.96	<0.001
LN (ng/mL)	67.46±20.41	87.66±21.86	107.38±22.14	124.82±23.45	17.13	<0.001

Note: BMI: body mass index; ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALB: albumin, GGT: glutamyl transferase, PLT: platelets, APRI: aspartate aminotransferase-to-platelet ratio index, FIB-4: fibrosis-4.

different liver fibrosis severities, it is shown that levels of ALT, AST, HBV-DNA, ALB, GGT, PLT, APRI index, FIB-4 index, PIIINP, IV-C, HA and LN were significantly different among HBV patients in S0-1, S2, S3 and S4 (p<0.05) (Table I).

Serum MiR-17 Expression Level in HBV Patients with Different Severities of Liver Fibrosis

QRT-PCR data showed that serum level of miR-17 was lower in healthy subjects than that in HBV patients. In addition, the serum level of miR-17 gradually decreased with the deterioration of liver fibrosis. The highest miR-17 level was found in S0-1 HBV patients; while the lowest miR-17 level was detected in S4 HBV patients (Figure 1). All these results showed that serum level of miR-17 in patients with hepatitis B induced liver fibrosis was negatively correlated with liver fibrosis severity.

Independent Risk Factors of Liver Fibrosis in HBV Patients

To illustrate the influence of miR-17 on liver fibrosis, 200 HBV patients were also classified into fibrosis group (S0-1+S2) and advanced fibrosis group (S3+S4). Multivariate Logistic regression analysis revealed that AST, HBV-DNA, ALB, PLT, APRI index, PIIINP, IV-C, HA, LN, and miR-17 were the independent risk factors of advanced liver fibrosis (OR = 1.213, 0.672, 0.788, 0.798, 1.338, 2.751, 2.381, 3.586, 1.954, 0.561, respectively) (p<0.05, Table II). All these results indicated that lowly expressed miR-17 was an unfavorable factor to liver fibrosis in HBV patients.

Predictive Value of Serum MiR-17 in Liver Fibrosis of HBV Patients

ROC curves were depicted for assessing the predictive value of miR-17 in HBV induced liver fibrosis. In assessing S0-1 HBV, AUC was 0.685

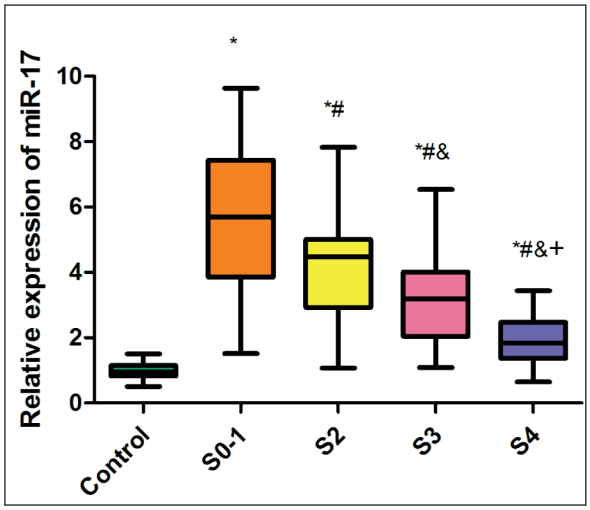

Figure 1. Serum miR-17 levels in healthy subjects and HBV patients with different severities of liver fibrosis (S0-1, S2, S3 and S4). *p<0.05, compared with control, $^{\#}p$<0.05, compared with S0-1, &p<0.05,compared with S2, ^{+}p<0.05,-compared with S3.

Table II. Multivariate logistic regression analysis of independent risk factors of HBV-induced liver fibrosis.

Variable	OR	95% CI	p
ALT	1.781	0.891-1.783	0.378
AST	1.213	1.008-2.672	0.009
HBV-DNA	0.672	0.288-0.808	0.014
ALB	0.788	0.701-0.923	<0.001
GGT	1.472	0.783-1.581	0.451
PLT	0.798	0.571-1.138	0.032
APRI index	1.338	1.093-3.073	0.036
FIB-4 index	1.235	0.984-2.673	0.067
PIIINP (ng/mL)	2.751	1.357-5.935	<0.001
IV-C (ng/mL)	2.381	1.452-3.972	0.006
HA (ng/mL)	3.586	2.431-6.448	0.017
LN (ng/mL)	1.954	1.531-4.034	0.024
MiR-17	0.561	0.481-0.793	0.002

Note: ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALB: albumin, GGT: glutamyl transferase, PLT: platelets, APRI: aspartate aminotransferase-to-platelet ratio index, FIB-4: fibrosis-4.

(95% CI=0.5813-0.7894, p=0.0015, Figure 2A). In addition, miR-17 was able to distinguish S2 HBV from healthy subjects (AUC=0.6716, 95% CI=0.5622-0.781, p=0.032, Figure 2B). Furthermore, miR-17 showed a better efficacy in predicting S3 (AUC=0.7932, 95% CI=0.7069-0.8796, p<0.001, Figure 2C) and S4 HBV (AUC=0.9169, 95% CI=0.8553-0.9784, p<0.001, Figure 2D). All these results showed that miR-17 could have a predictive value for patients with different degrees of HBV induced liver fibrosis, especially for advanced fibrosis group.

Discussion

At present, TBil, plasma prothrombin activity, and clinically used indicators (ALT, AST and GGT) contribute to reflect liver function defect. They are used to diagnose hepatitis B virus related acute-on-chronic liver failure (HBV-ACLF)[13]. In addition, detecting liver fibrosis markers in the serum is conductive to reflect the severity of liver inflammation and liver function. So, HA is an important component of connective tissues, which is a high molecular polysaccharide. During the chronic inflammation of the liver, the synthesis of HA in the liver stromal cells is accelerated. At the same time, due to the reduced ability of the liver to degrade HA, its serum level abnormally increases. PIIINP is a type III collagen precursor. During the formation of collagen fibers in the basement membrane, extracellular type III procollagen cleaves and forms PIIINP. IV-C is the main component of the basement membrane. Once the liver tissue and basement membrane are damaged, IV-C release is stimulated. LN is a non-collagenous structural glycoprotein, which is synthesized by endothelial cells and epithelial cells. Liver fibrosis triggers the deposition of LN in the liver sinus and the release into the blood[14-17]. Liver function is sensitive to the inflammation in the liver, manifesting as abnormally activated ALT immediately after liver inflammation, and AST abnormality following the aggravation of liver inflammation. Meanwhile, the production of HA and IV-C is a sensitive symbol for liver tissue damage caused by inflammation. With the deterioration of inflammatory response in the liver, liver fibrosis occurs and relevant markers are significantly upregulated. Consistently, our findings detected that serum levels of AST, HBV-DNA, ALB, PLT, APRI, and fibrosis markers (PIIINP, IV-C, HA, LN) were remarkably enhanced in patients with HBV induced liver fibrosis, and they were considered as risk factors for liver fibrosis. Serum miRNAs are highly specific and stable, and can be used as tumor hallmarks. Mitchell et al[13] reported that mature miRNAs are resistant to freeze thawing, RNase, and DNase. They proposed that serum level of miRNA-141 is able to distinguish prostate cancer patients from healthy subjects. Apoptotic and necrotic liver cells can release miRNAs synthesized in cells into the blood circulation system, showing a good stability[18]. Zhang et al[19] pointed out that several miRNAs are highly specific in serum of chronic HBV patients, which can accurately reflect types and severities of liver diseases. Through microarray analysis of 135 HBV-ACLF patients,

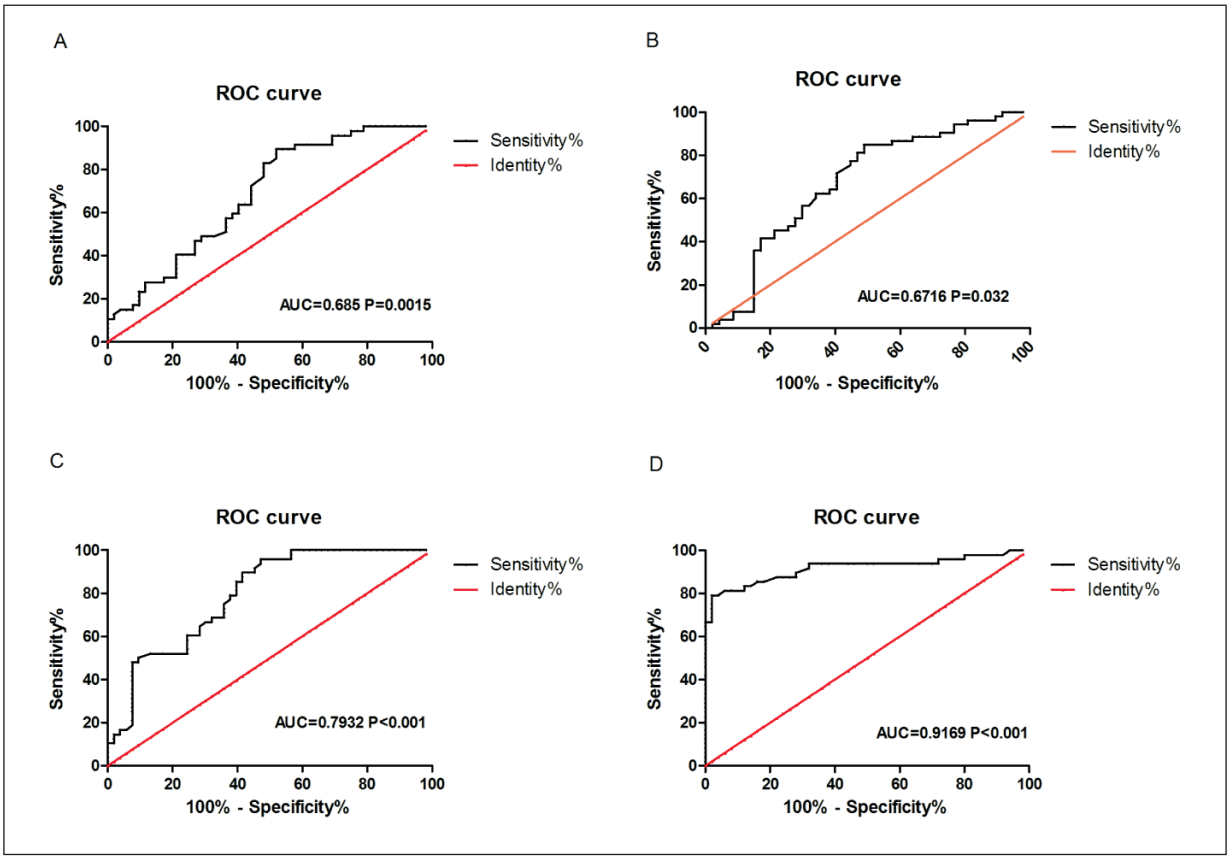

Figure 2. Predictive value of serum miR-17 in liver fibrosis of HBV patients. **A**, Predictive value of serum miR-17 in HBV induced liver fibrosis S0-1 (AUC=0.685, 95% CI=0.5813-0.7894, p=0.0015). **B**, Predictive value of serum miR-17 in HBV induced liver fibrosis S2 (AUC=0.6716, 95% CI=0.5622-0.781, p=0.032). **C**, Predictive value of serum miR-17 in HBV induced liver fibrosis S3 (AUC=0.7932, 95% CI=0.7069-0.8796, p<0.001). **D**, Predictive value of serum miR-17 in HBV induced liver fibrosis S4 (AUC=0.9169, 95% CI=0.8553-0.9784, p<0.001).

Ji et al[20] identified that miR-122 is upregulated in serum of HBV-ACLF patients, and it is capable of suppressing virus replication in Huh7 and HepG2 cells. Zhang et al[21] have shown that miR-122 is differentially expressed in serum of patients with viral or alcoholic liver diseases. Chen et al[22] conducted a clinical trial involving 107 chronic HBV patients and 76 HBV-ACLF patients. They demonstrated that miR-197 level in peripheral blood mononuclear cells of these patients is downregulated with the disease aggravation. In this paper, we found that miR-17 was upregulated in serum of HBV patients. With the deterioration of liver fibrosis, miR-17 was gradually downregulated accordingly.

Liver fibrosis is a pathological change caused by inflammatory reactions, persistent viral infections, alcoholicity, drug toxicity, or genetic factors[23]. Excessive deposition of ECM eventually results in liver fibrosis, and it will deteriorate into cirrhosis if active treatment is lacked. Serum miRNAs are closely related to liver fibrosis following HBV infection[24]. MiR-138 and miR-143 are upregulated in circulatory blood system following liver fibrosis induced by chronic HBV infection. MiR-138 is considered as an early stage fibrosis hallmark, while miR-138 combined miR-143 serve as hallmarks of advanced fibrosis[25]. MiR-33a is downregulated in the serum of patients with chronic HBV infection combined liver fibrosis, and its level is closely linked to fibrosis severity[26]. Trebicka et al[27] demonstrated that circulatory level of miR-122 is negatively correlated to fibrosis degree, which is co-mediated by miR-122 expressed in the serum and liver tissues. In this study, we clarified that miR-17 was the independent risk factor of HBV induced liver fibrosis. In addition, we further confirmed that differentially expressed miR-17 could determine fibrosis severity in HBV patients, especially advanced fibrosis. Lowly expressed miR-

17 indicated fibrosis development. The novelty of this study was that our findings could provide a novel hallmark in diagnosis of HBV-induced liver fibrosis. Nevertheless, Fibroscan test is not conducted in recruited patients, which requires for the further exploration.

Conclusions

In summary, serum level of miR-17 is highly expressed in HBV patients, and negatively correlated with liver fibrosis severity, which could be utilized as a non-invasive hallmark assessing liver fibrosis severity in HBV patients.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Liaw YF, Chu CM. Hepatitis B virus infection. Lancet 2009; 373: 582-592.
2) Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin AA, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CR, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De Leon FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. Lancet 2012; 380: 2095-2128.
3) Liu B, Yang XF, Liang XP, Wang L, Shao MM, Han WX, Wu YH. Expressions of miR-132 in patients with chronic hepatitis B, posthepatitic cirrhosis and hepatitis B virus-related hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2018; 22: 8431-8437.
4) Finch ML, Marquardt JU, Yeoh GC, Callus BA. Regulation of microRNAs and their role in liver development, regeneration and disease. Int J Biochem Cell Biol 2014; 54: 288-303.
5) Tagawa H, Karube K, Tsuzuki S, Ohshima K, Seto M. Synergistic action of the microRNA-17 polycistron and Myc in aggressive cancer development. Cancer Sci 2007; 98: 1482-1490.
6) Wang Y, Li J, Dai L, Zheng J, Yi Z, Chen L. MiR-17-5p may serve as a novel predictor for breast cancer recurrence. Cancer Biomark 2018; 22: 721-726.
7) Gruszka R, Zakrzewska M. The oncogenic relevance of miR-17-92 cluster and its paralogous miR-106b-25 and miR-106a-363 clusters in brain tumors. Int J Mol Sci 2018; 19:
8) Giray BG, Emekdas G, Tezcan S, Ulger M, Serin MS, Sezgin O, Altintas E, Tiftik EN. Profiles of serum microRNAs; miR-125b-5p and miR223-3p serve as novel biomarkers for HBV-positive hepatocellular carcinoma. Mol Biol Rep 2014; 41: 4513-4519.
9) Elshafei A, Shaker O, Abd EO, Salman T. The expression profiling of serum miR-92a, miR-375, and miR-760 in colorectal cancer: an Egyptian study. Tumour Biol 2017; 39: 1393383899.
10) Gong R, Lv X, Liu F. MiRNA-17 encoded by the miR-17-92 cluster increases the potential for steatosis in hepatoma cells by targeting CYP7A1. Cell Mol Biol Lett 2018; 23: 16.
11) Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S SM, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006; 43: 1317-1325.
12) Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003; 38: 518-526.
13) Mitchell AJ, Gray WD, Hayek SS, Ko YA, Thomas S, Rooney K, Awad M, Roback JD, Quyyumi A, Searles

CD. Platelets confound the measurement of extracellular miRNA in archived plasma. Sci Rep 2016; 6: 32651.

14) Roehlen N, Crouchet E, Baumert TF. Liver fibrosis: mechanistic concepts and therapeutic perspectives. Cells-Basel 2020; 9: E875

15) Dong S, Chen QL, Su SB. Curative effects of fuzheng huayu on liver fibrosis and cirrhosis: a meta-analysis. Evid Based Complement Alternat Med 2015; 2015: 125659.

16) Cao X, Shang QH, Chi XL, Zhang W, Xiao HM, Sun MM, Chen G, An Y, Lv CL, Wang L, Nan YM, Chen CY, Tan ZN, Liu XE, Zhuang H. Serum N-glycan markers for diagnosing liver fibrosis induced by hepatitis B virus. World J Gastroenterol 2020; 26: 1067-1079.

17) Wen Y, Peng SF, Fu L, Fu XY, Wu DX, Liu BJ, Tan DM, Ouyang Y. Serum levels of miRNA in patients with hepatitis B virus-associated acute-on-chronic liver failure. Hepatobiliary Pancreat Dis Int 2018; 17: 126-132.

18) Jin Z, Jia B, Tan L, Liu Y. MiR-330-3p suppresses liver cancer cell migration by targeting MAP2K1. Oncol Lett 2019; 18: 314-320.

19) Zhang H, Li QY, Guo ZZ, Guan Y, Du J, Lu YY, Hu YY, Liu P, Huang S, Su SB. Serum levels of microRNAs can specifically predict liver injury of chronic hepatitis B. World J Gastroenterol 2012; 18: 5188-5196.

20) Ji F, Yang B, Peng X, Ding H, You H, Tien P. Circulating microRNAs in hepatitis B virus-infected patients. J Viral Hepat 2011; 18: e242-e251.

21) Zhang Y, Jia Y, Zheng R, Guo Y, Wang Y, Guo H, Fei M, Sun S. Plasma microRNA-122 as a biomarker for viral-, alcohol-, and chemical-related hepatic diseases. Clin Chem 2010; 56: 1830-1838.

22) Chen L, Li C, Peng Z, Zhao J, Gong G, Tan D. MiR-197 expression in peripheral blood mononuclear cells from hepatitis B virus-infected patients. Gut Liver 2013; 7: 335-342.

23) Miao CG, Yang YY, He X, Huang C, Huang Y, Zhang L, Lv XW, Jin Y, Li J. Wnt signaling in liver fibrosis: progress, challenges and potential directions. Biochimie 2013; 95: 2326-2335.

24) Zhang Q, Xu M, Qu Y, Li Z, Zhang Q, Cai X, Lu L. Analysis of the differential expression of circulating microRNAs during the progression of hepatic fibrosis in patients with chronic hepatitis B virus infection. Mol Med Rep 2015; 12: 5647-5654.

25) El-Ahwany E, Nagy F, Zoheiry M, Shemis M, Nosseir M, Taleb HA, El GM, Atta R, Zada S. Circulating miRNAs as predictor markers for activation of hepatic stellate cells and progression of HCV-induced liver fibrosis. Electron Physician 2016; 8: 1804-1810.

26) Huang CF, Sun CC, Zhao F, Zhang YD, Li DJ. MiR-33a levels in hepatic and serum after chronic HBV-induced fibrosis. J Gastroenterol 2015; 50: 480-490.

27) Trebicka J, Anadol E, Elfimova N, Strack I, Roggendorf M, Viazov S, Wedemeyer I, Drebber U, Rockstroh J, Sauerbruch T, Dienes HP, Odenthal M. Hepatic and serum levels of miR-122 after chronic HCV-induced fibrosis. J Hepatol 2013; 58: 234-239.