HIF-1 α associated logistic regression model serves for predicting decompensation of hepatitis B cirrhosis

Jun-Hui Chen

The Third Affiliated Hospital of Sun Yat-sen University

Yu-Rong Gu

The Third Affiliated Hospital of Sun Yat-sen University

Zhe-Bin Wu

The Third Affiliated Hospital of Sun Yat-sen University

Yan-Yan Zhang

The Third Affiliated Hospital of Sun Yat-sen University

Shao-Zhuo Huang

The Third Affiliated Hospital of Sun Yat-sen University

Shui-Xian Yang

The Third Affiliated Hospital of Sun Yat-sen University

Lin-Dan Xie

The Third Affiliated Hospital of Sun Yat-sen University

Yi Wu

The Third Affiliated Hospital of Sun Yat-sen University

Xiang-Fu Liu

The Third Affiliated Hospital of Sun Yat-sen University

Xiu-Ting He

Guangdong Industry Technical College

Yu-Bao Zheng (✉ zhybao@mail.sysu.edu.cn)

The Third Affiliated Hospital of Sun Yat-sen University

Research Article

Keywords: HIF-1 α , hepatitis B cirrhosis, decompensation, nomogram

Posted Date: November 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1000616/v1>

Abstract

Background

HIF-1 α is relevant to inflammation and fibrosis in hepatitis B virus (HBV)-related liver diseases. Thus, we designed a predictive model for decompensated cirrhosis.

Methods

Peripheral plasma HIF-1 α levels were measured in 52 subjects, including 20 patients with HBV-related-compensated-cirrhosis (HBV-CC), 20 patients with HBV-related-decompensated-cirrhosis (HBV-DC) that underwent transjugular intrahepatic portosystemic shunt (TIPS), and 12 healthy controls (HC). Portal plasma HIF-1 α levels were detected in HBV-DC patients. The correlation between clinical data and HIF-1 α levels was assessed, logistic regression and nomogram were used to develop prediction model.

Results

Plasma HIF-1 α levels were significantly higher in HBV-DC patients than that in HBV-CC patients and healthy controls (DC: 656.34 ± 417.96 , CC: 294.23 ± 138.03 , HC: 194.63 ± 54.14 , pg/ml; $P = 0.0004$). Plasma HIF-1 α levels were positively correlated with total bile acid, total bilirubin, APRI, FIB-4, and MELD scores, and negatively correlated with albumin and platelets. Multivariate logistic regression manifested that total bilirubin (OR = 19.439; 95% CI: 1.486–254.320, $P = 0.024$), spleen thickness (OR = 75.144; 95% CI: 4.157–1358.440, $P = 0.003$) and HIF-1 α concentrations above 341.78 pg/ml (OR = 23.580; 95% CI: 1.842–301.781, $P = 0.015$) were markedly associated with HBV-DC and thus included in the nomogram. The terrific cut-off value for the probability of HBV-DC was > 45%, and area under the curve was 0.954 ($P < 0.001$), with 95% sensitivity and specificity.

Conclusions

HIF-1 α is related to biochemical liver parameters, cirrhosis grade, and progression to HBV-DC. Our model has preferable predictive value for HBV-DC.

Background

Hepatitis B virus (HBV) infection is a widespread chronic infection causing major public health problems worldwide. As many as 40% of men and 15% of women with perinatal HBV infection will reportedly die from liver cirrhosis or hepatocellular carcinoma [1–3]. It is widely acknowledged that 90% of patients with liver cirrhosis progress to the decompensated stage with concomitant portal hypertension (PHT), which is the common cause of death in this patient population [4]. PHT is caused by the obstruction of portal blood flow and/or increased circulating blood flow, which leads to increased portal pressure and

complications, including gastroesophageal varices, ascites, spontaneous bacterial peritonitis (SBP), and Hepatorenal syndrome (HRS) [5]. The pathogenesis of decompensation in liver cirrhosis is intricate, and patients with early stages of cirrhosis are largely asymptomatic [6]. Currently, clinical detection of hepatic venous pressure gradient (HVPG) is the recognized standard for determining portal vein pressure [7], having an essential value in staging cirrhosis and predicting the occurrence of decompensated complications. Nonetheless, this invasive method for the dynamic monitoring of portal vein pressure has many limitations. Although fiberendoscopy, color ultrasound, liver stiffness measurement (LSM), computed tomography (CT), and magnetic resonance elastography (MRE) have certain application values for non-invasive diagnosis of liver cirrhosis and assessment of portal hypertension, their accuracy, specificity, and sensitivity are largely influenced by the experience of the examiner. In addition, according to the incidence of complications such as ascites and upper gastrointestinal bleeding, the diagnosis of decompensated liver cirrhosis can be made clinically; however, at this stage, these patients have already progressed to advanced decompensated cirrhosis associated with increased mortality and poor prognosis [8]. Accordingly, early identification and dynamic monitoring of high-risk factors are essential for screening patients at risk of decompensated cirrhosis and providing optimal treatment.

Hypoxia-inducible factor (HIF) is a nuclear transcription factor that plays an active role in hypoxia, adjusts the expression of many functional genes at the transcriptional level, and participates in the maintenance of oxygen balance in cell tissues and the body. It is a heterodimer composed of α subunit (HIF-1 α , HIF-2 α , or HIF-3 α) and HIF-1 β subunit. The active HIF-1 α subunit degrades rapidly under normal oxygen conditions and remains stable under hypoxic conditions, playing a critical role in regulating oxygen balance in the microenvironment [9–11]. Interestingly, the level of HIF-1 α and HIF-2 α has been documented to be upregulated in chronic hepatitis B, liver cirrhosis, and liver cancer [12–14], which may be attributed to the significant increase in oxygen consumption of liver cells, infiltration of inflammatory leukocytes, and tissue hypoxia caused by an imbalance in metabolic demand and supply [15]. Vascular dysfunction, thrombosis, or fibrosis can also lead to a reduced oxygen supply. Our previous researches found that HIF-1 α was observably related to inflammation and fibrosis in HBV-associated liver disease and exhibited good diagnostic value in differentiating the compensatory and decompensated stage of cirrhosis [16]. Therefore, plasma HIF-1 α levels and clinical parameters were measured in this study, and a scoring model for predicting decompensation of hepatitis B cirrhosis was established based on the identified independent risk factors.

Methods

Participants

A total of 40 patients diagnosed with HBV-CC and HBV-DC between August 2020 and June 2021 were enrolled. The criteria for selecting research subjects: (1) Chinese citizens of 18–65 years of age; (2) HBsAg positive or negative, anti-HBcAb-positive, and a clear history of chronic HBV infection (HBsAg positive of > 6 months); (3) ultrasound, CT, or other imaging or liver pathology showing signs of cirrhosis; (4) decreased albumin levels (< 35 g/L) and/or INR > 1.3, or prolonged PT (stopping thrombolytic or

anticoagulant drugs for more than seven days, longer than the reference > 3s) and/or platelet count < $100 \times 10^9/L$, and other causes were excluded; (5) hepatitis B cirrhosis was divided into compensated and decompensated stages according to the occurrence of ascites, esophageal and gastric variceal bleeding, hepatic encephalopathy, and other serious complications and/or liver dysfunction, based on the diagnostic criteria in the Chinese guidelines for the management of liver cirrhosis [17] and guidelines for the prevention and treatment of chronic hepatitis B [18]. The criteria for excluding study subjects: (1) patients with liver cancer or combined with other tumors; (2) patients with liver disease complicated by other etiologies (drug liver disease, alcoholic liver disease, and autoimmune liver disease) or superinfection with other hepatitis viruses; (3) patients with severe respiratory, digestive, circulatory, and nervous system diseases; (4) patients with diabetes and thyroid diseases; (5) patients with serious mental and psychological diseases. Twelve healthy subjects with negative serological markers for viral hepatitis and normal liver function were enrolled in the control group during the same period.

Experimental operation

(1) The liver biochemical profile was detected using an Olympus automatic biochemical analyzer (Olympus AU640, Tokyo, Japan). Coagulation assays were performed using a Sta-Compact automatic analyzer (STAGO, France). (2) The human HIF-1 α enzyme-linked immunosorbent assay (ELISA) kit, provided by Wuhan Huamei Biological Engineering Company. (3) Preoperative and postoperative HVPg values were recorded during the TIPS.

Binary logistic regression model

Sixteen risk factors that may affect decompensation of cirrhosis were screened, including sex, age, aspartate transaminase (AST), alanine aminotransferase (ALT), total protein (TP), albumin (ALB), total bilirubin (TB), direct bilirubin (DB), total bile acid (TBA), red blood cell (RBC), hemoglobin (HGB), platelets (PLT), prothrombin time (PT), spleen thickness (ST), portal vein main diameter (PVD), and peripheral plasma HIF-1 α concentration. With the occurrence of cirrhosis decompensation as the dependent variable (0= no cirrhosis decompensation, 1= cirrhosis decompensation), the 16 factors included in the univariate analysis were considered as independent variables. If the continuous variable had a linear relationship with the result, the continuous variable was included in the regression formula; otherwise, the continuous variable was converted into a dichotomous or sequential variable [19]. Continuous independent variables were grouped and assigned to the corresponding order (Supplementary Table 1). Binary logistic regression was used to analyze and identify the risk factors that significantly affected decompensation of cirrhosis. Finally, a scoring model using a nomogram was established to predict the occurrence of cirrhosis decompensation.

Statistical analyses

SPSS 21 and GraphPad 8.0, were used for the statistical analyses. The research data conforming to a normal distribution were showed as mean $\pm$ standard deviation, and an independent sample *t*-test or analysis of variance was used. Data not normally distributed were described by median and range, and a rank-sum test was used. The relationship between continuous variables was examined using the Pearson

correlation analysis. Risk factors associated with HBV-DC using binary logistic regression, the odds ratio (OR), and 95% confidence interval (CI) of each factor for the risk of HBV-DC were calculated using multivariate analyses. The WALD stepwise screening method was used for binary logistic regression analysis of independent factors influencing cirrhosis decompensation [20]. R software (version 4.0.2) was used to construct the nomogram. Received operating characteristic (ROC) curves were compared using MedCalc (version 20.009) module ROC. Statistical significance was set at $P < 0.05$.

Results

Baseline Clinical data of enrolled subjects

This research included 12 cases of HC, 20 cases of HBV-CC, and 20 cases of HBV-DC; all patients with HBV-DC presented with symptoms and signs of portal hypertension. No significant difference in sex between the HC, CC, and DC groups ($P = 0.055$), while the mean age of the CC group is markedly lower than that of the DC group ($P = 0.007$). Liver biochemical parameters, including TB, DB, and TBA, are significantly higher in the DC group than in the CC group ($P < 0.001$). Moreover, the liver fibrosis index, model for end-stage liver disease (MELD) score, and portal vein main diameter are significantly higher in the DC group than in the CC group ($P < 0.001$, $P = 0.010$, $P = 0.049$, respectively) (Table 1).

Table 1
Baseline clinical data of subjects

Variables	HC group (n=12)	HBV-CC group (n=20)	HBV-DC group (n=20)	T/F	<i>P</i> vaule
Male/Female	5/7	17/3	14/6	3.252	0.055
Age,yr	43.92±9.89	47.70±9.05	54.35±8.66	5.486	0.007
ALT,U/L	-	31.60±11.82	25.80±7.47	1.855	0.071
AST,U/L	-	28.00±6.51	33.05±7.71	-2.238	0.031
TP,g/L	-	73.85±3.86	60.53±7.66	6.942	0.001
ALB,g/L	-	45.23±2.16	34.23±3.47	12.033	0.001
TB,umol/L	-	11.07±4.66	22.90±9.32	-5.076	0.001
DB,umol/L	-	2.84±1.13	9.74±5.62	-5.382	0.001
TBA,umol/L	-	9.18±5.32	48.59±18.99	-8.933	0.001
CysC,mg/L	-	1.03±0.22	1.23±0.34	-2.177	0.036
LY,%	-	0.32±0.07	0.20±0.14	3.25	0.003
RBC,10 ¹² /L	-	5.02±0.68	3.09±0.53	10.045	0.001
HGB,g/L	-	151.10±14.48	90.65±23.84	9.693	0.001
Hct,%	-	0.45±0.04	0.27±0.05	11.63	0.001
PLT,10 ⁹ /L	-	148.35±50.79	84.70±38.74	4.456	0.001
PT,sec	-	14.04±1.04	16.42±1.99	-4.732	0.001
INR	-	1.21±0.08	1.31±0.21	-2.132	0.043
ST,mm	-	36.35±5.05	57.35±12.88	-6.788	0.001
PVD,mm	-	11.81±1.30	13.02±2.31	-2.05	0.049
MELD score	-	8.90±0.97	10.70±2.71	-2.791	0.010

Note: Values are presented as mean±SD. Abbreviations: AST: Aspartate transaminase; ALT: Alanine aminotransferase; TP: Total protein; ALB: Albumin; TB: Total bilirubin; DB: Direct bilirubin; TBA: Total bile acid; CysC: Cystatin C; LY: Lymphocyte percentage; RBC: Red blood cell; HGB: Hemoglobin; Hct: hematocrit; PLT: Platelets; PT: Prothrombin time; INR: International normalized ratio; ST: Spleen thickness; PVD: Portal vein diameter; MELD:Model for end-stage liver disease; APRI: Aspartate aminotransferase-to-Platelet Ratio Index ; FIB-4: Fibrosis index based on the 4 factors; HIF-1α: Hypoxia inducible factor 1α; HVP: Hepatic venous pressure gradient.

Gender, Age, and Peripheral HIF-1α were analyzed by variance analysis while the remaining parameters were analyzed by *t*-test.

Variables	HC group (n=12)	HBV-CC group (n=20)	HBV-DC group (n=20)	T/F	<i>P</i> vaule
APRI	-	0.53±0.21	1.17±0.55	-4.913	0.001
FIB-4 score	-	1.92±0.96	5.16±2.71	-5.04	0.001
Peripheral HIF-1α,pg/ml	194.63±54.14	294.23±138.03	656.34±417.96	14.728	0.001
Portal HIF-1α,pg/ml	-	-	623.45±404.05	-	-
Preoperative HVPg,cm/H ₂ O	-	-	34.15±11.30	-	-
Postoperative HVPg,cm/H ₂ O	-	-	17.90±5.84	-	-
Decompression effect,cm/H ₂ O	-	-	16.25±8.72	-	-
Ascites,low/medium/large	-	0/0/0	6/4/7	-	-
Ascites/Gastrointestinal bleeding	-	0/0	17/8	-	-
<p>Note: Values are presented as mean±SD. Abbreviations: AST: Aspartate transaminase; ALT: Alanine aminotransferase; TP: Total protein; ALB: Albumin; TB: Total bilirubin; DB: Direct bilirubin; TBA: Total bile acid; CysC: Cystatin C; LY: Lymphocyte percentage; RBC: Red blood cell; HGB: Hemoglobin; Hct: hematocrit; PLT: Platelets; PT: Prothrombin time; INR: International normalized ratio; ST: Spleen thickness; PVD: Portal vein diameter; MELD:Model for end-stage liver disease; APRI: Aspartate aminotransferase-to-Platelet Ratio Index ; FIB-4: Fibrosis index based on the 4 factors; HIF-1α: Hypoxia inducible factor 1α; HVPg: Hepatic venous pressure gradient.</p>					
<p>Gender, Age, and Peripheral HIF-1α were analyzed by variance analysis while the remaining parameters were analyzed by <i>t</i>-test.</p>					

HIF-1α expression in hepatitis B cirrhosis patients and healthy controls

The mean plasma HIF-1α level is significantly higher in patients with hepatitis B cirrhosis (CC+DC group) than in healthy controls ($t = 2.690$, $P = 0.0097$) (Fig. 1A), while HIF-1α expression in the peripheral plasma of the DC group is significantly higher than that in the other groups ($P < 0.001$) (Fig. 1B). Even though HIF-1α levels in the peripheral plasma of the HBV-DC group are higher than those in the portal blood, the difference is not statistically significant ($t = 0.5041$, $P = 0.62$) (Fig. 1C). According to the Child-Pugh scoring criteria [17], patients in the compensated and decompensated stages of cirrhosis are divided into class A ($n = 23$), B ($n = 12$), and C ($n = 5$). HIF-1α levels in class A patients are markedly lower than those in class B and C patients ($P < 0.0001$) (Fig. 2A). Peripheral plasma HIF-1α levels are significantly higher in cirrhotic patients with ascites than in those without ascites ($t = 4.178$, $P = 0.0002$). However, there is no significant difference in peripheral plasma HIF-1α levels between patients with and without GI bleeding ($t = 0.0394$, $P = 0.9688$). Plasma HIF-1α levels in patients with both serious complications are significantly

higher than those in patients without complications ($t = 3.409$, $P = 0.0023$) (Fig. 2B). Interestingly, in the HBV-DC group, peripheral plasma HIF-1 α levels are negatively correlated with preoperative HVPg ($r = -0.2005$, $P = 0.3968$) (Supplementary Fig. 1A). The expression level of HIF-1 α in the peripheral blood of patients with hepatitis B cirrhosis is positively correlated with age ($r = 0.446$, $P = 0.0039$) (Supplementary Fig. 1B). No significant difference in HIF-1 α expression is found between the sexes in patients with hepatitis B cirrhosis ($P = 0.4872$) (Supplementary Fig. 3A). Furthermore, peripheral plasma HIF-1 α levels in the healthy control group are not markedly correlated with age ($r = -0.333$, $P = 0.290$) (Supplementary Fig. 1C), and gender stratification also shows no difference ($P = 0.5932$) (Supplementary Fig. 3B).

Correlation of HIF-1 α expression with liver biochemical metabolism and fibrosis parameters

Peripheral plasma HIF-1 α levels in patients with hepatitis B cirrhosis are positively correlated with TBA, TB, and DB ($r = 0.4059$, $P = 0.0094$; $r = 0.4520$, $P = 0.0034$; $r = 0.4517$, $P = 0.0034$, respectively) (Supplementary Fig. 1D,E,F), but not with AST and ALT ($r = 0.167$, $P = 0.304$; $r = -0.139$, $P = 0.394$) (Supplementary Fig. 1G,H). Peripheral plasma HIF-1 α levels in patients with elevated AST levels (> 40 U/L) are slightly higher than those with normal AST levels ($t = 1.784$, $P = 0.083$) (Supplementary Fig. 3C). Peripheral plasma HIF-1 α levels in patients with elevated ALT levels are slightly lower than those with normal ALT levels ($t = 0.832$, $P = 0.411$) (Supplementary Fig. 3D). Peripheral plasma HIF-1 α levels are negatively correlated with albumin (ALB) levels ($r = -0.518$, $P = 0.0006$) (Supplementary Fig. 1I). Furthermore, HIF-1 α levels in hepatitis B cirrhosis patients are positively correlated with APRI and FIB-4 scores ($r = 0.472$, $P = 0.0021$; $r = 0.493$, $P = 0.0012$) (Supplementary Fig. 2A,B) and negatively correlated with platelet (PLT) levels ($r = -0.404$, $P = 0.0097$) (Supplementary Fig. 2C).

Correlation between HIF-1 α expression and MELD, Child-Pugh classification, B-ultrasound index and Anemia indicators

Peripheral plasma HIF-1 α levels in patients with hepatitis B cirrhosis are positively correlated with the MELD score ($r = 0.335$, $P = 0.0346$) (Supplementary Fig. 2D). There is no correlation between plasma HIF-1 α levels and spleen thickness or portal vein diameter ($r = 0.165$, $P = 0.310$; $r = 0.035$, $P = 0.830$, respectively) (Supplementary Fig. 2E,F). Furthermore, peripheral plasma HIF-1 α levels are negatively correlated with RBC count ($r = -0.382$, $P = 0.015$) and hematocrit ($r = -0.341$, $P = 0.0312$) in patients with hepatitis B cirrhosis (Supplementary Fig. 2G,H). There is no correlation between HIF-1 α levels and hemoglobin concentration ($r = -0.280$, $P = 0.080$) (Supplementary Fig. 2I).

The above findings suggest that the peripheral plasma HIF-1 α levels of hepatitis B cirrhosis patients are closely related to the biochemical liver parameters, bile acid metabolism, cirrhosis grade, and progression of decompensated cirrhosis (Fig. 3A,B).

Risk factors for HBV-related decompensated cirrhosis

Univariate and multivariate logistic regression were performed to determine the significant risk factors for HBV-DC. Univariate logistic regression show that TB, PLT, PT, spleen thickness, and plasma HIF-1 α concentration are significant risk factors ($P < 0.05$) (Table 2). According to the multivariate logistic regression, elevated TB (OR, 19.439; 95% CI, 1.486–254.320; $P = 0.024$), elevated spleen thickness (OR, 75.144; 95% CI, 4.157–1358.440; $P = 0.003$), and HIF-1 α levels exceeding 341.78 pg/ml (OR, 23.580; 95% CI, 1.842–301.781; $P = 0.015$) were independent risk factors, logit (P) = $-5.411 + 2.967 \times (\text{total bilirubin}) + 4.319 \times (\text{spleen thickness}) + 3.160 \times (\text{HIF-1}\alpha)$. However, our small sample size did not meet the requirements of the event per variable (EPV); accordingly, the findings of our study were not robust enough. However, to our knowledge, few studies have successfully designed models to predict decompensation in HBV-induced cirrhosis.

Table 2

Univariate and multivariate logistic regression analysis associated with decompensation of hepatitis B cirrhosis

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age, yr,>50 vs ≤ 50	2.250 (0.635-7.973)	0.209	-	-
Gender, male vs female	0.412 (0.087-1.952)	0.264	-	-
ALT,U/L,>40 vs ≤40	0.211 (0.021-2.079)	0.182	-	-
AST,U/L,>40 vs ≤40	2019343580 (0.000-)	0.999	-	-
TP,g/L,<60 vs ≥60	2937227029 (0.000-)	0.999	-	-
ALB,g/L,<35(Adults under 60) or <34 (Adults over 60) vs 35-50 (Adults under 60) or 34-48 (Adults over 60)	3230949732 (0.000-)	0.999	-	-
TB,umol/L,>17.1 vs 1.7-17.1	6.000 (1.458-24.686)	0.013	19.439 (1.486-254.320)	0.024
DB,umol/L				
<1.71 vs 1.71-7.0	0.000 (0.000-)	0.999	-	-
>7.0 vs 1.71-7.0	3432884087 (0.000-)	0.998	-	-
TBA,umol/L,>10 vs ≤10	0.000 (0.000-)	0.999	-	-
RBC,10 ¹² /L				
<4.0 (Male) or <3.5(Female) vs 4.0-5.5(Male) or 3.5-5.0 (Female)	12116061494 (0.000-)	0.998	-	-
HGB,g/L,<130(Male) or <120 (Female) vs 130-185(Male) or 120-165 (Female)	16154748645 (0.000-)	0.998	-	-

AST: Aspartate transaminase, (0~40U/L); ALT: Alanine aminotransferase, (0~40U/L); TP: Total protein, (60~80g/L); ALB: Albumin, [<60 (35~50g/L), ≥60 (34~48g/L)]; TB: Total bilirubin, (1.71~17.1umol/L); DB:Direct bilirubin, (1.71~7umol/L); TBA:Total bile acid, (0~10umol/L); RBC:Red blood cell, [Male (4.0~5.0*10¹²/L), Female (3.5~5.0*10¹²/L)]; HGB: Hemoglobin, [Male (130~185g/L), Female (120~165g/L)]; PLT:Platelets, (100~300*10⁹/L); PT: Prothrombin time, (11.0~14.0s); ST: Spleen thickness, (3~4cm); PVD: Portal vein diameter, (0.6~1.0cm); Peripheral HIF-1α median: 341.78pg/ml.

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
PLT,10 ⁹ /L,<100 vs 100-300	16.000 (3.398-75.345)	0.001	-	-
PT,sec,>14.0 vs 11.0-14.0	12.667 (1.402-114.419)	0.024	-	-
ST,cm,>4.0 vs 3.0-4.0	27.000 (4.566-159.663)	0.001	75.144 (4.157-1358.440)	0.003
PVD,cm,>1.0 vs 0.6-1.0	0.706 (0.136-3.658)	0.678	-	-
Peripheral HIF-1 α ,pg/ml,>341.78 vs $\leq$ 341.78	9.000 (2.151-37.659)	0.003	23.580 (1.842-301.781)	0.015
AST: Aspartate transaminase, (0~40U/L); ALT: Alanine aminotransferase, (0~40U/L); TP: Total protein, (60~80g/L); ALB: Albumin, [<60 (35~50g/L), $\geq$ 60 (34~48g/L)]; TB: Total bilirubin, (1.71~17.1umol/L); DB:Direct bilirubin, (1.71~7umol/L); TBA:Total bile acid, (0~10umol/L); RBC:Red blood cell, [Male (4.0~5.0*10 ¹² /L), Female (3.5~5.0*10 ¹² /L)]; HGB: Hemoglobin, [Male (130~185g/L), Female (120~165g/L)]; PLT:Platelets, (100~300*10 ⁹ /L); PT: Prothrombin time, (11.0~14.0s); ST: Spleen thickness, (3~4cm); PVD: Portal vein diameter, (0.6~1.0cm); Peripheral HIF-1 α median: 341.78pg/ml.				

Nomogram development

We validated that HIF-1 α could independently affect the progression to decompensated cirrhosis. Subsequently, these three factors are used to draw a nomogram to construct a scoring model for HBV-DC (Fig. 4).

Application of the scoring model in evaluating HBV-DC

We compared the application value of plasma HIF-1 α level, scoring model, APRI, and FIB-4 score to assess the probability of HBV-DC. ROC curve analysis revealed that the optimal cut-off value for the probability of HBV-DC is > 45%, area under the curve was 0.954 ($P < 0.001$), with 95% sensitivity and specificity. In predicting the progression of HBV-DC, the scoring model achieves better specificity and sensitivity than the plasma HIF-1 α level, APRI, and FIB-4 scores (Fig. 5).

Comparison of ROC curve between scoring model and clinical non-invasive score

No noteworthy difference is observed between the scoring model, APRI, and FIB-4 scores in predicting HBV-DC. However, there is a significant difference between the scoring model and plasma HIF-1 α level alone in predicting decompensated cirrhosis ($P = 0.0356$) (Supplementary Table 2).

Discussion

HIF-1 α has been documented to maintain energy metabolism in myeloid cells under hypoxic conditions, promoting them toward inflammatory tissues [21] and play an anti-inflammatory and regulatory role in innate immunity. However, it is widely acknowledged that fluctuations in transaminase levels are highly predominant in HBV-induced cirrhosis patients who take antiviral and hepatoprotective drugs to lower transaminase levels. HIF-1 α may adjust the expression of many target genes encoding metabolic enzymes and improve cell metabolic adaptation to hypoxia [22], suggesting that HIF-1 α is involved in the metabolic functioning of the liver, which was verified by our statistical biochemical results. In our study, HIF-1 α levels were positively correlated with the APRI, FIB-4, and MELD scores. HIF-1 α can regulate the production of pro-fibrotic mediators to promote the development of liver fibrosis directly [23]. It has been found that hypoxia induces a series of angiogenic factors such as VEGF and PDGF- β to promote angiogenesis in primary human macrophages. PDGF- β can also promote the proliferation of hepatic stellate cells (HSCs), and continue to differentiate into myofibroblasts, which produce large amounts of collagen, leading to fibrosis and cirrhosis [24–26]. When cirrhosis continues to develop, it results in diffuse necrosis and regeneration of liver cells, destruction of the hepatic lobule structure, and formation of pseudolobules, whereas sinusoidal occlusion or perisinusoidal fibrosis causes intrahepatic vascular blockage and blood flow obstruction, consequently increasing portal pressure gradually [27]. In the development of portal hypertension, vasoconstriction causes tissue hypoxia, which leads to the phosphorylation of the NADPH oxidase subunit and promotes the oxidation-reducing coenzyme II (NADPH), which induces HIF-1 α upregulation in tissues [28]. In the presence of HIF-1 α , placental growth factor (PLGF) stimulates the growth and migration of endothelial cells and participates in the generation of pathological blood vessels, while liver sinusoidal endothelial cells (LSECs), HSCs, and Kupffer cells promote the generation and reconstruction of pathological blood vessels through direct or indirect pathways under hypoxic stimulation and injury [29]. In the process of fiber repair, these pathological microvessels, originating from the branches of blood vessels in the liver, bypass block the sinusoidal blood supply area, further exacerbating tissue hypoxia [30]. There were no distinct differences in plasma HIF-1 α levels between Child-Pugh class B and C patients, and preoperative HVPG were negatively correlated with HIF-1 α levels. We speculate that HIF-1 α may be a sensitive biomarker in the early stages of cirrhosis. However, the progression of cirrhosis is accompanied by collateral circulation formation and visceral and systemic vasodilation. High extrahepatic blood circulation may influence the peripheral plasma HIF-1 α levels.

Moreover, HIF-1 α is a transcription factor activated under hypoxia and regulates many genes involved in cell responses to hypoxia and other tissue environmental signals [31]. Therefore, HIF-1 α is inhibited under aerobic conditions, and any increase in HIF-1 α expression is suggestive of hypoxia. Furthermore, liver

cirrhosis patients often suffer from malnutrition, decreased liver synthesis, and hypersplenism, resulting in decreased hematopoiesis and increased hemolysis, thus substantiating our findings.

Regarding the prediction model, Bureau et al. [32] found that low bilirubin levels ($< 50 \mu\text{mol/L}$) and high platelet count ($> 75 \times 10^9/\text{L}$) predicted outcome in refractory ascites patients. In addition, the albumin-bilirubin score (ALBI score) provided a better evaluation of the severity and long-term survival of HBV-induced cirrhosis patients than the MELD and MELD-NA scores [33]. Splenomegaly is a sensitive indicator of portal hypertension with poor specificity. However, combining splenomegaly with non-invasive diagnostic indicators such as liver hardness test and platelet count can be used to detect esophageal varicose veins in patients with hepatitis B cirrhosis [34]. Spleen size can be quantified by measuring the longitudinal, anteroposterior, and transverse diameters; however, few studies have used spleen thickness [35]. Interestingly, we found that spleen thickness was an independent factor that influenced the progression of HBV-DC.

Furthermore, our scoring model exhibited better predictive ability, specificity, and sensitivity than other non-invasive, highly reliable metrics for liver fibrosis [36], including APRI [37] and FIB-4 [38]. Although there was no noteworthy difference during the non-inferiority test, APRI and FIB-4 scores were related to the degree of fibrosis in untreated HBV patients [39]. Indeed, fluctuations in transaminase levels are often observed in patients with cirrhosis who receive clinical treatment. Progression of liver fibrosis can lead to hypersplenism and further thrombocytopenia [40]. However, the APRI and FIB-4 scores are relatively inaccurate metrics. FIB-4 has been reported to have greater accuracy in excluding significant hepatitis fibrosis than in diagnosing severe hepatitis B fibrosis [41]. Therefore, the scoring model constructed in this study can reduce the interference of the clinical use of enzyme-lowering drugs on the prediction of HBV-DC and has better application value.

Conclusions

Plasma HIF-1 α levels were correlated with liver biochemical function, bile acid metabolism, liver cirrhosis stage, and decompensation progression. The logistic regression model constructed from total bilirubin, spleen thickness, and HIF-1 α has a high predictive value for HBV-DC and thus offers significant benefits such as dynamic non-invasive monitoring. Considering the small number of cases included in this single-center retrospective clinical study, prospective, large-sample, multi-center researches are needed to substantiate the application of our scoring model in predicting decompensation in patients with HBV-induced cirrhosis.

Abbreviations

HBV: Hepatitis B Virus; TIPS: Transjugular Intrahepatic Portosystemic Shunt; HVP: Hepatic venous pressure gradient; HIF-1 α : Hypoxia inducible factor 1 α ; AST: Aspartate transaminase; ALT: Alanine aminotransferase; TP: Total protein; ALB: Albumin; TB: Total bilirubin; DB: Direct bilirubin; TBA: Total bile acid; CysC: Cystatin C; LY: Lymphocyte percentage; RBC: Red blood cell; HGB: Hemoglobin; Hct:

hematocrit; PLT: Platelets; PT: Prothrombin time; INR: International normalized ratio; ST: Spleen thickness; PVD: Portal vein diameter; MELD: Model for end-stage liver disease; APRI: Aspartate aminotransferase-to-Platelet Ratio Index ; FIB-4: Fibrosis index based on the 4 factors.

Declarations

Acknowledgements

The authors thank all volunteers and patients recruited from the Third Affiliated Hospital of Sun Yat-sen University. We would like to thank Editage (www.editage.cn) for English language editing.

Authors' contributions

Chen JH, Gu YR, Wu ZB and Zheng YB conceived and designed the study, and wrote, edited and reviewed the manuscript. Chen JH and Zhang YY completed all experimental operations, Wu Yi, Yang SX and Xie LD collected clinical information of patients, Chen JH and Huang SZ analyzed the data in this paper and presented the results. Liu XF, He XT and Zheng YB provided experimental and methodological guidance and valuable advice. All the authors finally agreed to publish it. Chen JH and Zheng YB are responsible for the entire study results, including but not limited to study design, data acquisition, data analysis, results presentation, review and manuscript submission.

Funding

This study was funded by the Guangdong Basic and Applied Basic Research Foundation (No. 2020A1515010052), Natural Fund of Guangdong Province (No. 2016A030313237), Guangzhou City Science and Technology Project (No. 201607010064), National Science and Technology Major Project (2018ZX10302204), and National Natural Science Foundation of China (81974004).

Availability of data and materials

The data used and analysed in this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Helsinki. The research was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (approval number [2020]02-272-01). All study subjects have signed informed consent.

Consent for publication

Not applicable.

Competing interests

All authors disclose no potential conflicts (financial, professional, or personal) that are relevant to the manuscript.

Author details

¹Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

²Guangdong Provincial Key Laboratory of Liver Disease research, the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou , China.

³Department of Blood transfusion, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China.

⁴Institute of Light industry and chemical technology, Guangdong Industry Technical College, Guangzhou, China.

References

1. Liaw YF, Chu CM. Hepatitis B virus infection. *Lancet*. 2009;373:582–92.
2. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. *Lancet*. 2014;384:2053–2063.
3. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22707 men in Taiwan. *Lancet*. 1981;2:1129–33.
4. de Franchis R, Primignani M. Natural history of portal hypertension in patients with cirrhosis. *Clin Liver Dis*. 2001; 5: 645–663.
5. Bosch J, García-Pagán JC. Complications of cirrhosis. I. Portal hypertension. *J Hepatol*. 2000; 32: 141–156.
6. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet*. 2014;383:1749–61.
7. Bosch J, Abraldes JG, Berzigotti A, Garcia-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol*. 2009;6:573–82.
8. Arvaniti V, D'Amico G, Fede G, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology*. 2010;139:1246-1256,1256.e1-5.
9. Sutherland RM. Tumor hypoxia and gene expression—implications for malignant progression and therapy. *Acta Oncol*. 1998;37:567–74.
10. Zhu H, Bunn HF. Signal transduction. How do cells sense oxygen? *Science*. 2001;292: 449–451.
11. Choudhry H, Harris AL. Advances in Hypoxia-Inducible Factor Biology. *Cell Metab*. 2018;27:281–298.
12. Hu JL, Liu LP, Yang SL, et al. Hepatitis B virus induces hypoxia-inducible factor-2α expression through hepatitis B virus X protein. *Oncol Rep*. 2016;35:1443–8.

13. Corpechot C, Barbu V, Wendum D, et al. Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis. *Hepatology*. 2002;35:1010–21.
14. Li S, Yao D, Wang L, et al. Expression characteristics of hypoxia-inducible factor-1 α and its clinical values in diagnosis and prognosis of hepatocellular carcinoma. *Hepat Mon*. 2011;11:821–8.
15. Wilson GK, Brimacombe CL, Rowe IA, et al. A dual role for hypoxia inducible factor-1 α in the hepatitis C virus lifecycle and hepatoma migration. *J Hepatol*. 2012;56:803–9.
16. Zhang YY, Chen XT, Chen JH, et al. The expression of plasma HIF-1 α and its potential clinical significance in patients with HBV-related liver disease. *J Trop Med*. 2021 Vol.21,No.4.
17. Xu XY, Ding HG, Li WG, et al. Chinese guidelines on the management of liver cirrhosis. *J Clin Hepatol*. 2019;Vol.35,No.11.
18. Chinese Society of Infectious Diseases, Chinese Medical Association, Chinese Society of Hepatology, Chinese Medical Association. Guidelines for the prevention and treatment of chronic hepatitis B (version 2019). *J Clin Hepatol*. 2019;35: 2648–2669.
19. Zhou ZR, Wang WW, Li Y, et al. In-depth mining of clinical data: the construction of clinical prediction model with R. *Ann Transl Med*. 2019;7:796.
20. Zhang W. *Advanced Course of SPSS Statistical Analysis*. Beijing: Higher Education Press; 2004.
21. Cramer T, Yamanishi Y, Clausen BE, et al. HIF-1 α is essential for myeloid cell-mediated inflammation. *Cell*. 2003;12:645–657.
22. Mole DR, Blancher C, Copley RR, et al. Genome-wide association of hypoxia-inducible factor (HIF)-1 α and HIF-2 α DNA binding with expression profiling of hypoxia-inducible transcripts. *Journal of Biological Chemistry*. 2009;284:16767–16775.
23. Moon JO, Welch TP, Gonzalez FJ, Copple BL. Reduced liver fibrosis in hypoxia-inducible factor-1 α -deficient mice. *Am J Physiol Gastrointest Liver Physiol*. 2009; 296: G582-92.
24. White JR, Harris RA, Lee SR, et al. Genetic amplification of the transcriptional response to hypoxia as a novel means of identifying regulators of angiogenesis. *Genomics*. 2004;83:1–8.
25. Friedman SL, Arthur MJ. Activation of cultured rat hepatic lipocytes by Kupffer cell conditioned medium. Direct enhancement of matrix synthesis and stimulation of cell proliferation via induction of platelet-derived growth factor receptors. *J Clin Invest*. 1989;84:1780–5.
26. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepato*. 2017;14:397–411.
27. Kumar M, Sakhuja P, Kumar A, et al. Histological subclassification of cirrhosis based on histological-haemodynamic correlation. *Aliment Pharmacol Ther*. 2008;27:771–779.
28. Brandes RP, Miller FJ, Beer S, et al. The vascular NADPH oxidase subunit p47phox is involved in redox-mediated gene expression. *Free Radic Biol Med*. 2002;32:1116–1122.
29. Li X, Yao QY, Liu HC, et al. Placental growth factor silencing ameliorates liver fibrosis and angiogenesis and inhibits activation of hepatic stellate cells in a murine model of chronic liver

- disease. *J Cell Mol Med*. 2017;21:2370–2385.
30. Rappaport AM, MacPhee PJ, Fisher MM, Phillips MJ. The scarring of the liver acini (cirrhosis): tridimensional and microcirculatory consideration. *Virchows Arch A Pathol Anat Histopathol*. 1983;402:107–37.
 31. Ju C, Colgan SP, Eltzschig HK. Hypoxia-inducible factors as molecular targets for liver diseases. *J Mol Med (Berl)*. 2016;94:613–27.
 32. Bureau C, Métivier S, D'Amico M, et al. Serum bilirubin and platelet count: a simple predictive model for survival in patients with refractory ascites treated by TIPS. *J Hepatol*. 2011;54:901–7.
 33. Wang J, Zhang Z, Yan X, et al. Albumin-Bilirubin (ALBI) as an accurate and simple prognostic score for chronic hepatitis B-related liver cirrhosis. *Dig Liver Dis*. 2019;51:1172–1178.
 34. Kim BK, Han KH, Park JY, et al. A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. *Am J Gastroenterol*. 2010;105:1382–1390.
 35. Dănilă M. The ultrasound examination of the spleen. *Med Ultrason*. 2010;12:253–4.
 36. Thandassery RB, Kaabi SA, Soofi ME, et al. Mean Platelet Volume, Red Cell Distribution Width to Platelet Count Ratio, Globulin Platelet Index, and 16 Other Indirect Noninvasive Fibrosis Scores: How Much Do Routine Blood Tests Tell About Liver Fibrosis in Chronic Hepatitis C? *J Clin Gastroenterol*. 2016;50:518–523.
 37. Lin ZH, Xin YN, Dong QJ, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatology*. 2011;53:726–736.
 38. Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology*. 2007;46:32–36.
 39. Teshale E, Lu M, Rupp LB, et al. APRI and FIB-4 are good predictors of the stage of liver fibrosis in chronic hepatitis B: the Chronic Hepatitis Cohort Study (CHeCS). *J Viral Hepat*. 2014;21:917–20.
 40. Yada A, Iimuro Y, Uyama N, Uda Y, Okada, T, Fujimoto J. Splenectomy attenuates murine liver fibrosis with hypersplenism stimulating hepatic accumulation of Ly-6C(lo) macrophages. *J Hepatol*. 2015;63:905–16.
 41. Mallet V, Dhalluin-Venier V, Roussin C, et al. The accuracy of the FIB-4 index for the diagnosis of mild fibrosis in chronic hepatitis B. *Aliment Pharmacol Ther*. 2009;29: 409–415.

Figures

Figure 1

Hypoxia inducible factor 1 α (HIF-1 α) expression in all research subjects and the correlation with clinical parameters. (A) The mean plasma HIF-1 α levels (mean $\pm$ standard error of mean [SEM]) are significantly

increased in hepatitis B cirrhosis (CC+DC group) patients than that in healthy controls. (B) The mean plasma HIF-1α levels are significantly increased in HBV-DC patients than that in HBV-CC patients and healthy controls ($P = 0.0004$, $P < 0.001$). (C) Differences in plasma HIF-1α expression between peripheral and portal blood in patients with decompensated liver cirrhosis ($t = 0.5041$, $P = 0.62$). * $p < 0.05$, ** $p < 0.01$.

Figure 2

Comparison of HIF-1α level in different patients. (A) Violin plot analysis comparing the plasma HIF-1α levels in Child-Pugh A, B, and C patients. The vertical position is HIF-1 expression level (pg/ml). (B) Comparison of the mean plasma HIF-1α level (mean $\pm$ SEM) in Hepatitis B liver cirrhosis with different severity: with ascites vs. without patients, with gastrointestinal bleeding vs. without patients, with ascites and gastrointestinal bleeding vs. without patients. * $p < 0.05$, ** $p < 0.01$, *** $p \leq 0.001$.

Figure 3

Heat map of the correlation between plasma HIF-1 α level and other laboratory parameters. (A) Purple indicates positive correlation, green indicates negative correlation; p values are marked. (B) Relevant significant results are marked with red or blue, and the area of the circle indicates the value of the correlation coefficient. Non-significant results are marked with blanks. Significance was established as P $\leq$ 0.05.

Figure 4

Nomogram for predicting clinical outcomes in patients with hepatitis B cirrhosis.

Figure 5

Comparison of the ability to predict clinical outcomes. (A) The received operating characteristic (ROC) curve was used to evaluate the prognostic value of plasma HIF level, aspartate aminotransferase (AST)-to-platelet ratio index (APRI), fibrosis-4 (FIB-4) scores, and scoring model in patients with hepatitis B liver cirrhosis. (B) The scoring model was more sensitive and specific than FIB-4, APRI, and plasma HIF-1 α level in assessing the risk of decompensation of cirrhosis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigures.doc](#)
- [SupplementaryTables.doc](#)