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Total bile acid-to-cholesterol ratio as a novel noninvasive marker for significant liver fibrosis and cirrhosis in patients with non-cholestatic chronic hepatitis B virus infection

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Abstract

Although serum bile acids and total cholesterol (TC) are closely related to liver cirrhosis, the potential diagnostic value of total bile acid-to-cholesterol ratio (TBA/TC) for liver fibrosis is unclear. The present study aimed to evaluate the value of TBA/TC in the diagnosis of cirrhosis and the relationship between TBA/TC and significant liver fibrosis in chronic hepatitis B virus (HBV) infected patients without cholestasis.

667 patients with alkaline phosphatase (ALP) $\leq$ 1.5 upper limit of normal (ULN) and gamma-glutamyl transferase (GGT) $\leq$ 3 ULN were rigorously included in this cross-sectional study. Liver biopsy was performed in 32 patients and METAVIR scoring system was used to evaluate liver fibrosis stage. Liver ultrasound elastography was performed in 138 patients, significant fibrosis was defined as fibrosis $\geq$ F2. Multiple logistic regression as well as receiver operating characteristic (ROC) curves analyses were performed.

Compared to patients with non-cirrhosis, TBA and TBA/TC were significantly higher in cirrhosis while TC was significantly lower (all $P < .001$). In multivariate analysis, TBA/TC was also independently associated with cirrhosis [odds ratio (OR) = 1.102, 95% confidence interval (CI): 1.085–1.166]. The area under the curve (AUC) of TBA/TC (0.87) was almost equivalent to the aspartate aminotransferase to platelet ratio index (APRI, AUC = 0.84) and fibrosis 4 score (FIB-4, AUC = 0.80), and the optimal cut-off value for TBA/TC to diagnose cirrhosis was 2.70. Among the patients performed liver biopsy, TBA/TC were significantly higher both in significant fibrosis and cirrhosis as well as significantly correlated with fibrosis stage (all $P < .001$). Furthermore, In patients performed liver ultrasound elastography, TBA/TC was also independently associated with significant fibrosis (OR = 1.040, 95% CI: 1.001–1.078).

Assessment of TBA/TC could serve as an additional marker of significant liver fibrosis and cirrhosis in non-cholestatic chronic HBV infection.

Abbreviations: ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, APRI = aspartate aminotransferase to platelet ratio index, AST = aspartate aminotransferase, AUC = area under the curve, BAs = bile acids, CHE = cholinesterase, CI = confidence interval, FIB-4 = fibrosis 4 score, GGT = gamma-glutamyl transferase, HBV = hepatitis B virus, OR = odds ratio, PPV = positive predictive value, PTA = prothrombin activity, ROC = receiver operating characteristic, TBA/TC = total bile acid-to-cholesterol ratio, TBIL = total bilirubin, TC = total cholesterol, ULN = upper limit of normal.

Keywords: bile acids and salts, cholesterol, hepatitis B virus, liver cirrhosis

Editor: Sherief Abd-Elsalam.

LTY, LLW, and JY have contributed equally to this article and are considered to be the co-first author.

This study was supported by the National Natural Science Foundation (81800528); the Hospital Fund from the First Hospital of Lanzhou University (ldyyyn2017–17); the Gansu Health Industry Research Project (GSWSKY2018–24); the Science and Technology Development Project of Chengguan District (2018SHFZ0023); the National Science and Technology Key Project (2017ZX10201201, 2017ZX10203201–005, 2017ZX10202203–006–001 and 2017ZX10302201–004–002); the Beijing Municipal Administration of Hospital's Ascent Plan (DFL20151601); the research about clinical relevant factors and new treatment strategies for HBsAg clearance (No.2017ZX10202203–006); the clinical significance verification of new hepatitis B detection index (No.2017ZX10302201–004) and the National Science and Technology Key Project on "Major Infectious Diseases such as HIV/AIDS, Viral Hepatitis Preventon and Treatment" (2017ZX10302201–004, 2017ZX10202203–006)

The authors have no conflicts of interest to disclose.

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How to cite this article: Yan LT, Wang LL, Yao J, Yang YT, Mao XR, Yue W, Mao YW, Zhou W, Chen QF, Chen Y, Duan ZP, Li JF. Total bile acid-to-cholesterol ratio as a novel noninvasive marker for significant liver fibrosis and cirrhosis in patients with non-cholestatic chronic hepatitis B virus infection. Medicine 2020;99:8(e19248).

Received: 16 August 2019 / Received in final form: 4 January 2020 / Accepted: 17 January 2020

http://dx.doi.org/10.1097/MD.0000000000019248

1. Introduction

Chronic hepatitis B virus (HBV) infection affects an estimated 240 million persons worldwide,[1] making these patients at a high risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma.[2] Early diagnosis and intervention of liver fibrosis staging is essential. Currently, liver biopsy is considered as the 'gold standard' for determining the fibrosis.[3] However, liver biopsy is limited due to high sampling error and potential complications as an invasive examination.[3,4] Thus, noninvasive methods to assess the severity of hepatic fibrosis in chronic HBV infection is particularly needed, especially in resource-limited settings.

Several noninvasive fibrosis tests based on ultrasound principles or serum indicators are now available. Transient elastography (FibroScan) has been reported to accurately reflect liver fibrosis.[5] But the feasibility and reproducibility of the test may be affected by high body mass index.[6,7] In addition, the cost of acquiring, running and maintaining FibroScan is relatively high.[8] Serum indicators are simple and inexpensive noninvasive methods. Among them, aspartate aminotransferase to platelet ratio index (APRI) and fibrosis index based on the 4 factors (FIB-4) are recommended by clinical practice guidelines as commonly used indicators for evaluating liver fibrosis.[8,9] However, APRI and FIB-4 were initially used to diagnose hepatic fibrosis in patients with chronic hepatitis C and in patients co-infected with human immunodeficiency virus and hepatitis C virus, respectively.[10,11] The sensitivity and specificity of these two noninvasive indexes for detection of cirrhosis were low,[8,12] and their application in chronic HBV infected patients is controversial.[13,14]

Therefore, new noninvasive markers for predicting fibrosis stage may reduce the need for liver biopsy. According to the current researches, serum bile acids (BAs) and lipid metabolism have a close relationship with liver diseases.[15–18] Among them, total cholesterol (TC) is independently correlated with the mortality of cirrhosis and BAs is a valuable prognostic index for cirrhosis.[19,20] A combination of fasting and postprandial BAs measurements appear to be more sensitive for detection of cirrhosis in patients with normal transaminases than other biochemical tests of liver function.[16] While in patients with HBV infection, TBA and HBV have a co-receptor when entering hepatocytes,[21] binding of HBV to its cellular receptor affects the metabolism of BAs.[22] Since BAs are synthesized from cholesterol in hepatocytes and BAs metabolism plays an essential role in cholesterol homeostasis,[23] the abnormal of serum BAs levels may cause imbalance of BAs to cholesterol ratio.

In addition, the accumulation of serum BAs were also reported to be the main characteristic of cholestatic liver diseases that lead to necrosis and apoptosis of hepatocytes as well as progress of fibrosis.[24–26] The potential ability of the indicators related to BAs metabolism to differentiate non-cholestatic liver fibrosis has rarely been studied before. Therefore, the main purpose of the present study was to evaluate the value of total bile acid-to-cholesterol ratio (TBA/TC) in the diagnosis of liver fibrosis in chronic HBV infected patients without cholestasis. Based on a consecutive cohort, all subjects with serum alkaline phosphatase (ALP) $>$ 1.5 upper limit of normal (ULN) and gamma-glutamyl transferase (GGT) $>$ 3 ULN or with primary biliary cirrhosis, primary sclerosing cholangitis, obstructive jaundice and cholangiocarcinoma were strictly excluded. After eliminating the interference of cholestasis, the relationship between TBA/TC and liver fibrosis was truly reflected, and the true and convincing results of diagnostic value of TBA/TC for liver cirrhosis could be acquired based on our cohort.

2. Material and methods

2.1. Patients

A group of 667 consecutive chronic HBV infected patients from the first hospital of Lanzhou university were included from June 2016 to August 2017. Figure 1 summarized the flow diagram of the study population, and the inclusion criterion was the persistence of hepatitis B surface antigen for more than 6 months.[2,8] Identifying persons with cirrhosis was made by histological or combination of clinical signs (hepatomegaly and splenomegaly, ascites, caput medusae, spider naevi and others) and laboratory parameters or typical liver imaging signs (typical morphological changes of liver, portal hypertension signs in abdominal ultrasonography or computed tomography).[8] Clinical features of decompensated cirrhosis include Portal hypertension (ascites, variceal hemorrhage of esophageal or gastric and hepatic encephalopathy), coagulopathy, or liver insufficiency.[8] In addition, significant hepatic fibrosis was determined by liver ultrasound elastography.[27]

The exclusion reasons were as follows:

(1) less than 18 years old;
(2) insufficient clinical data (without hemocyte, liver chemistry and coagulation indicators);
(3) acute hepatitis B;
(4) combined with other liver diseases (hepatitis A, hepatitis C, hepatitis E, autoimmune hepatitis, alcoholic liver disease, steatohepatitis, drug-induced liver injury, Wilson's disease or metastatic liver cancer);
(5) co-infected with HIV;
(6) received liver transplantation or plasmapheresis;
(7) hepatocellular carcinoma received transcatheter arterial chemoembolization or radiofrequency ablation;
(8) with extrahepatic solid tumors received radiotherapy or chemotherapy;
(9) use of immune inhibitors, hepatotoxic drugs, ursodeoxycholic acid;
(10) co-existence of other serious diseases (shock, multiple organ failure, uremia and required dialysis, severe infection, hematologic malignancies);
(11) with cholestasis. Cholestasis is defined as serum ALP $>$ 1.5 ULN and GGT $>$ 3 ULN,[28] the ULN of ALP and GGT are 125U/L and 69U/L respectively. The patients with primary biliary cirrhosis, primary sclerosing cholangitis, obstructive jaundice and cholangiocarcinoma were also excluded.

The present study was anonymously analyzed and met the ethical requirements of the institutional review boards at the first hospital of Lanzhou university (Approval number: LDYYLL2019–211).

2.2. Laboratory tests

Serum hematological and fasting biochemical parameters within 24 hours of admission were tested. For patients performed liver biopsy, blood samples were collected before invasive operation. Haematological indicators, including white blood cell counts, lymphocyte, neutrophil, red blood cell counts, hemoglobin and platelet counts, were tested by fully automatic blood cell analyzer

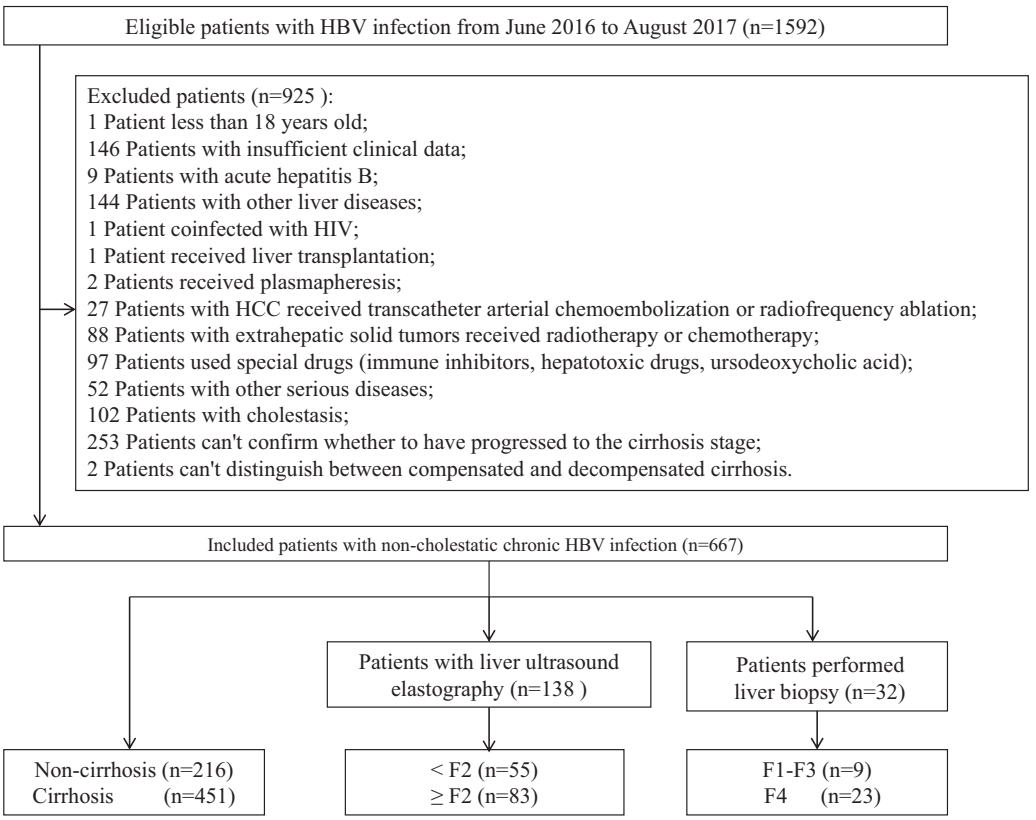

Figure 1. Flow diagram of the study population. HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HIV = human immunodeficiency virus.

(BC-5390 CRP, China). Biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), ALP, GGT, total bilirubin (TBIL), direct bilirubin, indirect bilirubin, TBA, TC, triglyceride, total protein, albumin (ALB), globulin, cholinesterase (CHE), creatinine and urea nitrogen, were tested by fully automatic biochemical analyzer (Olympos AU400, Japan). Virological parameters of HBV included hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B e antigen and HBV DNA.

APRI, FIB-4 were calculated as previously described: APRI = [AST (U/L)/its ULN]/PLT (10^9/L) × 100,[10] and FIB-4 = [Age (years) × AST (U/L)]/ PLT (10^9/L) × [ALT (U/L)$^{1/2}$],[11] respectively.

2.3. Liver biopsy and histological examination

Liver biopsies were performed using ultrasound localization. Each specimen was longer than 1.5 cm, containing at least 6 complete portal area. The samples were formalin-fixed and paraffin-embedded for histological analysis. Hepatic histology was interpreted by two senior pathologists who were blinded to the patients' clinical information. Hepatic fibrosis stage was assessed according to the METAVIR scoring system from F0 to F4: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4 was considered as cirrhosis.[29]

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (Chicago, IL). Continuous variables were presented as mean ± standard deviation, categorical values were expressed as frequencies. Data distribution were analyzed according to the Kolmogorov-Smirnov test. The differences of normally distributed data were analyzed using two-independent samples *t* test, non-normally distributed data were analyzed using Mann-Whitney test. Correlation analysis was evaluated using Spearman's rank correlation. The (LR) multivariate logistic regression analysis with stepwise forward selection was performed to identify predictors of cirrhosis and significant liver fibrosis, the *P* values of entry and removal were respectively set to .05 and .10. The diagnostic value of independent predictors were assessed according to the area under the receiver operating characteristic (ROC) curves and 95% confidence interval (CI). Sensitivity analyses were performed using MedCalc version 18.2 software (MedCalc Software, Mariakerke, Belgium). A two-sided $P < .05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

A group of 667 chronic HBV infected patients without cholestasis were included and the characteristics of included participants were shown in Table 1. The mean age was 48.80 ± 11.00 years and the proportion of men was 63.87%.

The progression stages of chronic HBV infection were divided into three parts: 216 patients (32.38%) without cirrhosis, 156 (23.39%) patients with compensated cirrhosis and 295 (44.23%) patients with decompensated cirrhosis. Among the 32 patients who performed liver biopsy, F4 accounted for the largest proportion (23/32, 71.87%), this was followed by F1 (4/32, 12.50%), F2 and

Table 1

Baseline characteristics of the included patients.

Parameter	Value
Age (years)	48.80 ± 11.00
Male, n (%)	426 (63.87)
WBC ($\times 10^9$/L)	4.67 ± 2.38
RBC ($\times 10^{12}$/L)	4.31 ± 2.02
Hb (g/L)	130.93 ± 28.43
PLT ($\times 10^9$/L)	109.78 ± 71.47
AST (U/L)	65.63 ± 101.32
ALT (U/L)	61.65 ± 107.83
ALP (U/L)	111.95 ± 43.75
GGT (U/L)	54.20 ± 55.21
TBIL (μmol/L)	43.85 ± 76.64
DBIL (μmol/L)	17.62 ± 50.77
IBIL (μmol/L)	26.32 ± 30.90
TBA (μmol/L)	35.58 ± 63.10
TC (mmol/L)	3.35 ± 1.09
TG (mmol/L)	1.06 ± 0.77
TP (g/L)	68.09 ± 9.47
ALB (g/L)	39.92 ± 6.64
CHE (KU/L)	5.11 ± 2.32
PT (s)	14.32 ± 4.47
PTA (%)	75.95 ± 21.94
APTT (s)	35.92 ± 10.34
HBeAg (+), n (%)	247 (37.03)
Ascites, n (%)	196 (29.30)
Esophageal or gastric varices, n (%)	288 (43.05)
Hepatic encephalopathy, n (%)	19 (2.84)
Progression stages of chronic HBV infection	
Non-cirrhosis, n (%)	216 (32.38)
Compensated cirrhosis, n (%)	156 (23.39)
Decompensated cirrhosis, n (%)	295 (44.23)
Fibrosis stages of liver ultrasound elastography	
F0	11 (7.97)
F1	44 (31.89)
F2	70 (50.72)
F3	8 (5.80)
F4	5 (3.62)
Liver biopsy	
F1, n (%)	4 (12.50)
F2, n (%)	2 (6.25)
F3, n (%)	3 (9.38)
F4, n (%)	23 (71.87)

Data are means ± SD or proportions (n [%]).

ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, APTT = partial thromboplastin time, AST = aspartate aminotransferase, CHE = cholinesterase, DBIL = direct bilirubin, GGT = gamma-glutamyl transferase, Hb = hemoglobin, IBIL = indirect bilirubin, PLT = platelet counts, PT = prothrombin time, PTA = prothrombin activity, RBC = red blood cell counts, TBA = total bile acid, TBIL = total bilirubin, TC = total cholesterol, TG = triglyceride, TP = total protein, WBC = white blood cell counts.

F3 were accounted for 6.25% (2/32) and 9.38% (3/32) respectively. Liver histological stages were divided into two groups of non-cirrhosis (F1-F3) and cirrhosis (F4).

In addition, 138 patients performed liver ultrasound elastography. F2 was presented in 70 patients, which accounted for the largest proportion (50.72%). This was followed by F1 (31.89%) and F0 (7.97%). F3 and F4 were found in 5.80% and 3.62% of the patients respectively.

3.2. TBA/TC as a serum marker for cirrhosis in chronic HBV infected patients without cholestasis

Compared to patients without cirrhosis, TBA, TBA/TC, AST, ALT, ALP, GGT and TBIL were significantly higher in cirrhosis,

Table 2

Indicators of cirrhosis in chronic hepatitis B virus infected patients without cholestasis.

Parameter	Progression stages		*P*
	Non-cirrhosis (n = 216)	Cirrhosis (n = 451)	
Age (years)	49.79 ± 12.74	48.33 ± 10.04	.095
WBC ($\times 10^9$/L)	5.92 ± 2.31	4.07 ± 2.18	< .001
RBC ($\times 10^{12}$/L)	4.89 ± 3.27	4.03 ± 0.84	< .001
Hb (g/L)	143.86 ± 23.20	124.74 ± 28.65	< .001
PLT ($\times 10^9$/L)	166.34 ± 61.53	82.69 ± 58.99	< .001
AST (U/L)	52.79 ± 87.24	71.78 ± 106.96	< .001
ALT (U/L)	59.65 ± 110.70	62.61 ± 106.53	< .001
ALP (U/L)	96.24 ± 31.05	119.47 ± 46.87	< .001
GGT (U/L)	40.32 ± 47.17	60.85 ± 57.54	< .001
TBIL (μmol/L)	23.71 ± 30.93	53.49 ± 89.16	< .001
TBA (μmol/L)	12.36 ± 35.05	46.70 ± 70.16	< .001
TC (mmol/L)	3.98 ± 0.94	3.04 ± 1.03	< .001
TP (U/L)	68.49 ± 8.45	67.89 ± 9.92	.425
ALB (g/L)	43.17 ± 4.75	38.37 ± 6.86	< .001
CHE (KU/L)	6.92 ± 1.95	4.24 ± 1.97	< .001
PTA (%)	91.67 ± 15.85	68.40 ± 20.41	< .001
TBA/TC	3.91 ± 14.12	27.42 ± 75.64	< .001

ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHE = cholinesterase, GGT = gamma-glutamyl transferase, Hb = hemoglobin, PLT = platelet counts, PTA = prothrombin activity, RBC = red blood cell counts, TBA/TC = total bile acid-to-cholesterol ratio, TBA = total bile acid, TBIL = total bilirubin, TC = total cholesterol, TP = total protein, WBC = white blood cell counts.

while TC, ALB, CHE and prothrombin activity (PTA) were significantly lower (Table 2, all $P < .001$), which were all significantly correlated with the progression stages of chronic HBV infection (all $P < .001$), as shown in Table 3. Subsequently, indicators related to bile excretion, including TBIL, ALP, GGT, TBA and TBA/TC, were entered into multivariate analysis. The selection of variables adopted stepwise forward procedures and the final results were shown in Table 4. TBA/TC has a larger OR value (OR = 1.102, 95% CI: 1.085–1.166) than ALP (OR =

Table 3

Correlation analysis between laboratory indicators and different degrees of liver fibrosis in chronic hepatitis B virus infected patients without cholestasis.

Parameter	Progression stages (n = 667)		F1, F2/F3, F4 (liver biopsy, n = 32)	
	r	*P*	r	*P*
PLT ($\times 10^9$/L)	−0.60	< .001	−0.63	< .001
ALB (g/L)	−0.49	< .001	−0.64	< .001
CHE (KU/L)	−0.66	< .001	−0.64	< .001
PTA (%)	−0.66	< .001	−0.51	.003
AST (U/L)	0.38	< .001	0.25	.169
ALT (U/L)	0.21	< .001	−0.09	.627
ALP (U/L)	0.24	< .001	0.04	.827
GGT (U/L)	0.18	< .001	−0.01	.938
TBIL (μmol/L)	0.49	< .001	0.25	.173
TBA (μmol/L)	0.63	< .001	0.57	.001
TC (mmol/L)	−0.50	< .001	−0.65	< .001
TBA/TC	0.67	< .001	0.62	< .001

ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHE = cholinesterase, GGT = gamma-glutamyl transferase, PLT = platelet counts, PTA = prothrombin activity, r = Correlation coefficient, TBA/TC = total bile acid-to-cholesterol ratio, TBA = total bile acid, TBIL = total bilirubin, TC = total cholesterol.

Table 4

Predictors of cirrhosis according to multiple logistic regression analysis.

Parameter	Non-cirrhosis	Cirrhosis	OR	95%CI	P
Total patients (n = 667)					
TBIL (μmol/L)	23.71 ± 30.93	53.49 ± 89.16			
ALP (U/L)	96.24 ± 31.05	119.47 ± 46.87	1.007	1.002–1.013	.008
GGT (U/L)	40.32 ± 47.17	60.85 ± 57.54	1.007	1.002–1.011	.003
TBA (μmol/L)	12.36 ± 35.05	46.70 ± 70.16			
TBA/TC	3.91 ± 14.12	27.42 ± 75.64	1.102	1.085–1.166	< .001
Patients with liver biopsy (n = 32)					
Age (years)	46.44 ± 5.34	45.96 ± 11.28			
ALB (g/L)	47.02 ± 3.66	38.90 ± 5.73			
CHE (KU/L)	7.93 ± 1.00	4.45 ± 2.47			
PTA (%)	91.28 ± 24.51	69.66 ± 15.95			
TBA (μmol/L)	4.87 ± 4.37	23.62 ± 15.56			
TBA/TC	1.13 ± 0.98	9.19 ± 6.31	2.145	1.035–4.442	< .001

ALB = albumin, ALP = alkaline phosphatase, CHE = cholinesterase, GGT = gamma-glutamyl transferase, PTA = prothrombin activity, TBA/TC = total bile acid-to-cholesterol ratio, TBA = total bile acid, TBIL = total bilirubin.

1.007, 95% CI: 1.002–1.013) and GGT (OR = 1.007, 95% CI: 1.002–1.011).

Furthermore, among the patients performed liver biopsy, TBA and TBA/TC were significantly higher both in significant fibrosis and cirrhosis (all $P < .001$), and ALB ($P = .009$ and $P < .001$), CHE (both $P < .001$) and PTA ($P = .028$ and $P = .006$) were significantly lower, while TC was only significantly lower in cirrhosis ($P < .001$, Table 5). In the subsequent Spearman's correlation analysis, significant correlations were also found between variables of TBA ($r = 0.57$, $P = .001$), TBA/TC ($r = 0.62$, $P < .001$), ALB ($r = -0.64$, $P < .001$), CHE ($r = -0.64$, $P < .001$), PTA ($r = -0.51$, $P = .003$) and the fibrosis stage of F1, F2/3 and F4 (Table 3). These five indicators together with age were then entered the multivariate analysis. The results were shown in Table 4, and TBA/TC was found to be independently correlated with cirrhosis in chronic HBV infected patients without cholestasis (OR = 2.145, 95% CI: 1.035–4.442).

3.3. TBA/TC diagnoses cirrhosis in chronic HBV infected patients without cholestasis

To evaluate the ability of TBA/TC in diagnosing cirrhosis in non-cholestatic chronic HBV infected patients, the ROC curves and sensitivity analyses were performed comparing with APRI and FIB-4, as shown in Figure 2A and Table 6. The area under the curve (AUC) of TBA and TBA/TC were 0.85 and 0.87 respectively (all $P < .001$), which were almost equivalent to APRI and FIB-4 (AUC = 0.84, $P < .001$ and AUC = 0.80, $P < .001$, respectively). The sensitivity, specificity and positive predictive value (PPV) of FIB-4 (80.49%, 71.76%, 85.60%, respectively) for diagnosing cirrhosis were lower than APRI (86.03%, 73.61%, 87.20%, respectively). But the specificity and PPV of TBA (85.19%, 91.70%) and TBA/TC (83.33%, 91.10%) were higher than those of APRI. The optimal cut-off value of TBA and TBA/TC were 10.40 and 2.70 respectively.

Table 5

Indicators of significant fibrosis and cirrhosis in non-cholestatic chronic hepatitis B virus infected patients performed liver biopsy.

Parameter	< F2 (n = 4)	≥F2 (n = 28)	P	≤F3 (n = 9)	F4 (n = 23)	P
Age (years)	45.50 ± 5.97	46.18 ± 10.40	.900	46.44 ± 5.34	45.96 ± 11.28	.870
WBC ($\times 10^9$/L)	4.41 ± 1.45	3.51 ± 2.23	.444	5.31 ± 1.76	2.97 ± 1.94	.004
RBC ($\times 10^{12}$/L)	4.65 ± 0.62	4.04 ± 0.84	.181	4.86 ± 0.47	3.83 ± 0.77	.001
Hb (g/L)	119.75 ± 32.68	118.29 ± 35.76	.939	144.11 ± 30.75	108.43 ± 31.58	.007
PLT ($\times 10^9$/L)	203.25 ± 68.56	84.75 ± 75.15	.017	180.00 ± 67.70	68.09 ± 66.76	< .001
AST (U/L)	27.03 ± 8.74	48.49 ± 55.41	.138	34.60 ± 20.31	50.19 ± 60.26	.213
ALT (U/L)	28.45 ± 7.64	40.89 ± 36.82	.819	46.21 ± 36.81	36.65 ± 34.30	.536
ALP (U/L)	74.80 ± 13.95	101.76 ± 36.07	.154	96.89 ± 28.28	98.97 ± 38.04	.883
GGT (U/L)	19.58 ± 6.07	62.85 ± 80.00	.082	83.50 ± 124.33	47.24 ± 46.36	.417
TBIL (μmol/L)	30.75 ± 34.32	27.33 ± 14.73	.856	24.71 ± 22.94	28.95 ± 15.23	.545
TBA (μmol/L)	5.23 ± 3.67	20.22 ± 16.02	< .001	4.87 ± 4.37	23.62 ± 15.56	< .001
TC (mmol/L)	3.92 ± 0.52	3.18 ± 1.09	.201	4.39 ± 0.60	2.84 ± 0.86	< .001
TP (U/L)	78.85 ± 4.65	68.06 ± 8.96	.026	74.64 ± 6.39	67.36 ± 9.47	.043
ALB (g/L)	48.70 ± 3.74	40.11 ± 5.95	.009	47.02 ± 3.66	38.90 ± 5.73	< .001
CHE (KU/L)	7.55 ± 0.21	5.13 ± 2.72	< .001	7.93 ± 1.00	4.45 ± 2.47	< .001
PTA (%)	96.83 ± 9.09	72.73 ± 20.32	.028	91.28 ± 24.51	69.66 ± 15.95	.006
TBA/TC	1.41 ± 1.02	7.71 ± 6.56	< .001	1.13 ± 0.98	9.19 ± 6.31	< .001

ALB = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHE = cholinesterase, GGT = gamma-glutamyl transferase, Hb = hemoglobin, PLT = platelet counts, PTA = prothrombin activity, RBC = red blood cell counts, TBA/TC = total bile acid-to-cholesterol ratio, TBA = total bile acid, TBIL = total bilirubin, TC = total cholesterol, TP = total protein, WBC = white blood cell counts.

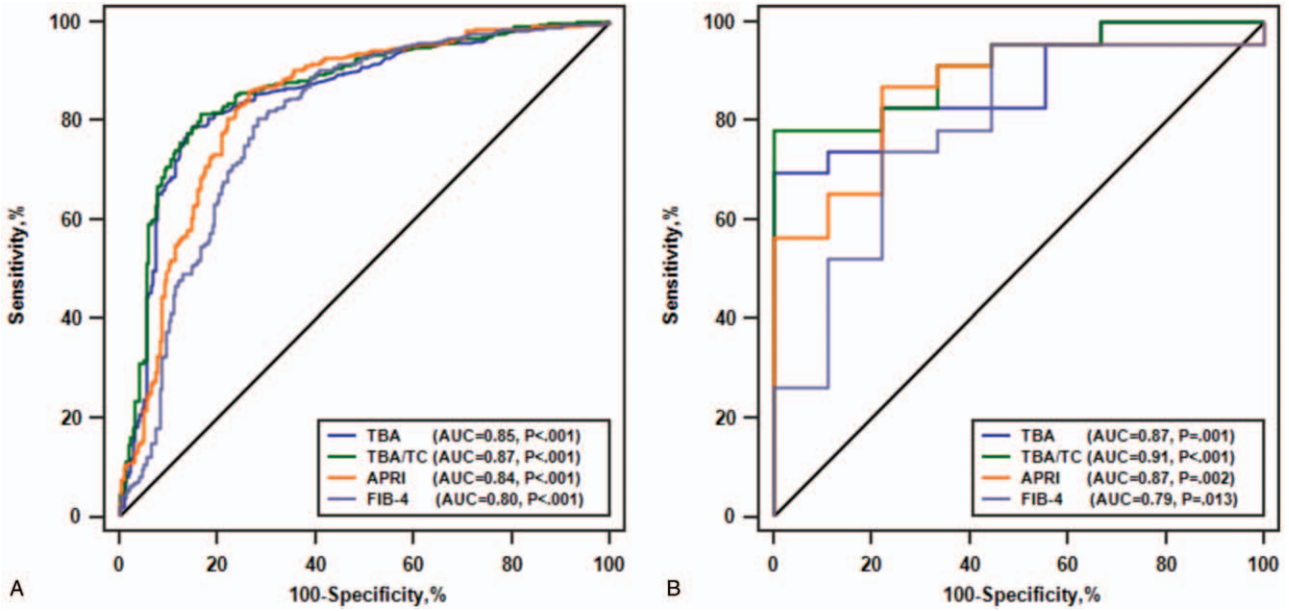

Figure 2. Receiver operating characteristic (ROC) curves of TBA, TBA/TC for diagnosing liver cirrhosis, in comparison to APRI, FIB-4. (A) In the general population. (B) In the population performed liver biopsy. APRI = aspartate aminotransferase to platelet ratio index, FIB-4 = fibrosis 4 score, TBA/TC = total bile acid-to-cholesterol ratio, TBA = total bile acid.

Furthermore, among the patients performed liver biopsy, The AUC of TBA and TBA/TC were 0.87 ($P = .001$) and 0.91 ($P < .001$) respectively (Fig. 2B), which were also almost equivalent to APRI and FIB-4 (AUC = 0.87, $P = .002$ and AUC = 0.79, $P = .013$, respectively).

3.4. TBA/TC as a serum marker for significant liver fibrosis in chronic HBV infected patients without cholestasis

TBA/TC was independently correlated with cirrhosis in chronic HBV infected patients without cholestasis. Consequently, we analyzed the correlation between TBA/TC and significant liver fibrosis, as shown in Table 7. TBA/TC was significantly increased in significant liver fibrosis as well as significantly associated with the fibrosis stages from F0 to F4 (both $P < .001$). In the subsequent multivariate analysis, after adjusting other factors related to liver injury and metabolism, including AST, ALT, ALP, GGT, TBA/TC was also independently correlated with significant fibrosis (OR = 1.040, 95% CI: 1.001–1.078). The AUC of TBA/TC that distinguishes significant liver fibrosis was 0.70 ($P < .001$) as shown in Figure 3.

Table 6

Diagnostic values of total bile acid, total bile acid-to-cholesterol ratio in liver cirrhosis, for comparison, diagnostic values of aspartate aminotransferase to platelet ratio index and fibrosis 4 score.

Parameter	Sensitivity%	Specificity%	PPV	NPV
Total patients (n = 667)				
TBA (μmol/L)	78.49	85.19	91.70	65.50
TBA/TC	81.37	83.33	91.10	68.20
APRI	86.03	73.61	87.20	71.60
FIB-4	80.49	71.76	85.60	63.80
Patients with liver biopsy (n = 32)				
TBA (μmol/L)	69.57	100.00	100.00	56.20
TBA/TC	78.26	100.00	100.00	64.30
APRI	86.96	77.78	90.90	70.00
FIB-4	73.91	77.78	89.50	53.80

APRI = aspartate aminotransferase to platelet ratio index, FIB-4 = fibrosis 4 score, TBA/TC = total bile acid-to-cholesterol ratio, TBA = total bile acid.

4. Discussion

BAs metabolism has a close relationship with cholestatic liver diseases.[24–26] In the study, all chronic HBV infected patients with cholestasis were excluded. Based on our cohort of included consecutive patients, the present study, for the first time, analyzed the relationship between the imbalance of TBA/TC ratio and liver fibrosis. According to the results, TBA/TC might be a novel noninvasive marker of significant fibrosis and cirrhosis in chronic HBV infected patients without cholestasis.

Currently, there is growing evidence determines the close relationship between TBA and cirrhosis, for example, TBA correlated strongly with hepatic venous pressure gradient,[30] and serum TBA levels may be elevated in portal hypertension bypassing the liver uptake via portosystemic shunt and intrahepatic shunt.[31–33] Since BAs metabolism plays an essential role in cholesterol homeostasis,[23] the elevation of serum BAs levels may cause imbalance of TBA/TC ratio. However, the association between TBA/TC and liver cirrhosis in chronic HBV infected patients without cholestasis remains to be elucidated. In the present study, the univariate analysis showed that TBA was significantly increased in cirrhosis in chronic HBV infected patients, which was consistent with previous studies.[17,34,35] In addition, TBA/TC was also significantly increased in cirrhosis and had an independent relationship with cirrhosis in non-cholestatic chronic HBV infected patients.

Of all noninvasive fibrosis tests based on serum indicators, APRI and FIB-4 are recommended by clinical practice guidelines

Table 7

Relationship between total bile acid-to-cholesterol ratio and significant liver fibrosis in non-cholestatic chronic hepatitis B virus infected patients performed liver ultrasound elastography.

Parameter	Univariate analysis			Correlation analysis (F0-F4)		Multivariate analysis		
	< F2 (n = 55)	≥ F2 (n = 83)	*P*	r	*P*	OR	95%CI	*P*
AST (U/L)	39.68 ± 23.30	99.62 ± 141.63	< .001	0.43	< .001			
ALT (U/L)	42.01 ± 36.60	95.56 ± 159.71	.005	0.23	.008			
ALP (U/L)	95.10 ± 36.02	122.54 ± 43.98	< .001	0.35	< .001	1.010	1.000–1.022	.051
GGT (U/L)	32.58 ± 28.64	63.09 ± 54.74	< .001	0.34	< .001	1.022	1.005–1.031	.007
TBA/TC	5.27 ± 9.12	16.27 ± 26.74	< .001	0.41	< .001	1.040	1.001–1.078	.046

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase, TBA/TC = total bile acid-to-cholesterol ratio.

as commonly used methods.[8,9] The APRI and FIB-4 were developed in patients with chronic hepatitis C virus infection,[10,36] and were also demonstrated to be suitable markers for detecting significant fibrosis and cirrhosis in patients with chronic hepatitis B.[37,38] However, the APRI as well as FIB-4 scores consist of indirect markers of fibrosis such as ALT, AST and platelet count,[10,11] while normal transaminase levels do not exclude the presence of significant fibrosis.[39,40] Furthermore, the PPV of APRI for detection of cirrhosis was low as detecting only one third of persons with cirrhosis.[8] In present study, we also compared the ability of TBA, TBA/TC and APRI, FIB-4 to differentiate cirrhosis. The AUC of TBA and TBA/TC were 0.85 and 0.87 respectively, which were equivalent to APRI and FIB-4 (AUC = 0.84 and AUC = 0.80, respectively). But the specificity and PPV of TBA (85.19%, 91.70%) and TBA/TC (83.33%, 91.10%) were higher than those of APRI (73.61%, 87.20%) and FIB-4 (71.76%, 85.60%). Similar results were found in liver biopsy patients. According to the results, the combination of these 4 noninvasive indicators may help to better distinguish the presence of cirrhosis.

Apart from cirrhosis, the presence of significant liver fibrosis is also a priority for antiviral therapy.[2,9] In our study, the univariate and multivariate analyses showed that TBA and TBA/TC were significantly increased in significant fibrosis in patients performed liver ultrasound elastography and TBA/TC was also found to be one of independent predictors of significant fibrosis (OR = 0.97, 95% CI: 0.95–0.10).

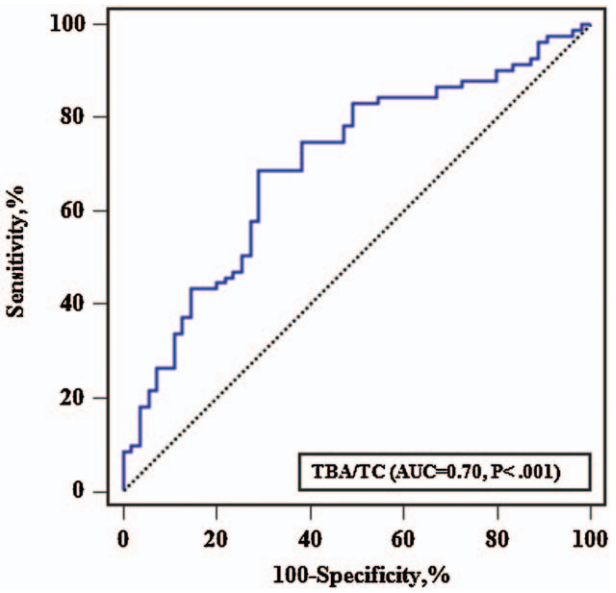

Figure 3. Receiver operating characteristic (ROC) curves of TBA/TC for distinguishing significant liver fibrosis. AUC = area under the ROC curves.

The limitations of the present study are as follows: firstly, although the retrospective study has been rigorously designed, there was a lack of animal or cell-based research to further elucidate the underlying mechanism of the imbalance of TBA/TC ratio in non-cholestatic chronic HBV infection. Secondly, the study was predominantly retrospective so that we did not analyze the dynamic changes of serum TBA and TC levels in non-cholestatic patients, and long-term prognostic value was not evaluated. Prospective studies need to be performed to explain the value of TBA/TC in the assessment of prognosis.

In conclusion, the calculated novel noninvasive indicator of TBA/TC could serve as an additional marker to distinguish significant liver fibrosis and cirrhosis in non-cholestatic chronic HBV infected patients. Further prospective studies are needed to confirm our findings.

Acknowledgments

The authors acknowledge all the staff of the First Hospital of Lanzhou University who have completed the serum parameter test, imaging and liver biopsy examination.

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