International Journal of Medical Sciences
2010; 7(5):272-277

Research Paper

Predictors of hepatic steatosis in HBeAg-negative chronic hepatitis B patients and their diagnostic values in hepatic fibrosis

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Received: 2010.05.05; Accepted: 2010.08.08; Published: 2010.08.11

Abstract

Objective: To investigate predictors of hepatic steatosis in HBeAg-negative chronic hepatitis B (CHB) patients and their diagnostic values in hepatic inflammation and fibrosis. **Methods**: A total of 106 HBeAg-negative CHB patients with clinically and pathologically proven steatosis and 98 patients without steatosis were recruited into this study. The levels of fasting blood glucose (FBG), fasting insulin (FINS), triglyceride (TG), cholesterol (CHOL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (Alb), globulin (Glb), HBV DNA, body mass index (BMI), homeostatic model assessment of insulin resistance (HOMA-IR) and pathological changes of the liver in inflammation, fibrosis and fatty deposition were examined in all patients. **Results**: The levels of BMI, HOMA-IR, FBG, insulin, TG, and CHOL were significantly higher in patients with steatosis than those without steatosis (all $P<0.05$). But ALT, AST and HBV DNA levels were significantly lower in patients with steatosis (all $P<0.05$). Logistic regression analysis showed that only FINS was a significant predictor for hepatic steatosis ($P<0.05$); FINS and Glb were significant predictors for hepatic inflammation (all $P<0.05$); BMI and TC were significant predictors for hepatic fibrosis (all $P<0.05$). **Conclusions**: Hepatic steatosis, a common disease in HBeAg-negative CHB patients, was positively associated with BMI, FBG, FINS, TG, TC, GGT, ALP and HOMA-IR. In these patients, the prevalence of hepatic inflammation and fibrosis was also increased.

Key words: HBeAg negative, chronic hepatitis B, nonalcoholic fatty liver disease, liver biopsy

Introduction

The prevalence of HBeAg-negative chronic hepatitis B (CHB) tends to increase in recent years (1). With the improvement of living standard and nutrition status, hepatic steatosis frequently occurs in CHB patients. It has been shown that the incidence of hepatic steatosis in CHB patients was about 32% (2). The distribution of hepatic triglyceride content (HTGC) in 2, 287 subjects from a multiethnic, population-based sample (32.1% white, 48.3% black, and 17.5% Hispanic) was previously examined and compared using proton magnetic resonance spectroscopy. Almost one third of the population had hepatic steatosis, and most subjects with hepatic steatosis had normal levels of serum alanine aminotransferase (ALT). The higher prevalence of hepatic steatosis in Hispanics was mainly due to the higher prevalence of obesity and insulin resistance in this ethnic group (3). But, that how does hepatic steatosis influence CHB still remains unclear (4), particularly in HBeAg-negative CHB patients. In contrast, a large body of evidence showed the incidence of hepatic steatosis in chronic hepatitis C (CHC) patients ranged from 31% to 72%. Moreover, it has been suggested that hepatic steatosis in CHC has correlations with obesity, disorder of fat

metabolism, insulin resistance and HCV genotypes (5). This study analyzed and compared the clinical and histological features of HBeAg-negative CHB patients with or without hepatic steatosis, so as to evaluate the predictors of clinical and pathological characteristics in these patients with steatosis and their diagnostic values in hepatic fibrosis.

Patients and methods

Patients

A total of 204 HBeAg-negative CHB patients were recruited from the Research and Therapy Center for Liver Diseases of China Southeast Hospital from May 2005 to March 2009. Most patients did not drink alcohol, and alcohol consumption in the remaining patients was less than 20g/day. These patients were divided in two groups according to presence of hepatic steatosis. Among all HBeAg-negative CHB participants, 106 were diagnosed as hepatic steatosis (83 men and 23 women, mean age: 41.08±10.23 years) and the remaining 98 patients were excluded from hepatic steatosis (79 men and 19 women, mean age 39.4±9.81 years). Their diagnoses were finally confirmed by clinical presentations and pathological features. The criteria for hepatic steatosis were based on the American Association for the Study of Liver Diseases Practice Guidelines (2007) (6). Hepatitis A virus (HAV), HCV, Hepatitis D virus (HDV) and Hepatitis E virus (HEV) infection, drug-induced hepatitis, alcoholic hepatitis and autoimmune hepatitis were all excluded. Clinical data were obtained and recorded immediately after enrollment.

Body mass index (BMI)

BMI was calculated as the individual's body weight divided by the square of his or her height. According to the new BMI criteria for Asians by the regional office for the western pacific region of WHO (WHO Technical Report Series No. 894, WHO, Geneva, 2000), normal weight, overweight, Obese Class I and Obese Class II were defined by BMI= 18.5-22.9 kg/m^2, 23.0-24.9 kg/m^2, 25.0-29.9kg/m^2 and ≥30 kg/m^2, respectively.

Serum markers of HBV

HBV markers, including HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe were measured by enzyme-linked immunosorbent assay (Livzon Group Reagent Factory, Guangdong, China).

HBV DNA

The HBV DNA level was determined by quantitative polymerase chain reaction (qPCR) (AcuGen HBV quantitative test; Biotronic Corp., Lowell, Mass.) with the fluorescent HBV DNA probes provided by the same company. Asymmetric primer 1 was 5′-TGTCTCGTGTTACAGGCGGGGT-3', asymmetric primer 2 was 5′-GAGGCATAGCAGCAGGA GAAGAG-3', and fluorescent primer was 5′-TCGCTGGAAGTGTCTGCGGCGT-3'.

Serum assays

Fasting blood was collected with a un-anticoagulated vacuum blood collection tube. Serum was separated by centrifugation at 4°C and stored in a sterile tube at -40°C within 4 h. The levels of fasting blood glucose (FBG), insulin, triglyceride (TG), cholesterol (CHOL), ALT, aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), alkaline phosphatases (ALP), albumin (Alb) and globulin (Glb) were determined.

Homeostatic model assessment of insulin resistance (HOMA-IR)

HOMA-IR was calculated by means of the homeostasis model assessment (HOMA-R) previously described, where HOMA-R=insulin/(22.5 $e^{-\ln \text{glucose}}$) (7).

Histological evaluation

All 204 specimens from liver biopsy were 1.0~2.5 cm in length. Liver biopsy was performed to obtain the specimens under the guidance of ultrasound within 1 week after admission, using a needle with an internal diameter of 1.4 mm (Quick-Cut; Hakko. Company, Japan). Each specimen was longer than 1 cm and had more than 6 portal areas. Specimens were fixed in buffered formalin, embedded in paraffin, and stained with hematoxylin-eosin-safran and Masson's trichrome. Hepatic steatosis, stage of fibrosis and grade of disease activity were determined according to the Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region (8). Microvesicular steatosis was also graded as: F0 (<5% hepatocytes with microvesicular steatosis), F1 (5~30% hepatocytes involved), F2 (31~50% hepatocytes involved), F3 (51~75% involved) and F4 (>75% hepatocytes involved). Fibrosis stage was defined as S0 (no fibrosis), S1 (mild fibrosis), S2 (moderate fibrosis), S3 (severe fibrosis), and S4 (cirrhosis), and grade of disease activity was defined as G0 (no activity), G1 (mild activity), G2 (moderate activity), and G3 (severe activity). All the sections were blindly and independently assessed by 3 pathologists and the results were processed by the Kappa concordance test. The inter- and intra-observer agreements were excellent.

Statistical analysis

Data were analyzed with the SPSS 12.0 statistical package (SPSS Inc., Chicago, IL, USA). Baseline characteristics and anthropometric indices were expressed as means ± standard deviation (SD) or percentage frequency, if necessary. The baseline characteristic and anthropometric indices were compared between HBeAg-negative CHB patients with steatosis and those without steatosis by independent *t* test for continuous variables and Chi-square test for categorical variables. A binary logistic regression model was used to determine predictors and their odds ratios for hepatic steastosis among HBeAg-negative CHB patients. To screen the predictors for both hepatic inflammation and fibrosis stages, multivariate logistic regression models with adjustment for age and gender were employed. For all comparisons, two-tailed P values of less than 0.05 were considered statistically significant.

Results

A total of 106 patients were diagnosed as hepatic steatosis (83 men and 23 women, mean age: 41.08±10.23 years), and 98 patients excluded from hepatic steatosis (79 men and 19 women, mean age 39.4±9.81 years) served as controls. There was no significant difference in age or gender between both groups (P>0.05). Clinical characteristics and some anthropometric indices of HBeAg-negative CHB patients with or without steatosis are listed in Table 1. HBeAg-negative CHB patients with steatosis had significantly higher levels of BMI, FBG, FINS, TG, TC, GGT, ALP, Glb and HOMA-IR (*all* P<0.05) than did those without steatosis. However, the HBV DNA, AST, ALT and Alb levels were significantly lower in patients with steatosis (all P <0.05).

Histological features of HBeAg-negative CHB patients with or without steatosis were summarized in Table 2.

Table 1 Clinical characteristics and anthropometric indices of HBeAg-negative CHB patients with and without steatosis

	CHB with steatosis	CHB without steatosis	*P* value
Number	106	98	
BMI (kg/m^2)	28.66±1.62	20.74±1.01	0.0078
FBG (mmol/L)	6.79±0.84	4.37±0.26	0.0371
FINS (U/L)	16.31±1.27	11.62±0.84	0.0013
TG (mmol/l)	3.99±0.22	2.65±0.10	0.0064
TC (mmol/l)	5.87±0.62	3.70±0.57	0.0216
ALT (U/L)	110.82±21.59	366.90±86.87	0.0014
AST (U/L)	92.61±15.38	157.62±23.31	0.0291
GGT (U/L)	79.99±11.70	48.63±6.72	0.0116
ALP (U/L)	157.514±14.72	83.46±14.72	0.0268
Alb (g/L)	41.03±4.06	47.89±4.73	0.0307
Glb (g/L)	41.84±11.73	31.22±7.49	0.0053
HOMA-IR	5.86±1.03	3.02±0.91	0.0396
HBV DNA ($\log_{10}$ copies/ml)	3.29±1.08	5.61±0.91	0.0081

Table 2 Histological features of HBeAg-negative CHB patients with and without steatosis

	CHB with steatosis (%)	CHB without steatosis (%)	*P* value
Number	106	98	
Steatosis			
F0	0	98(100)	
F1	23(21.70)	0	
F2	25(23.58)	0	
F3	34(32.08)	0	
F4	24(22.64)	0	
Inflammation activity			
G0	17(16.04)	4(4.08)	0.019
G1	51(48.11)	13(13.27)	0.000
G2	23(21.70)	47(47.96)	0.002
G3	15(14.15)	34(34.69)	0.010
Fibrosis stage			
S0	34(32.08)	2(2.04)	0.000
S1	49(46.22)	15(15.31)	0.000
S2	11(10.38)	23(23.47)	0.046
S3	6(5.66)	42(42.85)	0.000
S4	6(5.66)	16(16.33)	0.054

The results of binary logistic regression are shown in Table 3. Among all indices and laboratory characteristics, FINS was the only characteristic that strongly associated with hepatic steatosis in HBeAg-negative CHB patients. The OR of FINS (every 1-unit increase) was 31.757 [95% confidence interval (CI) 6.899~45.454, P<0.001]. The regression function for predicting hepatic steatosis among HBeAg-negative CHB patients could be defined as $P=e^{-240.827+17.165FINS}/(1+ e^{-240.827+17.165FINS})$. The results of multivariate regression for hepatic inflammation are shown in Table 4. TG, GGT, Glb, and FINS were all associated with hepatic inflammation in each stage among HBeAg-negative CHB patients, but only FINS and Glb were strong predictors tested by likelihood ratio test (P=0.014, and P=0.013, respectively). Taken G0 stage as reference, each regression model could be expressed as follows: G_1: $P_1=e^{-3.3-2.347TG-0.057GGT+0.234Glb+0.461FINS}/(e^{-3.3-2.347TG-0.057+0.234Glb+0.461FINS}+e^{18.690-1.698TG-0.023GGT+0.083Glb-0.082FINS}+e^{8.537-2.448TG-0.057GGT+0.034Glb+0.207FINS})$; G_2: $P_2=e^{18.690-1.698TG-0.023GGT+0.083Glb-0.082FINS}/(e^{-3.3-2.347TG-0.057+0.234Glb+0.461FINS}+e^{18.690-1.698TG-0.023GGT+0.083Glb-0.082FINS}+e^{8.537-2.448TG-0.057GGT+0.034Glb+0.207FINS})$; G_3: $P_3=e^{8.537-2.448TG-0.057GGT+0.034Glb+0.207FINS}/(e^{-3.3-2.347TG-0.057+0.234Glb+0.461FINS}+e^{18.690-1.698TG-0.023GGT+0.083Glb-0.082FINS}+e^{8.537-2.448TG-0.057GGT+0.034Glb+0.207FINS})$; G_0: $P_0=1/(e^{-3.3-2.347TG-0.057+0.234Glb+0.461FINS}+e^{18.690-1.698TG-0.023GGT+0.083Glb-0.082FINS}+e^{8.537-2.448TG-0.057GGT+0.034Glb+0.207FINS})$. Similarly, the results of multivariate regression for hepatic fibrosis were shown in Table 5. BMI and TC were strongly predictors of hepatic fibrosis among HBeAg-negative CHB patients tested by likelihood ratio test (P=0.033 and P=0.025, respectively). Regression function for each stage could be expressed as follows: S_1: $P_1=e^{-22.942+0.087BMI+4.203TC}/(e^{-22.942+0.087BMI+4.203TC}+e^{-14.352-0.390BMI+5.146TC}+e^{-18.024-0.253BMI+5.224TC}+e^{-39.445+0.199BMI+6.543TC})$; S_2: $P_2= e^{-14.352-0.390BMI+5.146TC}/(e^{-22.942+0.087BMI+4.203TC}+e^{-14.352-0.390BMI+5.146TC}+e^{-18.024-0.253BMI+5.224TC}+e^{-39.445+0.199BMI+6.543TC})$; S_3: $P_3= e^{-18.024-0.253BMI+5.224TC}/(e^{-22.942+0.087BMI+4.203TC}+e^{-14.352-0.390BMI+5.146TC}+e^{-18.024-0.253BMI+5.224TC}+e^{-39.445+0.199BMI+6.543TC})$; S_4: $P_4= e^{-39.445+0.199BMI+6.543TC}/(e^{-22.942+0.087BMI+4.203TC}+e^{-14.352-0.390BMI+5.146TC}+e^{-18.024-0.253BMI+5.224TC}+e^{-39.445+0.199BMI+6.543TC})$; S_0: $P_0=1/(e^{-22.942+0.087BMI+4.203TC}+e^{-14.352-0.390BMI+5.146TC}+e^{-18.024-0.253BMI+5.224TC}+e^{-39.445+0.199BMI+6.543TC})$.

Table 3 Binary logistic regression analysis was performed to screen predictors for hepatic steatosis in HBeAg-negative CHB patients

	B	*SE*	*Wald* χ^2	*P*	*OR*	95% *CI*
FINS	17.165	1432.081	19.148	1.210E-3	31.757	6.899~45.454
Constant	-240.827	20161.091	20.373	6.374E-6	0.107	
Cox and Snell R^2	0.750					
Nagelkerke R^2	0.830					
Overall percentage	82%					

Table 4 Multinomial logistic regression analysis was performed to screen predictors for hepatic inflammation grades in HBeAg-negative CHB patients. Categorical variables were defined as follows: G_0: 0, G_1: 1, G_2: 2, G_3: 3.

	G	*Wald* χ2	*P*	*OR*	95%*CI*	Cox and Snell R^2	Model fitting test
TG	1	7.004	0.008	0.096	0.017~0.544	0.271	*P*=0.001
	2	2.547	0.111	0.183	0.023~1.473	0.271	*P* =0.001
	3	4.331	0.037	0.086	0.009~0.867	0.271	*P* =0.001
GGT	1	4.330	0.037	0.945	0.896~0.997	-	-
	2	0.620	0.431	0.977	0.922~1.035	-	-
	3	3.246	0.072	0.944	0.887~1.005	-	-
Glb	1	5.542	0.019	1.263	1.040~1.535	-	-
	2	0.570	0450	1.087	0.875~1.350	-	-
	3	0.076	0.782	1.034	0.814~1.314	-	-
FINS	1	0.955	0.328	1.585	0.629~3.995	-	-
	2	2.681	0.102	0.435	0.161~1.178	-	-
	3	0.137	0.711	1.230	0.411~3.677	-	-

Table 5 Multinomial logistic regression analysis was performed to screen predictors for hepatic fibrosis stages in HBeAg-negative CHB patients. Categorical variables were defined as follows: S0: 0, S1: 1, S2: 2, S3: 3, S4: 4.

S	Index	*Wald* $\chi 2$	*P*	*OR*	*95% CI*	Cox and Snell R^2	Model *P*
S1	BMI	0.019	0.890	1.090	0.320~3.717	0.170	0.011
	TC	1.796	0.180	66.911	0.143~31279.998		
S2	BMI	0.404	0.525	0.677	0.203~2.256	0.170	0.011
	TC	2.720	0.099	171.712	0.379~77702.459		
S3	BMI	0.174	0.677	0.776	0.236~2.556	0.170	0.011
	TC	2.838	0.092	185.683	0.426~80963.996		
S4	BMI	0.098	0.754	1.220	0.352~4.220	0.170	0.011
	TC	4.314	0.038	694.314	1.446~333294.121		

Discussion

HBeAg-negative and HBeAg-positive hepatitis are two different types of chronic hepatitis B with distinct clinical features (9). The prevalence of HBeAg-negative hepatitis as well as non-alcoholic fatty liver disease (NAFLD) has been increasing in the past decades (4,10). Increasing studies on chronic hepatitis C with hepatic steatosis have been conducted, but little is known about CHB with steatosis. Hepatic steatosis may have different influences on the liver affected by other diseases. Therefore, it cannot always be considered as a "benign" condition and simply ignored. On the contrary, it has to be recognized as a "co-factor" capable of affecting the gravity and progression and also therapeutic perspectives of liver diseases.

We compared the clinical and histological characteristics between HBeAg-negative CHB patients with and without steatosis, and the results demonstrated significant increases in BMI, FBG, FINS, TG, TC, GGT, ALP, Glb and HOMA-IR in patients with steatosis, implying that obesity, diabetes and hyperlipemia appeared to be the risk factors in patients with steatosis, and insulin resistance might play an important role (12). HBeAg-negative CHB is characterized by low spontaneous remission, frequent ALT flare, easy progression to cirrhosis, low HBV DNA titer and curative difficulty, and thus hepatic steatosis will definitely increase the difficulty of therapy in HBeAg-negative HB patients (13).

In comparison to HBeAg-negative CHB with hepatic steatosis, the ALT, AST and HBV-DNA levels were higher in patients without steatosis, indicating that the ALT and AST flares may be associated with HBV DNA titer in our study, while in patients with hepatic steatosis, these parameters are more likely related to hepatic steatosis. Thus, for the treatment of HBeAg-negative CHB with hepatic steatosis, in addition to antivirus therapy and liver protection therapy, insulin resistance reduction, lipid modulation, diet restriction and exercise for prevention and control of risk factors are also important. Some HBeAg-negative CHB patients with hepatic steatosis may even progress into fibrosis and cirrhosis (14). In HBeAg-negative CHB patients with hepatic steatosis, the activity of hepatic inflammation may be associated with NAFLD in the presence of slightly high ALT level and low HBV DNA level. Clinically, it is very difficult to conclude whether hepatitis is from steatosis and/or HBV infection through detecting ALT, HBeAg and HBV DNA levels (15). Under such condition, in addition to detection of the ALT, HBeAg and HBV DNA levels, BMI, FBG, FINS, TG, TC, GGT, ALP, Glb and HOMA-IR are also critical for diagnosis. If these parameters are abnormal, liver biopsy is strongly recommended in order to assess histology and prognosis. Our study demonstrated the significance of liver biopsy in determining the causes of high ALT levels. The most important limitation of this study is the lack of long-term follow-up and evaluation of response to antiviral therapy in HBeAg-negative CHB patients with steatosis.

Acknowledgment

This work was supported by the grant from Science and Technology Commission of Shanghai Municipality (No.054119618) and Technology Fund of Zhangzhou (No. Z04094). We appreciate Dr. Qianglin Duan from Tongji Hospital of Tongji University for critical reading of the manuscript.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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