Chronic hepatitis B associated with hepatic steatosis, insulin resistance, necroinflammation and fibrosis.

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Abstract
Background: The effect of hepatitis B virus (HBV) infection on fatty liver disease is unclear..

Objectives: The aim of this study was to investigate the viral and host causes of fatty liver in chronic hepatitis B (CHB) patients. This study included 88 CHB patients of which 17 were not treated. Liver biopsy was performed in each patient. Group 1 included those with hepatic steatosis (n=28) and group 2 those without hepatic steatosis. The groups were compared in terms of age, body mass index (BMI), Homeostasis Model Assessment- Insulin Resistance (HOMA-IR), viral load, biochemical parameters and histological findings. Patients in group 1 were subdivided according to the degree of steatosis as follows: grade 1 (15 patients, 53.6%), grade 2 (6 patients, 21.4%),and grade 3 (7 patients, 25%).

Results: In group 1 (n=28), mean age, BMI, cholesterol, and HOMA-IR were found to be significantly higher than in group 2 (n=60). There were no significant differences in the positivity of viral load, HbeAg, treatment, fibrosis and other laboratory parameters between the two groups. HOMA-IR was the only independent predictive factor of liver steatosis in patients with CHB in logistic regression analysis.

Conclusion: Hepatic steatosis in CHB patients was associated with host metabolic factors.

Keywords: Chronic hepatitis B, liver steatosis, HOMA-IR, BMI, biopsy

Introduction

Hepatic steatosis (HS) and chronic viral hepatitis are both common causes of chronic liver disease. Chronic hepatitis B is a major cause of liver disease. Most recent estimates indicate that 350 million patients are chronically infected with hepatitis B virus (HBV), worldwide[1]. HS is defined as fat deposition in the liver that exceeds 5% of the gross total weight of the liver, or with more than 5% of hepatocytes containing fat deposits based on microscopic examination[2]. Many agents are capable of inducing HS. Most common cause include drugs, toxins, infections, and metabolic alterations. Non- alcoholic fatty liver disease (NAFLD) is another common chronic liver disease that affects more than a quarter of the general population and has been strongly associated with obesity and metabolic syndrome. Insulin resistance (IR) and metabolic syndrome have also been linked to liver disease[3].

Previous studies have clearly demonstrated that hepatitis C infection is associated with HS. Moreover the relationship between HS and hepatitis C genotype 3 is well established[4]. The prevalence of HS in patients with chronic hepatitis C(CHC) ranges from 35%-81%[5]. Host metabolic factors and the direct cytopathic effect of hepatitis C virus(HCV) have been shown to be significant risk factors for HS[6], although the association between HBV and HS remains unclear. Numerous large series have failed to demonstrate that there is an association between HS and CHB[7,8] whereas some studies

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have shown that HS in CHB patients is associated with the co-presence of metabolic syndrome[9-11].

This study aimed to investigate the histological prevalence of HS in CHB patients undergoing liver biopsy, and to investigate the association of clinical data, IR, severity of liver fibrosis, and necroinflammation with the degree of occurence. We also examined the factors associated with HS and their interaction with viral factors in CHB patients.

Materials and methods

The study included 88 patients with CHB that underwent liver biopsy for diagnostic purposes during a 2-year period. The HS group included 28 patients and the non-HS group included 60 patients. In all cases the diagnosis of CHB was based on the presence of serum HBsAg , elevated liver transaminase for >6 months, and/or HBV DNA more than 10000 copies/ml. Patients taking hepatotoxic drugs that could potentially cause steatosis, those that consumed alcohol regularly, those diagnosed with diabetes and cirrhosis, patients with anti-HCV, anti-HIV or anti-HDV-antibody positivity, and those diagnosed with an autoimmune disease or other known metabolic liver disease were excluded from the study..

Liver biopsy was performed on each patient. Biopsy specimens were evaluated by an experienced hepatopathologist,blinded to the patient's clinical data. Biopsy specimens were considered adequate if they were 1,5 cm long and/or contained at least 4 to 5 portal tracts. Determination of the histological activity index (HAI) and staging of the biopsy specimens were performed according to Knodell's classification[12]: HAI was scored as portal inflammation (0-4), lobular degeneration (0-4), or periportal necrosis (0-10). Grading ranged between 0-3 points (minimal), 4-8 points (mild), 9-12 points (moderate) and 13-18 points (severe). Fibrosis was staged separately on a scale of: 0-6, corresponding to no fibrosis (0), mild (1-2), moderate (3-4), and severe or cirrhosis (5-6). HS was graded according to the Brunt classification as[13]: 0 (no hepatocytes involved with macrovesicular steatosis), 1 (0-33% involved hepatocytes), 2 (33-66% involved), and 3 (>66% involved hepatocytes). In steatosis classification, less than 5% was considered normal, and ≥5% was considered as steatosis. The height and weight of all patients were measured and the BMI was calculated. Based on the BMI, patients were classified as normal (BMI=18.5-24.9), over-weight (BMI=25-29.9), obese (BMI=30-34.9), or extremely obese (BMI= 35-39.9). IR was diagnosed based on the homeostasis model assessment (HOMA-IR) method, using the following equation: IR (HOMA) = (fasting insulin (μU/ml) $\times$ fasting glucose (mmol/L))/22.5[14]. Laboratory data of all patients, including liver function tests, fasting glucose, cholesterol, triglyceride, iron, iron binding capacity, ferritin, alpha fetoprotein (AFP), insulin, C-peptide, and IGF-1 levels, were also determined at the time of liver biopsy.In each patient HBsAg and HBeAg were measured using the macro ELISA method, anti-HDV and anti-HIV were measured with a micro ELISA method. TaqMan real-time polymerase chain reaction assay was used to measure the HBV DNA level.

Statistical analysis

Statistical analysis was performed using SPSS 15.0 for Windows. Demographic, biochemical, virological and histological parameters were compared between the 2 groups.. Descriptive data are expressed as median (range) or mean (standard deviation). Comparison of parametric data between the 2 groups was performed using Student's t-test. Correlation studies were performed using Pearson's correlation coefficient. Categorical variables were compared using the chi-squared test. A two-tailed P value < 0.05 was considered statistically significant.

Results

The 88 CHB included in the study were divided into the HS group(n=28) and non-HS group (n=60) according to liver biopsy findings (Table 1). Among the 28 patients in the HS group, 15 (17%) had grade 1, 6 (7%) grade 2, 7(8%9) grade 3, based on the Brunt classification. In all, 61 of the patients were male and 27 were female and mean age was 31±1.1 years. Among the patients, 4 (14%) of the females and 24 (39%) of the males had HS. Male gender was associated with an increased risk for HS; the difference between genders was significant (p = 0.02).

Table 1. Demographical, laboratory, metabolic and virological features of 88 patients univariate analysis with chronic hepatitis B

Variable			
n	60	28	
Mean age(year)	29.2±10.1	36.8±11.0	0.002
Gender (Male/Female)	37 (61.7)/23 (38.3)	24 (85.7%)/4 (14.3%)	0.02
BMI			
<25	43 (71.7)	9 (32.1)	0.001
25-29.9	15 (25.0)	14 (50.0)	
≥30	2 (3.3)	5 (17.9)	
Virological status			
Viral load	3200 copy/ml (0-7x10^8)	15000 copy/ml (01.1x10^8)	0.95
HBeAg(+)	19 (31.7)	9 (32.1)	0.96
Treatment(+)	14 (23.3)	3 (10.7)	0.16
ALT	70.5 (15-542)	56 (17-320)	0.74
AST	43.5 (15-266)	37 (19-150)	0.59
GGT	28.5 (10-190)	29 (17-499)	0.77
ALP	77 (33-354)	84 (35-130)	0.77
Cholesterol	149.1±29.2	168.1±36.8	0.01
Triglyceride	88 (13-394)	107.5 (43-462)	0.08
Glucose	86±15.2	97±29.4	0.07
Insulin	5.9 (1.2-49)	7.9 (1.2-802)	0.004
C-peptide	2.06 (0.6-11.7)	2.18 (0.2-6.2)	0.63
IGF-1	125 (31-431)	122 (45-262)	0.53
HOMA-IR	1.16 (0.25-5.6)	1.8 (0.14-5.37)	0.004

Mean age, BMI, cholesterol, and average HOMA-IR levels in the HS group were significantly higher than those in the non- HS group. In all, 28 patients were HBeAg (+), of which 9 (33%) patients had HS. 60 patients were HBeAg (-) and 28 were HBeAg (+). In total, 19 (32%) of the HBeAg (-) patients and 9(33%) of the HBeAg (+) patients had HS. Hepatitis serologies were not different between the HS and non-HS groups (P>0.05).

Necroinflammatory activity and fibrosis staging were not associated with HS. Among all the patients, 17 received treatment for CHB, of which 3 had HS, and. 71 patients were untreated, of which 25 had HS. The difference in HS occurrence between the treated and untreated was not significant. Logistic regression analysis utilizing age, sex, BMI, cholesterol and HOMA-IR as variables revealed that only HOMA-IR (OR=1.66, 95% CI: 1.0-2.7) was significantly and independently predictive of hepatic steatosis in patients with Chronic hepatitis B (Table 2).

Table 2. Multivariate logistic regression analysis for hepatic steatosis in patients with Chronic hepatitis B

	OR	95%Cl	P
Age	1.04	0.99-1.1	0.09
Sex	2.48	0.63-9.75	0.19
BMI	1.12	0.96-1.3	0.14
Cholesterol	1.02	0.99-1.04	0.062
HOMA-IR	1.66	1.0-2.7	0.041

Discussion

In the present study, it was found that presence of steatosis in CHB patients does not cause a difference regarding histopathological findings and HBeAg status is not important in the presence of steatosis. Insulin resistance, which is known to be an important factor for steatosis, was identified as the only independent predictor of hepatic steatosis in CHB patients in this study. In recent years, IR has increased in importance and has been linked to chronic liver diseases. IR is associated with complications similar to those associated with chronic HBV and HCV infection, including cirrhosis and hepatocellular carcinoma[15]. In addition, IR is predictive of a poor response to antiviral therapy targeting HCV[16]. HCV infection has been associated with the development of IR. Fasting insulin and the HOMA-IR score are high in HCV-infected patients[17]. In HCV genotype 3-related HS patients, the, HCV replication level has a direct role in the pathogenesis of HS. On the other hand, the association between HBV and HS is unclear. Our literature review revealed only a small number of studies on the prevalence of HS in patients with CHB[8-11]. Wang et al.[18] reported that HBV infection is not associated with the development of HS. Other studies have indicated that accumulation of lipids via activated SREBP-1and PPAR γ[19] is the cause of HS.

This aimed to investigate the histological prevalence of HS in CHB patients undergoing liver biopsy, and to investigate the association of clinical data, IR, degree of liver fibrosis, and necroinflammation with the degree of occurrence. We also examined the factors associated with HS and their interactions with viral factors in CHB patients.. One study reported that HS was observed in 27% of CHB patients[20]. In the present study age , gender, BMI, serum cholesterol, HOMA-IR and hypertension were strongly associated with HS. Interestingly HS was more common in male patients. Moreover, viral load and the severity of HS were not correlated. Furthermore, HS was not correlated with HBeAg positivity. Unfortunately, genotyping of HBV could not be conducted in the present study for various reasons. Another interesting finding was that triglyceride levels were not associated with HS.

A relationship was observed in the present study between liver damage and HS in CHB patients. This finding is in agreement with previously reported literature [21]. Moreover , the severity of HS was not associated with the severity of fibrosis, which is in agreement with

earlier reports[9,22]. The primary determinant of HS in the present study was HOMA-IR. HS and non-HS patients did not have significant differences in the treatments that were provided. Recently Machado et al. and Lesmana et al. reported their results on hepatosteatosis in CHB. Similar to our study, Machado found that hepatosteatosis was linked to metabolic alterations and was lower in rate when compared to hepatitis C patients[23]. Lesmana on the other hand specifically found a correlation with central obesity[24].

The limitations of the present study are the small number of patients and use of univariate for most analysis.

Conclusion

HS was observed in 33% of cases of HBV-related chronic liver disease. In patients infected with HBV, HS was primarily associated with metabolic factors, such as obesity, IR, and dyslipidemia rather than viral factors. Additional large-scale studies are needed to further evaluate the effect of HS on the outcome of antiviral therapy in CHB patients.

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