

RESEARCH ARTICLE

Hepatic Steatosis: Prevalence and Host/Viral Risk Factors in Iranian Patients with Chronic Hepatitis B Infection

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Abstract

Background: In chronic hepatitis B (CHB), the presence of hepatic steatosis (HS) seems to be associated with known host and viral factors which may influence the long-term prognosis of chronic hepatitis B (CHB), probably leading to cirrhosis and hepatocellular carcinoma (HCC). Different from chronic hepatitis C (CHC), factors associated with HS in CHB are not clearly explored. **Materials and Methods:** 160 CHB patients were divided into two groups depending on the results of liver biopsy. Group I consisted of 71 patients with confirmed steatosis. Group II comprised 89 patients without steatosis. The groups were compared in terms of basal characteristics, body mass index (BMI), liver enzymes (ALT, AST, ALP), serum fasting blood sugar (FBS) and lipids, hepatitis B e antigen (HBeAg), viral load, and histological findings. **Results:** In terms of host factors, male gender, older age, BMI, high serum FBS and lipid levels were associated with HS. On the other hand, ALT levels, the HAI scores of necroinflammation and stage of fibrosis did not associate with HS. On multivariate analysis, parameters of sex, BMI, cholesterol and FBS levels were independently associated with HS. Regarding viral factors, HBeAg negativity was significantly associated with HS (81.7%, p value 0.006), but not HBV DNA level (p value 0.520). **Conclusions:** HS in CHB appears to be unrelated to the status of HBV replication. However, fibrosis progression in CHB is related to variable host factors. HS may be enhanced through these factors in HBV chronic patients.

Keywords: HBV - steatosis - host factors - fibrosis

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Introduction

Hepatitis B virus (HBV) chronic infection frequently leads to undesirable complications like cirrhosis and hepatocellular carcinoma (HCC). One of the potential risk factors for the progression to cirrhosis includes hepatic steatosis (HS). HS is characterized by the deposition of lipid droplets, mainly triglycerides, in hepatocytes that exceed 5% of the total weight of liver, or excessive fat accumulation in more than 5% of hepatocytes cytoplasm under light microscopic examination (Schiff et al., 1999; Powell et al., 2005). The accumulation of lipids within the hepatocytes exposes the liver to injury from a variety of causes (Yang et al., 1997; 2001; Wan et al., 2000), which make them vulnerable to factors associated with further hepatic injury by their increased sensitivity to oxidative stress and to cytokine-mediated hepatic damage (Ates et al., 2011). HS is also shown to be associated with higher programmed cell death by apoptosis with stellate cell activation (Walsh et al., 2004; Basaranoglu and Basaranoglu, 2011). Moreover, HS can influence the

progression of chronic liver diseases to non-alcoholic steatohepatitis (NASH) with development and acceleration of fibrosis, which is associated with an increased risk of cirrhosis or even a cause for hepatocellular carcinoma (Matteoni et al., 1999; Tsochatzis et al., 2007; Starley et al., 2010). Concerning the latter, lines of evidence support the notion that HS may account for a large proportion of cirrhosis (and also idiopathic or cryptogenic cirrhosis), which predisposes these patients to the development of HCC (Bugianesi, 2007; Kim et al., 2010). Moreover, HS could influence the response to given therapy (Jiang et al., 2006; Ates et al., 2011).

Hepatic steatosis may occur concurrently in the setting of viral hepatitis B and C with variable frequencies ranging from 4.5% to 76% and from 31% to 72%, (Bach et al., 1992; Scheuer et al., 1992; Roberts et al., 1993; Gordon et al., 2005; Sass et al., 2005; Minakari et al., 2009; Jin et al., 2012; Lesmana et al., 2012). In patients with chronic hepatitis C (CHC), the source of steatosis can be related to the host factors (metabolic syndrome) and/or related to the virus itself (complicated with HCV genotype 3). Also,

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serum and intra-hepatic HCV RNA/HCV core protein titers have been correlated reportedly with steatosis in genotype 3 suggesting a viral aetiology (Fujie et al., 1999; Lagging et al., 2002; Gordon et al., 2005). HS has been noted in up to 70% of HCV biopsies in particular common with genotype 3 (Lonardo et al., 2004; Ratziu et al., 2004; Harrison et al., 2005; Yoon and Hu, 2006).

However, preliminary data from HBV patients also suggest that hepatic steatosis in these patients may be related to host factors rather than viral factors (Altıparmak et al., 2005; Gordon et al., 2005; Thomopoulos et al., 2006; Bondini et al., 2007). Accumulating data show that while the association between steatosis and HCV is specific, this is not the case in HBV-infected patients (Ates et al., 2011).

The aim of this study was to compare CHB patients with steatosis in terms of demographic, virological, biochemistry and histological parameters with control group (CHB patients without steatosis) and to determine the factors that could be potentially associated with steatosis in CHB patients.

Materials and Methods

Patients

160 HBsAg-positive chronic carrier patients who were referred to the Iranian Hepatitis Network and Digestive Disease center for GI, Tehran during (2010-2011) were enrolled in a cross-sectional study. They had no evident of co-infection with hepatitis C virus, human immunodeficiency virus (HIV), and they were treatment-naïve. None had history of alcohol consumption, drug-induced hepatitis or other known diseases. The diagnosis of chronic CHB disease was made by clinical, biochemical, radiological and endoscopic criteria. The stages and scores of liver pathology and fibrosis were based on biopsy results. All patients gave their informed consent prior to liver biopsy. The clinical data of all patients were retrospectively reviewed in order to collect information about previous laboratory tests and demographic (gender and age) data.

Demographic information including age, gender, weight, height, and body mass index (BMI, calculated the body weight in kilograms divided by the height in meter squared) were obtained from all patients. The study was approved by the Local Committee of Ethics and conformed to the ethical guidelines of Tehran Hepatitis Center.

Serum assay

Venous blood samples were taken in the morning after a 12 hours overnight fasting and the following parameters were determined: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), fasting blood sugar (FBS), cholesterol (CHOL), triglyceride (TG) levels, as well as prothrombin time (PT), total bilirubin (TBIL) and direct bilirubin (DBIL).

HBV serological tests

Serological markers for HBV including HBsAg, HBeAg, and anti-HBe, as well as anti-HDV were confirmed in stored serum samples from all patients. All

serological markers were measured by enzyme linked immunosorbent assay (ELISA) using a commercial kit (Enzygnost® HBS Ag, USA).

HBV DNA quantification

HBV DNA was extracted from 200- μ L serum samples using the QIAamp DNA mini-kit (Qiagen, Hilden, Germany) and eluted in 100- μ L according to the manufacturer's instructions. Total HBV DNA was determined by the COBAS-TaqMan real-time PCR (Roche Molecular Diagnostics, NJ, USA) and was expressed in copy/ml according to the manufacturer's instructions.

Histological evaluation

Liver biopsy was performed on all patients (stained with H&E, Masson's trichrome, and Reticulin). For the purpose of this study, the microscopic slide sets of 160 hepatitis B patients were reviewed by two independent pathologist's expert in histological diagnosis of liver diseases. Necroinflammation of liver lesions (grading) were evaluated according to the Knodell score for activity: periportal bridging necrosis (0-10), intralobular degeneration (0-4) and focal necrosis (0-4). Thus, 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). The four histological criteria of hepatic steatosis: ballooning degeneration, lobular and portal inflammation were scored zero to 3 according to the existence and severity of each criterion (grade 0 without steatosis, grade 1 mild, grade 2 moderate and grade 3 severe). According to histopathological finding based on biopsy results, 71 (44.4%) of CHB patients had steatosis (Group I), whereas 89 (55.6%) of those patients did not (Group II). The latter group was chosen as control group.

Statistical analysis

Data were analyzed with the Statistical Program for Social Sciences (SPSS-16, SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as the mean \pm standard deviation (SD) or median and were compared using independent t-test or Mann-Whitney U-test. Categorical variables were expressed as percentages, and differences between groups were judged for significance using the chi-squared test or Fisher's exact test. Multivariate logistic regression was performed to establish independent predictors of steatosis and high fibrosis stage. For all comparisons, p value <0.05 was considered as statistically significant.

Results

General characteristics

160 HBsAg-positive chronic patients were enrolled in this study, all were chronic carriers, HBV DNA positive and treatment-naïve with a mean ALT level of 76.81 \pm 74.14 IU/L. The median HBV DNA level was 2.8 \times 10⁵ (range 0 to 5 \times 10⁸) copy/mL. The main characteristics of patients shown in Table 1. 71 (44.4%) had biopsy proven steatosis (Group I), whereas 89 (55.6%) were without

steatosis (Group II). In total, 116 (72.5%) were males and 44 (27.5%) were females with a mean age of 35.89 (12.33±SD). Group I was composed of 58 (50%) male and 13 (29.5%) female, whereas, Group II was consisted of 58 (50%) male and 31 (70.5%) female (p=0.02), indicating that male gender was more susceptible for acquiring steatosis (Table 1). The mean age values for group I and II were 39.13±12.56 and 33.31±11.60 years, respectively (p=0.03) (Table 1), highlighting that steatosis prevalence increased with advanced ages.

The total BMI (kg/m²) values for groups I and II were 26.67±4.06 and 22.92±3.14, respectively (p>0.001) (Table 1), therefore, patients with increased weight showed a more chance for acquiring liver steatosis.

Virological features

Of all patients, 113 (70.6%) and 47 (29.4%) were positive for anti-HBe and HBeAg, respectively. 13 (18.3%) and 34 (38.2%) were positive for HBeAg in group I and II, respectively, on the other hand, 58 (81.7%) and 55 (61.8%) were positive for anti-HBe in group I and II, respectively. Results of chi-square tests showed that there was a significant associations between both groups in terms of HBeAg negativity (p=0.006), indicating the presence of steatosis with anti-HBe positivity in CHB patients. On the other hand, when HBeAg/anti-HBe positivity was compared with the age classification: <25, 25-29, 30-34 and >35 years old, the same statistical methodology showed no strong associations between

HBeAg/anti-HBe status of patients and steatosis (results not shown).

The mean HBV viral load of patients in group I and II were 2.85×10⁵ (range 427 to 4×10⁸) and 2.74×10⁵ (range 0 to 5×10⁸) copies/mL, respectively, however, these values did not reach statistically significant (p=0.52, Table 1).

Five patients in each of both groups (7% and 5.6% in groups I and II, respectively) were positive for HDV antibody. There was no significant association between HDV positivity and the presence of steatosis (Table 1).

Biochemistry features

The mean levels for liver enzyme tests (including: ALT, AST, Alkaline phosphatase, bilirubin (total and direct) and prothrombin time (PT) were not significantly different between both groups (Table 1). However, the fasting blood glucose and serum lipids including cholesterol and triglycerides levels were higher in group I (with steatosis) compared with control group with strong associations (p values for all three ≤0.001).

Histological features

Liver histological examination showed that among the total 160 CHB patients, 30 (18.8%) contained low inflammation (HAI score 0-3), 93 (58.1%), 34 (21.2%) and 3 (1.9%) patients had mild (HAI score 4-8), moderate (HAI score 9-12) and severe (HAI score 13-18) histopathologic changes, respectively (Table 1). As it shown in table 1, the mean HAI scores for groups I and II, (6.11±2.78 versus

Table 1. Univariate Analysis of the Association of Steatosis with Clinical and Virologic Parameters in Patients with CHB

Parameter	All patients No (%)	Steatosis (-) n=89	Steatosis (+) n=71	p value
Male, n (%)	116 (72.5)	58 (50)	58 (50)	0.020
Female, n (%)	44 (27.5)	31 (70.5)	13 (29.5)	
Age	35.89±12.33	33.31±11.60	39.13±12.56	0.03
BMI (kg/m ²), n (%)	24.58±4.0394	22.92±3.14	26.67±4.06	<0.001
<25	94 (58.8)	73 (82)	21 (29.6)	<0.001
≥25	66 (41.2)	16 (18)	50 (70.4)	
CHOL (mg/dL)	161.68±47.88	142.69 ± 42.97	185.49±44.27	<0.001
TG (mg/dL)	114.23±58.26	100.39±44.97	131.58±67.96	0.001
FBS (mg/dL)	92.91±30.13	83.56±15.47	104.79±38.96	<0.001
ALT (IU/L)	76.81±74.14	67.70±47.10	84.07±89.72	0.166
AST (IU/L)	52.97±55.79	52.80±41.53	53.18±70.02	0.966
ALP (IU/L)	192.59±89.35	194.48±93.76	190.21±84.10	0.765
PT (second)	12.90±1.37	12.87±1.59	12.94±1.05	0.741
TBIL (mg/dL)	1.10±0.62	1.07±0.65	1.13±0.59	0.550
DBIL (mg/dL)	0.27±0.16	0.27±0.15	0.28±0.17	0.521
Median viral load (copies/mL)	2.80×10 ⁵ (range 0 to 5×10 ⁸)	2.74×10 ⁵ (range 0 to 5×10 ⁸)	2.85×10 ⁵ (range 427 to 4×10 ⁸)	0.520
HAI score	6.18±2.97	6.24±3.12	6.11±2.78	0.792
0-3	30 (18.8)	17 (19.1)	13 (19.3)	0.504
4-8	93 (58.1)	52 (58.4)	41 (57.7)	
9-12	34 (21.2)	17 (19.1)	17 (23.9)	
13-17	3 (1.9)	3 (3.4)	0 (0.0)	
Stage	1.92±1.31	1.76±1.19	2.11±1.44	0.104
0	20 (12.5)	10 (11.2)	10 (14.1)	
1-2	83 (51.9)	54 (60.7)	29 (40.8)	
3-4	52 (32.5)	23 (25.8)	29 (40.8)	
5-6	5 (3.1)	2 (2.2)	3 (4.2)	
HDV, n (%)	Negative	84 (94.4)	66 (93.0)	0.752
	Positive	10 (6.2)	5 (7)	
HBeAg, n (%)	Negative	113 (70.6)	58 (81.7)	0.006
	Positive	47 (29.4)	13 (18.3)	

*BMI: body mass index; CHOL: cholesterol; TG: triglycerides; FBG: fasting blood glucose; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; PT: prothrombin time; TBIL: total bilirubin; DBIL: direct bilirubin; HDV: hepatitis D virus; HBeAg: hepatitis B e antigen

Table 2. Multivariate Analysis of the Association of Steatosis with Clinical and Virologic Parameters in Patients with CHB

Parameter	Odds ratio	Lower to upper p5% CI	p value
Male sex	0.393	0.077-0.714	0.011
Cholesterol	1.02	1.007-1.029	0.001
FBS	1.049	1.020-1.079	0.001
BMI	1.345	1.170-1.546	<0.001

6.24±3.12, respectively [p=0.792]), indicating that there was no significant association between patients with and without steatosis in terms of liver grade scores.

Ishak stages of fibrosis for 160 patients were: 0 (no fibrosis) for 20 (12.5%), 1-2 (mild) for 83 (51.9%), 3-4 (moderate) for 52 (32.5%) and 5-6 (severe) for 5 (3.1%) (Table 1). As it shown in Table 1, no significant association were found between groups I and II in terms of Ishak biopsy results for the stages of liver fibrosis (mean stages: 2.11 ±1.44 and 1.76±1.19 for groups I and II, respectively, p=0.104). However, by multivariate logistic regression analysis, when the cut off of >3 was used as criterion decision for severe fibrosis, we found such strong relationship between fibrosis and steatosis for stages >3 (p=0.026-results not shown).

Also, the severity of steatosis were compared within the group I (steatosis patients) and results showed that 45 (63.3%), 20 (28.2%) and 6 (8.5%) of patients were classified as being mild (Grade 1), moderate (Grade 2), and sever steatosis (Grade 3), respectively (results not shown).

Multivariate analysis

The final multivariate model for predictors of hepatic injury is shown Table 2. Sex (odds ratio [OR] 0.393, 95% confidence interval [CI] 0.077-0.714), BMI (OR 1.345, 95% CI 1.170-1.546), Cholesterol (OR 1.020, 95% CI 1.007-1.029) and FBS (OR 1.049, 95% CI 1.020-1.079) levels were independently influence HS. The Cox and Snell and Nagelkerke R Square of this model were 0.418 and 0.560, respectively.

Discussion

In patients with chronic hepatitis C, steatosis of the liver increases the severity of fibrosis and adversely influences the response to given therapy; however, the association of liver steatosis and CHB is less clear.

Our study evaluated the frequency of hepatic steatosis in liver biopsies of 160 adult CHB patients to determine whether the presence of steatosis was associated with viral and host metabolic factors and its potential impact on the severity of necroinflammation and stage of fibrosis was also evaluated. Previous studies showed that hepatic steatosis is a common phenomenon in CHB patients. In the present study, the prevalence of hepatic steatosis was 44.4% in CHB patients, in agreement with most published reports and higher than that in Iranian general population of 27-51% (Sass et al., 2005; Jin et al., 2012), hinting its potential effects in CHB.

On univariate analysis we found that the presence of

steatosis was significantly associated with male gender, older age, BMI index, high serum glucose (FBS) and lipid levels and HBeAg negativity, similar to other studies (Malhotra et al., 2000; Bondini et al., 2007; Minakari et al., 2009). Indeed, sex, BMI, Cholesterol and FBS levels was found on multivariate analysis to be the parameters to independently were associated with the presence of steatosis. On the other hand, we did not find any significant association between steatosis and HBV DNA and ALT levels and the HAI scores of necroinflammation. In accordance to previous reports, we found that hepatic steatosis was significantly associated with higher body mass index and waist circumference. Many studies have reported the association of hepatic steatosis and metabolic factors such as obesity or high body-mass index index (Thomopoulos et al., 2006; Cindoruk et al., 2007; Zheng et al., 2010; Lesmana et al., 2012), waist circumference (Bondini et al., 2007; Lesmana et al., 2012) hypertriglyceridemia (Cindoruk et al., 2007; Zheng et al., 2010; Rastogi et al., 2011; Nascimento et al., 2012), hyperglycemia (Thomopoulos et al., 2006; Zheng et al., 2010). The FBS level in hepatic steatosis group was significantly higher, supporting the coexistence of dys-regulated glucose metabolism. Previous works found that BMI and TG were independent factors for hepatic steatosis (Peng et al., 2008; Minakari et al., 2009; Zheng et al., 2010; Ates et al., 2011).

There was no significant association between patients with and without steatosis in terms of liver grade scores. Likewise, in other reports, no significant association was found between the stage of fibrosis and the severity of steatosis (Bondini et al., 2007; Peng et al., 2008; Rastogi et al., 2011). However, by univariate analysis, when the cut off of >3 was used as criterion decision for severe fibrosis, a strong relationship between fibrosis and HS was achieved (p=0.026), a finding similar to two reports (Shi et al., 2008; Petta et al., 2011). Moreover, on multivariate analysis when we compared the HIA scores with the stages of fibrosis in both groups of patients, higher stages of fibrosis (>3) were associated with HAI scores of necroinflammation (P value<0.001), consistent with other reports of patients with CHC and CHB (Altıparmak et al., 2005; Peng et al., 2008). Serum triglyceride level was found on multivariate analysis to be the only parameter to independently influence HS. The blood sugar, serum leptin, c-peptide levels, and the waist circumference did not reveal any statistically significant association. These parameters associated with superimposed NASH in patients with CHB are known components of metabolic syndrome.

In terms of viral factors, a majority of CHB patients who had HS were HBeAg-negative (81.7%, p value 00.6) with approximately 16.7% of them having at least moderate HS. However, other studies did not confirm a strong association between either the presence or the severity of HS and HBeAg status of CHB patients (Bondini et al., 2007; Tsochatzis et al., 2007; Ates et al., 2011). Further, no significant association was found between HBV-DNA and HS. In fact, previous reports showing that HBV-DNA titre, HBeAg status and liver enzymes did not associate with the presence of hepatic

steatosis or NASH (Altıparmak et al., 2005; Bondini et al., 2007; Tsochatzis et al., 2007; Rastogi et al., 2011). A possible explanation for the lack of association between HBeAg negativity (and also HBV DNA levels) in all studies so far, could be the fluctuation of HBV replication in HBeAg-negative CHB patients together with bias on patients selection (as a majority of CHB patients included in investigations were HBeAg negative). However, this hypothesis does need the ability to differentiate active CHB status from non-active CHB status in longitudinally or perhaps cohort studies to elucidate the relationship between HBV-DNA levels and the severity of fibrosis.

On the other hand, a role of steatogenic effect for HBx protein in the pathogenesis of HCC has been described (Kim et al., 2001; 2007). In a study by Na et al, this role has been attributed to the expression and transcriptional activity of liver X receptor α (LXR α), a regulatory protein in the expression of genes involved in the metabolism of lipids and cholesterol which was increased in HBV-associated HCC in transgenic mice (Na et al., 2009).

The cause and clinical importance of hepatosteatosis accompanying CHB are not well defined. Therefore, we cannot draw any conclusions about the ability of HBV to cause steatosis directly or indirectly. As a result, the progression of steatosis in CHB may be primarily due to the host metabolic status of the host, not directly affect the development of steatosis. These findings probably imply that HBV does not induce accumulation of hepatic steatosis and we believe that steatosis in HBeAg-negative CHB is due to host metabolic risk factors and is not related to the virus per se.

In the present study, HBV genotyping could not be performed due to lack of enough materials for further molecular studies. Regarding the association between HS and HBV genotypes, in one report, authors found that hepatic steatosis tended to present more among patients with viral genotype C (37.9%) compared to genotype B (24.0%) (Lesmana et al., 2012), while other study did not reveal statistically significant differences between genotype A and D (Rastogi et al., 2011). Similarly, two studies from Turkey, a region with HBV genotype D predominance, showed no association between HS and viral genotypic factors (Altıparmak et al., 2005; Ates et al., 2011). Hepatitis B infection in Iran is accepted to be virtually all genotype D (almost 100%); hence, a genotype effect is not expected to influence HS in our CHB patients. It seems that these genotypes do not seem to be steatogenic per se. Nonetheless, there is little data in the literature addressing this relationship. This is in contrast to CHC, in which genotype 3 directly involved in the development of steatosis (Gordon et al., 2005; Lonardo et al., 2006; Yoon and Hu, 2006).

In conclusion, the presence of steatosis associate with various host factors and HBeAg negativity, but not with viral load. Additional studies on molecular aspects and prospective epidemiological studies are needed to explore the real impact of HBV infection on the pathogenesis of steatosis in CHB patients.

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