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STEATOSIS HAS A PROTECTIVE EFFECT AND IS NOT ASSOCIATED WITH MORE SEVERE DISEASE IN HBEAG POSITIVE CHRONIC HBV WITH RAISED ALANINE AMINOTRANSAMINASE

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ABSTRACT

Introduction: The impact of steatosis on hepatitis B e antigen (HBeAg)-positive chronic hepatitis B virus (HBV) infection with raised serum alanine aminotransaminase (ALT) remains unknown. **Aim:** To determine the effect of steatosis in 112 treatment naive HBeAg-positive chronic HBV patients with raised serum ALT. **Methods:** Sixty-one of the 112 patients (54.5%) had steatosis on liver biopsy (HBV plus Steatosis Group) while 51 of the 112 patients (45.5%) did not (HBV without Steatosis Group). Significant chronic hepatitis was defined as having a modified histologic activity index 4-16. **Results:** Fibrosis stage was similar between the HBV plus Steatosis Group and HBV without Steatosis Group (mean 3.03 vs. 3.00; p=NS). Significant chronic hepatitis was also similar between the HBV plus Steatosis Group and HBV without Steatosis Group [53/61 (86.9%) vs. 38/51 (74.5%), p=NS]. The HBV plus Steatosis Group had a lower intrahepatic hepatitis B surface antigen (HBsAg) when compared with the HBV without Steatosis Group (mean 0.49 vs. 1.00, respectively; p<0.001). Steatosis correlated negatively with intrahepatic HBsAg (r=-0.43, p<0.001). There was a trend that the HBV plus Steatosis group had a lower median serum HBV DNA level when compared with the HBV without Steatosis Group (6.73 vs. 7.62 Log IU/ml; p=0.07). **Conclusion:** In HBeAg positive chronic HBV infection with raised serum ALT levels, steatosis is not associated with an increased severity of liver disease. Steatosis with a lower intrahepatic HBsAg may offer protective effect against HBV.

KEYWORDS

INTRODUCTION

Hepatic steatosis, defined as collections of triglyceride within hepatocytes, is an important histological finding in chronic hepatitis C virus (HCV) infected patients.[1-4] The effect of steatosis on the liver, whether by directly causing liver injury or as a marker of increased oxidative stress, is still uncertain.[5,6] Obesity and type 2 diabetes mellitus contributes to steatosis in a variety of liver diseases. This includes metabolic-associated fatty liver disease (MAFLD), nonalcoholic fatty liver disease (NAFLD), chronic HCV and alcoholic liver disease.[1,2,7,8]

HCV-infected patients are more likely to have risk factors for MAFLD and NAFLD, like high body mass index and serum triglyceride level.[1] There is significant association between steatosis and inflammation and fibrosis stage on liver biopsy in those with chronic HCV infection. Furthermore, HCV genotype 3a is more strongly linked to steatosis than other genotypes.[4,9]

NAFLD is an important cause of chronic liver disease, cryptogenic cirrhosis and hepatocellular carcinoma.[10] The histological spectrum of NAFLD ranges from pure fatty liver to the more extreme form of steatohepatitis. In addition to simple steatosis, histological characteristics of non-alcoholic steatohepatitis (NASH) include ballooning degeneration, lobular neutrophilic infiltration, presence of Mallory hyaline and perisinusoidal fibrosis.[11,12] Patients with NASH are at higher risk of progressive disease when compared to patients with only simple steatosis. There is a strong association between NAFLD and metabolic syndrome, which is characterized by obesity, hypertension, hypertriglyceridemia, type II diabetes mellitus and insulin resistance.[13]

Although metabolic syndrome has traditionally been linked to the affluent Western lifestyle, its prevalence among the Oriental population is increasing. Therefore, it is conceivable that NAFLD and its complication will constitute a significant healthcare burden in this part of the world in the future. However, unlike the situation in Western countries where chronic HCV infection predominates, the Asia-Pacific region has a high prevalence of chronic hepatitis B virus (HBV) infection.[14-16] The impact of steatosis on hepatitis B e antigen (HBeAg) positive chronic HBV infection with raised serum alanine aminotransaminase (ALT) remains to be elucidated.

In order to determine the effect of steatosis on liver disease in chronic HBV infected patients, we conducted a cross-sectional study on patients with HBeAg positive chronic HBV infection with raised serum ALT levels with and without steatosis.

Patients and Methods

Patients

One hundred and twelve HBeAg positive chronic HBV patients with raised serum ALT who underwent a liver biopsy before starting anti-HBV therapy were retrospectively identified from our database and included into this study. All patients were seen at the Centre for Digestive Diseases from 2005 to 2010. All patients fulfilled the following inclusion criteria: 1) hepatitis B surface antigen (HBsAg) and HBeAg positive for more than 12 months before liver biopsy; 2) persistently raised serum ALT levels (normal value 7-53 U/L for males and 7-33 U/L for females) on four consecutive readings three months apart before liver biopsy; 3) serum HBV DNA more than 10^3 IU/ml; 4) alcohol intake less than 5 gram/day as previously defined [2]; 5) negative for antibody to hepatitis C virus, antibody to hepatitis delta virus and antibody to human immunodeficiency virus by enzyme immunoassays (Abbott Laboratories, Chicago, IL, USA); and 6) treatment naive.

Virological Studies

HBsAg, HBeAg, HBeAb (Abbott Laboratories, North Chicago, Ill, USA) and anti-HCV (Ortho Diagnostics System, Raritan, New Jersey, USA) were assayed with the second-generation enzyme-linked immunosorbent assay. Serum HBV DNA was quantified with the Abbott real time HBV assay (Abbott Laboratories, North Chicago, Ill, USA) with a linear range of 10-10^9 IU/ml.[17,18] Quantification of HBeAg was performed retrospectively on frozen sera as previously described.[18]

Histology

Percutaneous liver biopsy was performed on the patients with a 16G Temno needle. Liver histology was assessed by a pathologist specializing in liver diseases who was blinded to the clinical data. A sample was considered adequate if it was longer than 1.5 cm and contained six portal tracts or more. Liver biopsy specimens were prepared with haematoxylin and eosin stain, Masson trichrome stain, Prussian blue stain, reticulin stain, orcein stain and periodic acid-Schiff stain.

Liver biopsies were scored using the modified histology activity index (HAI) score for inflammation and the Ishak fibrosis stage for staging of fibrosis. Steatosis was scored according to an accepted scoring system: 0, no steatosis; 1, <33% of hepatocyte with steatosis; 2, 33-66% of hepatocytes affected; 3, >66% of hepatocytes affected. The steatosis observed was predominantly macrovesicular.[19-21] Liver biopsies were also stained for HBsAg and hepatitis B core antigen (HBcAg) as previously described.[22]

Modified HAI score was classified as being consistent with normal

pattern, minimal chronic hepatitis (0-3), mild chronic hepatitis (4-8), moderate chronic hepatitis (9-12), and severe chronic hepatitis (13-16) as previously defined.[19,23] Subjects with at least stage 4 fibrosis on liver biopsy were considered as having severe fibrosis. Subjects with fibrosis stage 5 and 6 on liver biopsy were defined as liver cirrhosis. Significant chronic hepatitis was defined as modified HAI score 4-16.

This study was approved by the local Institutional Review Board.

Statistical Analysis

All statistical analyses were performed using the SPSS software (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp, Armonk, New York, USA). The Student t test or Mann-Whitney U test was used for comparing two continuous variables as appropriate. Categorical variables were compared using the chi-square with Yates' correction for continuity or Fisher's exact test. The degree of association between modified HAI score, fibrosis stage and steatosis were assessed using Spearman's correlation. Continuous variables were expressed as mean [standard deviation (SD)] or median [interquartile range 25-75% (IQR)] if data is not normally distributed. All statistical analyses were performed on an intention-to-treat basis. Statistical significance was defined as $p<0.05$ (2 tailed).

RESULTS

Patients Demographics

Sixty-one of the 112 (54.5%) HBeAg positive chronic HBV patients had steatosis on liver biopsy (HBV plus Steatosis Group) while 51 of the 112 (45.5%) HBeAg positive chronic HBV patients did not have steatosis on liver biopsy (HBV without Steatosis Group). The baseline characteristics between these two groups are shown in Table 1 (all p=NS).

The HBV plus Steatosis Group had a lower HBeAg level when compared with the HBV without Steatosis Group (p=0.04) [Table 1]. There was a trend that the HBV plus Steatosis group had a lower median serum HBV DNA level when compared with the HBV without Steatosis Group (p=0.07) [Table 1].

Liver Histology on Biopsy

A comparison of histological features between the 2 groups of subjects is shown in Table 2.

None of the subjects from the 2 groups had normal liver histology. In the HBV plus Steatosis Group, 8 (13.1%) had minimal chronic hepatitis, 42 (68.9%) had mild hepatitis, 11 (18.0%) had moderate hepatitis, and none had chronic hepatitis with severe activity [Table 2]. On the other hand, in the HBV without Steatosis Group, 13 (25.5%) had minimal chronic hepatitis, 23 (45.1%) had mild hepatitis, 11 (21.6%) had moderate hepatitis, and 4 (7.8%) had chronic hepatitis with severe activity (all p=NS) [Table 2]. Fifty-three of the 61 patients (86.9%) from the HBV plus Steatosis Group when compared with 38 of the 51 patients (74.5%) from the HBV without Steatosis Group had significant chronic hepatitis on liver biopsy (p=0.10).

Fibrosis stage was similar between the 2 groups [mean SD 3.03 (1.89) vs. 2.96 (1.81), p=NS]. Twenty-seven of the 61 patients (44.3%) in the HBV plus Steatosis Group had severe liver fibrosis when compared with 20 of the 51 patients (39.2%) in the HBV without Steatosis Group (p=0.59). There was no significant difference in liver cirrhosis between the 2 groups [18/61 (29.5%) in the HBV plus Steatosis Group vs. 11/51 (21.6%) in the HBV without Steatosis Group respectively, p=0.31].

HBV plus Steatosis Group had a Lower Intrahepatic HBsAg staining

The HBV plus Steatosis Group had a lower intrahepatic HBsAg staining when compared with the HBV without Steatosis Group (mean SD 0.490.79 vs. 1.19 0.82, respectively) (p<0.001).

However, there was no significant difference in intrahepatic HBcAg staining when the HBV plus Steatosis Group was compared with the HBV without Steatosis Group (mean SD 0.96 1.15 vs. 0.710.94, respectively) (p=NS).

Higher Steatosis Score was Associated with Less Intrahepatic HBsAg Staining in the HBV plus Steatosis Group

The distribution of steatosis score for the 61 patients in the HBV plus Steatosis Group is shown in Table 2.

Intrahepatic HBsAg staining in the HBV plus Steatosis Group is shown in Table 3. Those with a higher steatosis score had lower intrahepatic HBsAg stain (p=0.04).

Steatosis Correlated Negatively with HBsAg Staining

Steatosis score also correlated negatively with HBsAg staining (r= -0.43, p<0.001) [Figure 1A] but was not correlated with HBcAg staining (p=NS).

Modified HAI Score Correlated with Fibrosis Stage

Modified HAI correlated with fibrosis (r= 0.50, p<0.001) [Figure 1B]. Steatosis did not correlate with either modified HAI, age or fibrosis stage (all p=NS).

DISCUSSION

The significance of steatosis in chronic HBV infection remains to be elucidated. Because hepatic steatosis is primarily associated with diabetes mellitus and alcohol in the general population, we excluded patients with moderate to heavy alcohol intake as defined by Monto et. al. from this study. Contrary to the prevailing belief that alcohol is the primary cause of steatosis in liver diseases, 61 of the 112 patients (54.5%) in our cohort of chronic HBV patients with alcohol intake of less than 5 gram/day had evidence of hepatic steatosis.

Although statistically insignificant, patients with HBV plus steatosis are older than those in the HBV without steatosis Group. Previous studies on chronic HCV infection and steatosis have repeatedly shown old age to be associated with steatosis and is an independent predictor of steatosis.[2] It is postulated that older age patients have increased susceptibility to insulin resistance and its associated metabolic consequences, including hepatic steatosis.[24,25]

This study showed there was a trend that HBV plus Steatosis Group has a lower HBV DNA, lower intrahepatic HBsAg and lower serum HBeAg, when compared with the HBV without Steatosis Group. Moreover, within the HBV plus Steatosis Group, those with higher steatosis score had lower intrahepatic HBsAg (Table 3). This inverse relationship may be due to decreased viral replication or decreased intrahepatic HBV in those with co-existing steatosis. There was also a negative correlation between steatosis score and HBsAg staining [Figure 1A].

Therefore, steatosis may offer some “protection” against HBV replication in those who are HBeAg positive with raised serum ALT levels. The lower intrahepatic HBsAg, serum HBeAg level and serum HBV DNA level in those with steatosis may signify less active disease in this subgroup of chronic HBV patients. This less active liver disease may explain why those with steatosis have a higher rate of HBsAg seroclearance as a less active disease was found to be a significant factor associated with HBsAg seroclearance.[15,16,26-28] The lower serum HBeAg level may also help to facilitate HBeAg seroconversion, the first step required for HBsAg seroclearance.

Steatosis did not correlate with either modified HAI score or fibrosis stage in HBeAg positive chronic HBV patients with raised serum ALT. This means that steatosis does not result in more severe liver inflammation or fibrosis in HBeAg positive chronic HBV with raised serum ALT levels, unlike in chronic HCV infection.[1-3,29]

Histological inflammation has a well-established association with fibrosis, but the process and the circumstances under which inflammation impact on fibrosis remains controversial.[19,20] Although this study found that fibrosis stage correlated positively with the modified HAI score, that is, severe liver inflammation leads to greater liver fibrosis, it failed to demonstrate an association between steatosis and modified HAI score. This implies that firstly, steatosis in HBeAg positive chronic HBV with raised serum ALT levels does not result in worsening of liver inflammation, unlike in those with chronic HCV infection.[3,4] Secondly, in HBeAg positive chronic HBV with raised serum ALT levels, the degree of liver inflammation, rather than steatosis, plays a major role in fibrosis progression. Therefore, early commencement of antiviral therapy should be considered in this subgroup of patients to decrease liver inflammation before liver fibrosis develops.

Due to the retrospective nature of this study, it has several limitations. Firstly, subjects in this study are not representative of community-based cohorts. They are a tertiary clinic based population of HBeAg positive patients with raised serum ALT levels. Selected subjects should not be used to predict steatosis at a community level. Secondly,

the lack of a significant difference does not imply equivalence because of the undetermined power of statistical tests. Thirdly, serum lipid levels are not available for comparison. Whether serum lipid levels are abnormal in patients with chronic HBV is unknown. NASH may have a relationship with lipid abnormalities, most commonly hypertriglyceridemia, with approximately 20% of patients affected.[30] Finally, while still considered a gold standard in diagnosing NAFLD, NASH and for measuring fibrosis stage, histological scoring systems can be unreliable due to potential sampling error and inter-observer variability among pathologists.[16,31] Newer noninvasive and reproducible methods for diagnosing steatosis or measuring liver stiffness such as serum biomarkers and magnetic resonance elastography may be diagnostically superior.[16]

In conclusion, in HBeAg positive chronic HBV infection with raised serum ALT levels, steatosis is not associated with an increased severity of liver disease. Steatosis with a lower intrahepatic HBsAg, may in fact, offer protective effect against HBV.

Table 1. Baseline Demographics Between The Two Groups.

	HBV plus Steatosis Group. (n=61)	HBV without Steatosis Group. (n=51)	P-value.
Age, years	41 (29-57)	38 (25-59)	0.78
Sex; M:F	34:27	32:19	0.45
Albumin, g/dl	42 (38-48)	43 (38-51)	0.63
Bilirubin, mol/L	11 (8-45)	13 (6-65)	0.13
Alanine aminotransaminase, U/L	186 (65-357)	175 (67-430)	0.57
Aspartate aminotransaminase, U/L	125 (45-258)	135 (43-440)	0.37
Prothrombin time, seconds	12.6 (10.2-15.8)	12.8 (9.0-15.8)	0.60
Platelet, X 109 cells/mm3	198 (164-339)	186 (162-334)	0.93
HBV DNA, log IU/ml	6.73 (5.82-7.10)	7.62 (6.12-8.78)	0.07
HBeAg, log PEIU/ml	2.78 (2.17-5.02)	3.88 (3.16-5.17)	0.04

Table 2. Liver Histology In The HBV Plus Steatosis And HBV Without Seatosis Group.

	HBV plus Steatosis Group (n=61)	HBV without Steatosis Group (n=51)	P-value
Mean SD modified HAI score	5.93 2.50	6.47 3.71	0.78
Modified HAI score:			-
1	0	2	
2	6	4	
3	2	7	
4	11	6	
5	7	3	
6	11	7	
7	5	3	
8	8	4	
9	6	6	
10	5	1	
11	0	3	
12	0	1	
13	0	2	
14	0	1	
15	0	0	
16	0	1	
Mean SD Fibrosis stage	3.03 1.89	2.96 1.81	0.65
Fibrosis stage:			-
0	6	3	
1	12	12	
2	6	6	
3	10	10	
4	9	9	
5	13	5	
6	5	6	
Steatosis:			
1	20	NA	
2	34	NA	
3	7	NA	

Table 3. Distribution Of Intrahepatic HBsAg Staining And Steatosis Score In The HBV Plus Steatosis Group.

Steatosis (n)	Intrahepatic HBsAg staining score			
	0	1	2	3
1 (20)	10 (50%)	9 (45%)	0 (0%)	1 (5%)
2 (34)	23 (67.6%)	5 (14.7%)	5 (14.7%)	1 (2.9%)
3 (7)	7 (100%)	0 (0%)	0 (0%)	0 (0%)

HBsAg- hepatitis B surface antigen.

HBsAg- hepatitis B surface antigen; HAI- histologic activity index

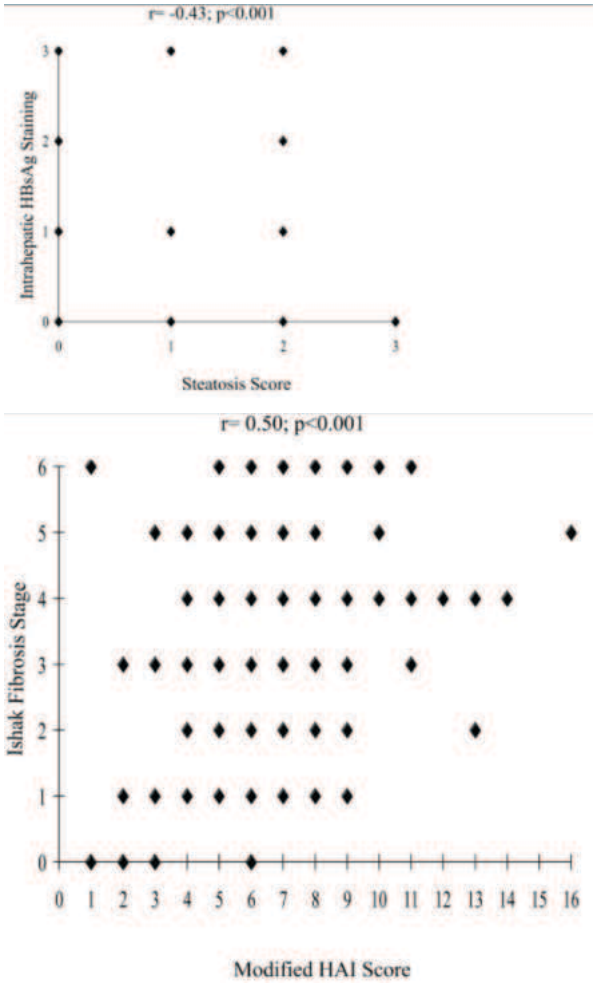

Figure 1. (A) Graph showing correlation between steatosis and intrahepatic hepatitis B surface antigen staining on liver biopsy and (B) Graph showing correlation between modified histology activity index and fibrosis stage. (Total number of data points are 112 in both graphs).

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