Original Article

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Significance of Hepatic Steatosis in Chronic Hepatitis B Infection

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ABSTRACT

Background: Significance of liver steatosis (LS) in patients with chronic viral hepatitis B (CHB) was less clear in terms of prevalence, factors in association, relation to HBV-related liver injury, and pathologic characteristics. This study was aimed to explore these problems.

Results: A total of 60 patients with CHB, liver steatosis were present in 19 patients (31.7%). All viral factors had shown no significant association with liver steatosis ($p>0.05$). Body mass index and fasting HDL-cholesterol showed significant correlation with steatosis ($p=0.007$ for BMI and $p=0.02$ for fasting HDL-cholesterol). Different histologic parameters according to METAVIR was not found among patients with and without liver steatosis ($p>0.05$). Compared to patients without liver steatosis, patients with liver steatosis had more ballooning hepatocytes (89% vs. 32%; $p<0.0001$), Mallory bodies (68.4% vs. 9.8%; $p<0.0001$), large lipogranuloma (31.6% vs. 0%; $p=0.001$), and glycogenated nuclei (26.3% vs. 4.9%; $p=0.028$). Mean NAS was also higher among patients with liver steatosis ($p<0.00001$). Ballooning hepatocytes, degree of lobular inflammation, portal inflammation, fibrosis according to Kleiner's classification, and any Mallory bodies were found to be associated with significant HBV-related liver disease ($p<0.05$). Limitations of these preliminary results were mainly due to its relatively small sample size and inability to perform multivariate analysis. More patient enrollment is needed to finalize the results.

Key words : Liver steatosis, chronic viral hepatitis B, NAFLD

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INTRODUCTION

Liver steatosis (LS) and chronic infection with hepatitis B virus (HBV) are two common causes of chronic liver disease. LS is defined as fat deposition in the liver that exceeds 5% of the total weight of liver, or with more than 5% of hepatocytes containing fat deposits under light microscopic examination. Common causes of LS are alcohol, drugs, nutritional disorders, and metabolic diseases[1-4]. LS is also a common histopathological feature of chronic hepatitis C (CHC) and chronic hepatitis B (CHB). Prevalence of NAFLD has increased in the past couple years in relation to the increasing number of obesity, type 2 diabetes mellitus, and dyslipidemia[5].

There are some studies which described pathological characteristics of liver steatosis and steatohepatitis. Steatosis and pericellular fibrosis tends to occur primarily in zone 3. Additional features for

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strengthening the diagnosis of steatohepatitis include Mallory's hyaline perisinusoidal fibrosis, portal inflammation, acidophilic bodies, glycogenated nuclei, periodic acid stain after diastase Kupffer cells and lipogranulomas[6,7]. In contrast, the pathological features of chronic viral hepatitis B are primarily confined to portal and periportal area (zone1).

The frequency of steatosis in CHB ranges from 27 to 51%, which is higher than that of normal population[8-15]. Association of chronic HBV infection and LS has not been extensively studied. Few studies showed only the role of host factors on LS. Some studies showed that the steatosis in CHB seems to be a result of metabolic factors of the host rather than the effect of viruses and no correlation exists between the presence of steatosis and stage of fibrosis in CHB[16-20], but other in vitro study showed that increased X protein expression from HBV can induce lipid accumulation in hepatocytes[21].

This controversial result that could not summarize whether host or viral factors is the cause of steatosis, and the lack of pathological description of steatosis in patient with chronic viral hepatitis, leads us to investigate the prevalence of histologic evidence of steatosis in a group of patients with chronic HBV infection undergoing liver biopsy and to compare clinical data, laboratory features, and severity of hepatic fibrosis between patients both with and without steatosis. Pathologic description of steatosis in these patients was also investigated.

Patients and methods

A total of 60 patients with CHB, who underwent liver biopsy for evaluation of possibility to receive treatment, at Siriraj hospital were enrolled during the 6-month ongoing study. In all cases, diagnosis of CHB was carried out in the presence of serum HBsAg for at least 6 months and/or HBV-DNA more than 2000 IU/mL.

The study protocol had been approved by Siriraj Institutional Review Board of Mahidol University. Patients signed the written consent to participate in this study. All patients were investigated to assess clinical features, anthropometric parameters, presence of conditions associated with hepatic steatosis, liver biochemical tests, and serologic markers of HBV replication.

The following patients were excluded from the study. Those receiving antiviral treatment within 1 year before the study, those on hepatotoxic drugs that potentially can cause steatosis (i.e. valproic acid, amiodarone, tetracycline, metotrexate), those consuming alcohol regularly (20 g/day for men and 10 g/day for women), those diagnosed with decompensated cirrhosis, anti-HCV, anti-HIV or anti- HDV-positive patients, those diagnosed as having autoimmune or other known metabolic liver diseases, such as Wilson's disease, hereditary hemochromatosis, α-antitrypsin deficiency, etc.

A transcutaneous liver biopsy was performed through a lateral intercostal approach using a needle with a diameter of 16 mm by, and liver specimens were formalin fixed and paraffin embedded for histological evaluation. One experienced pathologists (A.M.) who was blinded to the clinical and laboratory findings assessed all biopsies on Haematoxylin-eosin and Masson trichrome. Pathological characteristics of hepatic steatosis were described according to NAFLD activity score (NAS) proposed by Kleiner DE, et al[7]. Briefly, grade of steatosis was scored as 0 = <5%; 1 = 5% to 33%; 2 = >33% to 66%; 3 = >66%. Grade of lobular inflammation was scored as 0 = no foci; 1 = <2 foci/200x field; 2 =2 to 4 foci/200x field; 3 = >4 foci/200x field. Ballooning was scored as 0 = none; 1 = few ballooned hepatocytes; 2 = many cells/prominent ballooning. The grade of steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2) were then combined to determine the NAS (0-8) as proposed. Fibrosis was scored as 0 = none; 1 = zone 3 perisinusoidal fibrosis (1a, delicate, 1b, dense) or portal/periportal (1c); 2 = perisinusoidal and portal/periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis.

Statistical analysis

Prevalence of steatosis was reported as percent and 95% CI. To determine factors associated with steatosis Pearson's chi-square test or Fisher's exact test was employed for qualitative variables whereas 2-sample t-test or Mann-Whitney U test for normally and non-normally distributed quantitative variable respectively.

Results

Patient characteristics

From June to December 2010, there were 60 chronic viral hepatitis B patients enrolled in the study. Patients with positive serum HBeAg accounted for 27%

of all patients, and another 73% had negative serum HBeAg. All patients enrolled in the study had serum HBV-DNA of more than 2000 IU/mL. Male patients were accounted for 58% of the patients. Mean age was 45 ± 12 years (range 24 - 73 years). Mean body mass index was 23.6 ± 3.6 kg/m^2. Fourteen patients (37.8%) had underlying diseases of diabetes mellitus or impaired fasting plasma glucose, whereas 28 patients (47%) had essential hypertension, and 26 patients (67%) had dyslipidemia. Overweight and obesity (body mass index of more than 23%) was present in 52% of patients (31 patients). All patients had serum albumin of more than 3.0 g/dL and normal level of serum total bilirubin.

Prevalence of liver steatosis

The histological features of the patients with liver steatosis included in the present study are summarized in Table 1 and 2. Liver steatosis was present in 19 patients (31.7%), of whom 11 (57.9%) had grade 1, 5 (26.3%) had grade 2, and 3 (15.8%) had grade 3.

Association of liver steatosis with chronic viral hepatitis B

Liver steatosis was presented in any type of chronic viral hepatitis B. Presence of positive serum HBeAg, level of HBV viral load, baseline prothrombin time, total bilirubin, alanine aminotransferase, aspartate aminotransferase, albumin, and globulin had

Table 1. Grading of liver steatosis.

Steatosis grade	No. of patients	Percent	Cumulative percent
<5%	41	68.3%	68.3%
5-33%	11	18.3%	86.7%
>33-66%	5	8.3%	95.0%
>66%	3	5.0%	100%
Total	60	100%	

Table 2. Association of liver steatosis with chronic viral hepatitis B.

	Number (%) or Mean (±SD) or Median (range)		
	No Steatosis (<5%)	Steatosis	*p*-value
HBeAg status			
Negative	27 (67.5%)	16 (84.2%)	0.18
Positive	13 (32.5%)	3 (15.8%)	
Median HBV viral load (IU/mL)	291000 (3390 - 20000000)	34900 (2250 - 110000000)	0.260
HBV viral load ≥6 log IU/mL	14 (34.1%)	5 (26.3%)	0.572
Prothrombin time (sec)	12.2 ± 0.88	11.73 ± 0.89	0.056
Prothrombin time more than ULN	9 (22%)	2 (10.5%)	0.476
Total bilirubin (mg/dL)	0.68 ± 0.33	0.55 ± 0.19	0.252
ALT (IU/mL)	34 (14-257)	29 (9-137))	0.667
ALT >1 time ULN	27 (65.9%)	11 (57.9%)	0.552
ALT >2 times ULN	14 (34.1%)	4 (21.1%)	0.303
AST (IU/mL)	31 (16-185)	27 (17-71)	0.247
Alkaline phosphatase (IU/mL)	66 (33-144)	65 (39-77)	0.512
Albumin (g/dL)	4.32 ± 0.37	4.45 ± 0.26	0.196
Globulin (g/dL)	3.19 ± 0.57	3.23 ± 0.50	0.794

ULN = upper limit of normal

shown no significant association with liver steatosis, $p>0.05$ (Table 2).

Host factors contributed to liver steatosis

Patients with steatosis were not significantly older (46.6 ± 9.8) than patients without steatosis (45.3 ± 12.5 years; $p=0.674$). Presence of steatosis did not show any difference between both sex, $p=0.28$ (Table 3).

No significant association between underlying disease of diabetes mellitus, dyslipidaemia, and essential hypertension was found. However, BMI and fasting HDL-cholesterol showed significant correlation with steatosis ($p=0.007$ for BMI and $p=0.02$ in fasting HDL-cholesterol). Another laboratory markers (including platelet count, prothrombin time, total bilirubin, alanine aminotransferase, aspartate aminotransferase, albumin, globulin, fasting blood sugar, total cholesterol, triglyceride, and LDL-cholesterol) were not found to have association with liver steatosis ($p>0.05$).

Association between liver steatosis and extent of HBV-related liver injury

Table 4 showed comparison of histologic parameter according to METAVIR among patients with and without liver steatosis. No difference degree of piecemeal necrosis, lobular inflammation, fibrosis, or significance of necroinflammation (according to METAVIR) was found ($p>0.05$).

Histological characteristics of patient with steatosis

Among patients with liver steatosis, mixed pattern of micro- and macrovesicular steatosis were found in all patients. The most common distribution of steatosis were zone 3 in 8 patients (42.1%). Azonal and panacinar distribution were seen in 5 patients each (26% and 26%, respectively). Zone 1 distribution of steatosis was seen in only one (5%) cases. Ballooning hepatocytes were seen in 89% of patients, but only 2

Table 3. Host factors contributed to liver steatosis.

	Number (%) or Mean (±SD) or Median (range)		
	No Steatosis (<5%)	**Steatosis**	***p*-value**
Age	45.3 ± 12.5	46.6 ± 9.8	0.674
Gender			
Female	19 (46.3%)	6 (31.6%)	0.28
Male	22 (53.7%)	13 (68.4%)	
BMI (kg/m^2; mean ±SD)	22.71 ± 3.49	25.39 ± 3.36	0.007
BMI (kg/m^2)			
Underweight (<18.5)	3 (7.3%)	0	0.026
Normal (18.5 - 22.9)	22 (53.7%)	4 (21.1%)	
Overweight (23.0 - 27.4)	13 (31.7%)	11 (57.9%)	
Obesity (≥27.5)	3 (7.3%)	4 (21.1%)	
Diabetes status			
Impaired fasting glucose	4 (16.7%)	3 (23.1%)	1.000
Diabetes mellitus	5 (20.8%)	2 (15.4%)	
Dyslipidaemia	19 (76.0%)	7 (50%)	0.16
Essential hypertension	19 (46.3%)	9 (47.4%)	0.94
Fasting blood sugar (mg/dL)	94.5 (80-223)	94 (85-134)	0.992
Total cholesterol (mg/dL)	201 ± 30	195 ± 37	0.630
Triglyceride (mg/dL)	73.5 (31-293)	94 (52-168)	0.341
HDL-cholesterol (mg/dL)	65 (26-108)	49.5 (40-77)	0.020
LDL-cholesterol (mg/dL)	115 (72-164)	117 (82-197)	0.931
Metabolic syndrome*	5 (12.2%)	2 (10.5%)	1.000

*Metabolic syndrome is defined according to all parameters in NCEP ATPIII panel except fasting plasma glucose that we change the cut off level to ≥100mg/dL.

Table 4. The association between the presence of steatosis and the histological activity and severity of CHB.

	Number (%)		
	No Steatosis (<5%)	**Steatosis**	***p*-value**
Piecemeal necrosis			
None	12 (29.3%)	6 (31.6%)	0.5610
Mild	20 (48.8%)	11 (57.9%)	
Moderate	9 (22.0%)	2 (10.5%)	
Lobular inflammation			
None or mild	33 (80.5%)	15 (78.9%)	1.00
Moderate	6 (14.6%)	4 (21.1%)	
Severe	2 (4.9%)	0	
Fibrosis			
F0	15 (36.6%)	8 (42.1%)	0.90
F1	16 (39.0%)	9 (47.4%)	
F2	7 (17.1%)	2 (10.5%)	
F3	2 (4.9%)	0	
F4	1 (2.4%	0	
METAVIR of ≥A2 or ≥F2	13 (31.7%)	6 (31.6%)	0.992

of these 17 patients showed large numbers of ballooning (11%). No patients showed significant amount of acidophilic bodies, megamitochondria, pigmented macrophage, or Mallory-Denk bodies. Large lipogranuloma was seen in 32%, and glycogenated nuclei was found in 26% of patients. Lobular inflammation and portal inflammation (greater than mild) were found in 21% and 16% of patients, respectively. Perisinusoidal fibrosis (stage 1a or 1b) was found in only 5% of patients, whereas most patients (42%) had portal or periportal fibrosis (stage 1c). Significant fibrosis (≥F2) was found in 16% of patients.

Compared to patients without liver steatosis, patients with liver steatosis had more ballooning hepatocyte (89% vs. 32%; $p<0.0001$), Mallory bodies (68.4% vs. 9.8%; $p<0.0001$), large lipogranuloma (31.6% vs. 0%; $p=0.001$), and glycogenated nuclei (26.3% vs. 4.9%; $p=0.028$). Mean NAS was also higher among patients with liver steatosis ($p<0.00001$).

Among patients with non-significant HBV-related liver injury (i.e. cases with METAVIR of <A2 and <F2), Serum level of alanine transferase was not elevated to significant degree in patients with liver steatosis compared to patients without liver steatosis (Table 6.) Other baseline patient characteristics were not significantly associated with the presence of live steatosis except NAFLD activity score (NAS), which was found to be associated with liver steatosis.

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) is an important cause of chronic liver disease. The prevalence of NAFLD ranges from an estimated 3-33% and is expected to increase in association with the global increase of obesity, insulin resistance, type 2 diabetes, and other component of metabolic syndrome[22-24]. In patients with chronic viral hepatitis C, the presence of steatosis can be associated with more advanced fibrosis and also with lower rates of response to antiviral therapy[25]. Despite aforementioned knowledge in HCV, the significance of steatosis in patients with chronic viral hepatitis B is less clear.

These preliminary results reported the prevalence of steatosis in patients with chronic viral hepatitis B to about 31.6% which was comparable to other studies[8-15].

Liver steatosis was found not to be associated with any viral hepatitis B parameters such as HBeAg status, level of HBV viral load, and liver biochemical tests. These results were in line with Minakari's study[27].

Table 5. Type, grade, zonality, and histological features of steatosis/steatohepatitis among CHB patients with or without steatosis.

	No. of patients (%)		
	No Steatosis (<5%)	**Steatosis**	***p*-value**
Grade of macrovesicular steatosis			
Grade 0 (<5%)	41 (100%)	0	<0.0001
Grade 1 (5 –<33%)	0	11 (57.9%)	
Grade 2 (33-66%)	0	5 (26.3%)	
Grade 3 (>66%)	0	3 (15.8%)	
Microvesicular steatosis	20 (48.8%)	19 (100%)	<0.0001
Distribution of steatosis			
Zone 3	4 (9.8%)	8 (42.1%)	<0.001
Zone 1	0	1 (5.3%)	
Azonal	20 (48.8%)	5 (26.3%)	
Panacinar	0	5 (26.3%)	
No any fat droplet	17 (41.5%)	0	
Ballooning hepatocyte			
None	28 (68%)	2 (11%)	<0.0001
Few	13 (32%)	15 (78%)	
Many	0	2 (11%)	
Lobular inflammation			
No foci	6 (14.6%)	1 (5.3%)	0.5996
<2 foci/200x field	25 (61%)	14 (73.7%)	
2-4 foci/200x field	8 (19.5%)	4 (21.1%)	
>4 foci/200x field	2 (4.9%)	0	
Portal inflammation			
None to minimal	10 (24.4%)	3 (15.8%)	0.45
Greater than minimal	31 (75.6%)	16 (84.2%)	
Fibrosis			
No fibrosis	15 (36.6%)	7 (37%)	0.474
Perisinusoidal (1a or 1b)	0	1 (5%)	
Portal/ Periportal (1c)	21 (51.2%)	8 (42%)	
Fibrosis ≥2	5 (12.2%)	3 (15.8%)	
Mallory bodies	4 (9.8%)	13 (68.4%)	<0.0001
Megamitochondria	1 (2.4%)	0	1.00
Acidophil bodies	1 (2.4%)	0	1.00
Pigmented macrophage	1 (2.4%)	0	1.00
Large lipogranuloma	0	6 (31.6%)	0.001
Glycogenated nuclei	2 (4.9%)	5 (26.3%)	0.028
Microgranuloma	6 (14.6%)	7 (36.8%)	0.089
NAS			
Not NASH	35 (85.4%)	2 (10.5%)	<0.00001
Borderline	6 (14.6%)	11 (57.9%)	
NASH	0	6 (31.6%)	

Table 6. The association between the presence of steatosis, liver biochemical abnormality, and baseline patient characteristic of non-significant HBV-related liver injury.

	No. of patients (%)		
	No Steatosis (<5%)	**Steatosis**	***p*-value**
Age	46 ± 12.5	47 ± 10.5	0.333
Gender			
Female	14 (50%)	5 (38.5%)	0.491
Male	14 (50%)	8 (61.5%)	
BMI	22.4 ± 3.0	25.2 ± 3.8	0.675
Diabetes status			
No DM	12 (63.2%)	6 (75%)	0.563
IFG or IGTT	3 (15.8%)	2 (25%)	
DM	4 (21.1%)	0	
Dyslipidaemia	17 (89.5%)	6 (66.7%)	0.290
Essential hypertension	13 (46.4%)	6 (46.2%)	0.987
ALT >1x ULN	16 (57.1%)	6 (46.2%)	0.511
ALT >2x ULN	7 (25%)	0	0.077
NAS			
Not NASH	27 (96.4%)	2 (15.4%)	<0.00001
Borderline	1 (3.6%)	9 (69.2%)	
NASH	0	2 (15.4%)	

Body mass index was associated with other features of metabolic syndrome, in which steatosis is its liver manifestation. Wang CC, et al had demonstrated that BMI was positively correlated with the presence of ultrasonographic fatty liver[28]. Our study, similar to that of Wang's, was also showed the correlation of BMI with the pathological liver steatosis. Serum triglyceride was found to be significantly correlated with liver steatosis in other studies[27], but in our study, we found serum HDL-cholesterol to be associated with liver steatosis instead. This different result may be due to low sample size, and multivariate analysis is needed to confirm this result.

The pathological characteristics of liver steatosis in patients with CHB, in which to our knowledge, no another study had documented before, was also described. The major distribution of steatosis in our study patients was zone 3, which is similar to other study[6]. In contrast to previous studies, we found mixed pattern of micro- and macrovesicular fat in all patients. The significance of microvesicular fat is less clear, some study show more severe cases of steatohepatitis associated with this kind of fat[26].

According to METAVIR which was used to defined degree of liver necroinflammation and fibrosis, presence of liver steatosis did not influence degree of various parameters.

We found histopathological featiures of steatohepatitis such as, hepatocyte ballooning, Mallory-Denk bodies, lipogranuloma, and glycogenated nuclei more common in patients with steatosis than without steatosis. These findings is in line with other studies, but other pathological markers such as acidophil bodies, megamitochondria, pigmented macrophage, and microgranuloma were not found to be significantly associated with liver steatosis. This may be due to small sample size of the preliminary result. We also found lobular and portal inflammation to be insignificant association with liver steatosis. These results may be due to the similar pathological finding of chronic viral hepatitis B at portal & lobular area.

Among patients with non-significant HBV-related liver injury, surprisingly, our results demonstrated that the cause of elevated serum alanine transferase in some patients was not associated with the presence of liver steatosis. This may be due to small number of patients enrolled to the analysis which need to be corrected in the full paper in the future.

Limitations of our preliminary results were mainly due to its relatively small sample size and inability to perform multivariate analysis. More patient enrollment is needed to finalize the results.

REFERENCES

1. Ludwig J, Viggiano TR, McGill DB, *et al*. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434-8.
2. Marchesini G, Bugianesi E, Forlani G, *et al*. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917-23.
3. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 1990;12:1106-10.
4. Diehl AM, Goodman Z, Ishak KG. Alcohollike liver disease in nonalcoholics. A clinical and histologic comparison with alcohol-induced liver injury. Gastroenterology 1988;95:1056-62.
5. Sass DA, Chang P, Chopra KB. Nonalcoholic fatty liver disease: a clinical review. Dig Dis Sci 2005;50:171-80.
6. Brunt EM, Neuschwander-Tetri BA, Oliver D, *et al*. Nonalcoholic steatohepatitis: histologic features and clinical correlations with 30 blinded biopsy specimens. Hum Pathol 2004;35:1070-82.
7. Kleiner DE, Brunt EM, Van Natta M, *et al*. Design and validation of a histologic scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41: 1313-21.
8. Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992;15:572-7.
9. Czaja AJ, Carpenter HA. Sensitivity, specificity, and predictability of biopsy interpretations in chronic hepatitis. Gastroenterology 1993;105:1824-32.
10. Czaja AJ, Carpenter HA, Santrach PJ, *et al*. Host- and disease-specific factors affecting steatosis in chronic hepatitis C. J Hepatol 1998;29:198-206.
11. Gerber MA, Krawczynski K, Alter MJ, *et al*. Histopathology of community acquired chronic hepatitis C. The Sentinel Counties Chronic Non-A, Non-B Hepatitis Study Team. Mod Pathol 1992;5:483-6.
12. Hwang SJ, Luo JC, Chu CW, *et al*. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. J Gastroenterol Hepatol 2001;16:190-5.
13. Lefkowitch JH, Schiff ER, Davis GL, *et al*. Pathological diagnosis of chronic hepatitis C: a multicenter comparative study with chronic hepatitis B. The Hepatitis Interventional Therapy Group. Gastroenterology 1993;104:595-603.
14. Roberts JM, Searle JW, Cooksley WG. Histological patterns of prolonged hepatitis C infection. Gastroenterol Jpn 1993;28 (Suppl 5):37-41.
15. Scheuer PJ, Ashrafzadeh P, Sherlock S, *et al*. The pathology of hepatitis C. Hepatology 1992;15:567-71.
16. Czaja AJ, Carpenter HA. Sensitivity, specificity, and predictability of biopsy interpretations in chronic hepatitis. Gastroenterology 1993;105:1824-32.
17. Bondini S, Kallman J, Wheeler A, *et al*. Impact of non-alcoholic fatty liver disease on chronic hepatitis B. Liver Int 2007; 27:607-11.
18. Tsochatzis E, Papatheodoridis GV, Manesis EK. Hepatic steatosis in chronic hepatitis B develop due to host metabolic factors: a comparative approach with genotype 1 chronic hepatitis C. Dig Liver Dis 2007;39:936-42.
19. Cindoruk M, Karakan T, Unal S. Hepatic steatosis has no impact on the outcome of treatment in patients with chronic hepatitis B infection. J Clin Gastroenterol 2007;41:513-17.
20. Shi JP, Fan JG, Wu R, *et al*. Prevalence and risk factors of hepatic steatosis and its impact on liver injury in Chinese patients with chronic hepatitis B infection. J Gastroenterol Hepatol 2008;23:1419-25.
21. Kim KH, Shin HJ, Kim K,et al. Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPAR gamma. Gastroenterology 2007;132: 1955-67.
22. Ong JP, Younossi ZM. Approach to the diagnosis and treatment of nonalcoholic fatty liver disease. Clin Liver Dis 2005; 9:617-737.
23. Brunt EM, Ramrakhiani S, Cordes BG, *et al*. Concurrence of histologic features of steatohepatitis with other forms of chronic liver disease. Mod Pathol 2003;16:49-56.
24. Powell EE, Jonsson JR, Cloudston AD. Steatosis: cofactor in other liver diseases. Hepatology 2005;42:5-13.
25. Bressler BL, Giundi M, Tomlinson G, *et al*. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. Hepatology 2003;38:639-44.
26. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis 2001;21:3-16.
27. Minakari M, Molaei M, Shalmani HM, *et al*. Liver steatosis in patients with chronic hepatitis B infection: host and viral risk factors. Eur J Gastroenterol Hepatol 2009;21:512-6.
28. Wang CC, Hsu CS, Liu CJ, *et al*. Association of chronic hepatitis B virus infection with insulin resistance and hepatic steatosis. J Gastroenterol Hepatol 2008;23:779-82.