Turk J Gastroenterol *2016; 27: 42-6*

Impact of fatty liver on hepatitis B virus replication and virologic response to tenofovir and entecavir

LIVER

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ABSTRACT

Background/Aims: We aimed to evaluate the impact of non-alcoholic fatty liver disease (NAFLD) on viral kinetics and virologic response to tenofovir and entecavir treatment in patients with chronic hepatitis B virus (HBV) infection.

Materials and Methods: This study was designed as a retrospective multicenter cohort study. The impact of hepatosteatosis on pre-treatment serum HBV DNA levels and also on the virologic response to either tenofovir or entecavir at 6 and 12 months of therapy was investigated.

Results: A total of 145 cases were involved in the study [median age 40 (18–73) years, 90 (62%) males]. In multivariate analysis, it was detected that patients with NAFLD were older and had a higher body mass index (BMI) [Odds ratio (95% confidence interval) and p-value for age were 1.040 (1.003–1.079) and 0.033 and for BMI were 1.348 (1.190–1.528) and 0.0001, respectively]. When only the 43 patients who were younger than 35.5 years old and who had a BMI less than 27.59 were investigated, serum high-density lipoprotein (HDL) levels and serum HBV DNA levels were lower in patients with NAFLD in multivariate analysis [Odds ratio (95% confidence interval) and p-values for serum HDL level and HBV DNA level were 0.864 (0.061–0.980) and 0.023 and 0.995 (0.990–0.999) and 0.025, respectively]. Totally, 57 and 75 of the patients had received entecavir and tenofovir, respectively.

Conclusion: Viral replication decreases in patients with chronic HBV infection in the presence of NAFLD, and NAFLD had no impact on the virologic response to entecavir and tenofovir treatment.

Keywords: Entecavir, fatty liver, hepatitis B virus, tenofovir

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by a chronic liver pathology that may progress to cirrhosis and hepatocellular carcinoma (1). Approximately one-third (13.6-44.4%) of patients with chronic hepatitis B virus (HBV) infection have been reported to have NAFLD (2-8). Despite studies that claim an increase in NAFLD in the presence of active viral replication in patients with chronic HBV infection, there are studies that report suppression of viral replication in NAFLD (9,10). Many epidemiological studies have reported no difference regarding HBV replication between patients with NAFLD and those without NAFLD (3-6,8,10-13). However, a negative correlation between NAFLD and serum HBV DNA levels has also been reported in a meta-analysis (7). When the literature regarding the relation between NAFLD and chronic HBV infection was reviewed, the result seems to be inconclusive (3-8,10-18). We think that the cause of the results that were obtained in those studies may relate to the lack of subgroup analysis.

We have found only one study that has investigated the effect of fatty liver on virologic response to entecavir treatment (19). We could not discover any study that investigated the relation between NAFLD and tenofovir treatment in patients with chronic HBV infection in the medical literature. In the present study, we aimed to evaluate the impact of NAFLD on viral kinetics and virologic response to tenofovir and entecavir treatment in patients with chronic HBV infection.

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Received: September 16, 2015 **Accepted:** October 31, 2015 **Available Online Date:** December 17, 2015
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MATERIALS AND METHODS

Study design and patients

This was a retrospective multicenter cohort study that included the data of patients with chronic HBV infection from three different medical centers between December 2001 and December 2012. Patients older than 18 years who had been diagnosed with chronic HBV infection, confirmed by liver biopsy, and who had been treated with either tenofovir or entecavir for at least 6 months were included in the study. Patients whose liver biopsy had been assessed with a scoring system other than the Ishak scoring system and who had no pre-treatment serum HBV DNA levels measured, subjects with diabetes mellitus or alcohol consumption, and those who were treated for dyslipidemia were excluded. Patients who did not have serum HBV DNA levels measured at 6 and 12 months of either tenofovir or entecavir treatment were also excluded from the analysis of the effect of NAFLD on virologic response to therapy.

Histopathologic evaluation of liver

The presence of inflammation and fibrosis in liver biopsy specimens was defined according to the Ishak scoring system. NAFLD was evaluated according to the Brunt classification (20).

Evaluation of virologic response

Virologic response was defined as undetectable serum HBV DNA levels under treatment with either tenofovir or entecavir.

Measurement of serum HBV DNA levels

Serum HBV DNA levels before and during the course of treatment were measured by a real-time polymerase chain reaction (PCR) method (1: BioRad iCycler iQ System, Qiagen DNA isolation kit, Germany: detection limit: 20 IU/mL; 2: COBAS TaqMan 48 HBV assay, Roche Diagnostics, lower limit of HBV DNA quantification: 20 IU/mL).

Parameters evaluated in the study

Cases with and without NAFLD were compared with respect to age; gender; body mass index (BMI); HBeAg status; histologic activity index (HAI) and fibrosis scores on liver biopsy; serum cholesterol, triglyceride, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels, alanine aminotransferase (ALT), and serum HBV DNA levels, and independent predictors of NAFLD were then investigated.

Cases with and without virologic response to treatment with tenofovir and entecavir at 6 and 12 months of therapy were compared in terms of the variables above and the percentage of fatty hepatocytes and independent predictors of response to treatment were investigated.

Statistical analyses

The Statistical Package for the Social Sciences (SPSS)-17 (SPSS Inc.; Chicago, IL, USA) package program was used for statistical analyses. Categorical variables were presented as the number of cases and percentages, continuous variables with a normal distribution were presented as mean±standard deviation, and continuous variables without a normal distribution were presented as median (minimum-maximum). Categorical variables were compared using a chi-squared test. Continuous variables with and without a normal distribution were compared using a two-tailed Student's t-test and a Mann-Whitney U-test, respectively. Logistic regression analysis was used for multivariate analysis. Receiver operating characteristic (ROC) curve analysis was used to estimate the best cut-off levels for age and BMI for predicting NAFLD. A p-value of <0.05 was considered statistically significant.

Ethics committee approval was obtained from the ethics committee of İstanbul Medipol University.

RESULTS

A total of 145 cases were involved in the study [median age 40 (18-73) years, 90 (62%) males]. Among these, 76 (52.4%) patients had NAFLD. In univariate analysis, patients with NAFLD were older and had a higher BMI, their serum cholesterol levels were higher, and their serum HBV DNA levels were significantly lower (Table 1).

Table 1. Comparison of data of chronic HBV patients with and without non-alcoholic fatty liver disease

	Patients without NAFLD (n=69, 47.6%)	Patients with NAFLDd (n=76, 52.4%)	p
Age (years)[a]	33 (18–63)	46 (21–73)	0.0001
Gender (male)[b]	42 (60.9)	48 (63.2)	0.777
Body mass index[a]	23 (15–37)	29 (15–40)	0.0001
HBeAg-positive status[b]	26 (37.7)	23 (30.3)	0.346
Histologic activity index on liver biopsy[a,c]	8 (2–14)	9 (3–16)	0.566
Fibrosis on liver biopsy[a,c]	3 (0–5)	3 (0–6)	0.161
Serum cholesterol level (mg/dL)[d]	168±35	186±34	0.002
Serum low-density lipoprotein level (mg/dL)[a]	100 (36–160)	114 (39–377)	0.0001
Serum high-density lipoprotein level (mg/dL)[a]	47 (3–96)	47 (17–71)	0.497
Serum triglyceride level (mg/dL)	81 (38–389)	94 (32–424)	0.106
Serum alanine aminotransferase level (mg/dL)[a]	106 (14–985)	81 (22–360)	0.146
Serum HBV DNA level (x10^6 copies/mL)[a]	263.650 (0.002042–2940)	67.512 (0.797–2700)	0.012

[a]Median (minimum–maximum)
[b]Number of cases (%)
[c]Ishak scoring system
[d]Mean±standard deviation
HBeAg: hepatitis B virus e antigen; NAFLD: non-alcoholic fatty liver disease; HBV: hepatitis B virus

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In multivariate analysis, patients with NAFLD were still significantly older and had a higher BMI [Odds ratio (95% confidence interval) and p-value for age were 1.040 (1.003-1.079) and 0.033 and for BMI were 1.348 (1.190-1.528) and 0.0001, respectively]. When only the 43 patients who were younger than 35.5 years old and who had a BMI less than 27.59 were investigated, serum HDL levels and serum HBV DNA levels were lower in patients with NAFLD in both univariate and multivariate analyses [in multivariate analyses, the odds ratio (95% confidence interval) and p-values for serum HDL level and HBV DNA level were 0.864 (0.061-0.980) and 0.023 and 0.995 (0.990-0.999) and 0.025, respectively] (Table 2).

Table 2. Results of multivariate logistic regression analysis evaluating the predictors of non-alcoholic fatty liver disease (NAFLD) in patients less than 35.5 years old with a body mass index less than 27.59

	Odds ratio	95% confidence interval	p
Serum high-density lipoprotein level	0.864	0.061–0.980	0.023
Serum HBV DNA level	0.995	0.990–0.999	0.025

HBV: hepatitis B virus

In total, 132 of 145 patients had serum HBV DNA measurements made at 6 and 12 months of therapy. These cases were compared for predictors of virologic response at 6 and 12 months of entecavir or tenofovir therapy. Of these, 57 and 75 had received entecavir and tenofovir, respectively.

Virologic response was detected in 25/57 (44%) and 36/48 (75%) patients at 6 and 12 months of entecavir treatment, respectively. In the tenofovir treatment arm, virologic responses were observed in 23/75 (30.7%) and 53/68 (77.9%) patients at 6 and 12 months, respectively. No independent predictors of response to entecavir treatment at 6 and 12 months and tenofovir treatment at 12 months were detected. NAFLD had no effect on virologic response to either entecavir or tenofovir at 6 and 12 months of treatment. The presence of steatohepatitis (SH) was found to predict a more favorable response to treatment at 6 months in the tenofovir arm than in patients without SH in univariate analysis (p=0.046) [only 2 (3.8%) patients without virologic response and 4 patients (17.4%) with virologic response had steatohepatitis, p=0.046] (Table 3).

Table 3. Comparison of patients with and without virologic response at 6 months of tenofovir treatment

	Non-responder to tenofovir treatment (n=52, 69.3%)	Responder to tenofovir treatment (n=23, 30.7%)	p
Age (years)[a]	39 (18–66)	42 (20–67)	0.434
Gender (male)[b]	34 (65)	12 (52)	0.279
Body mass index[a]	25 (15–35)	27 (20–40)	0.091
HBV DNA level (x10^6 copies/mL)[a]	271.06 (10.83–640.20)	82.64 (12.59–873)	0.381
HBeAg-positive status[b]	22 (42.5)	6 (26.5)	0.181
Serum alanine aminotransferase level (IU/mL)[a]	96 (16–515)	84 (27–985)	0.459
Histologic activity index[a,c]	8 (2–15)	8 (3–15)	0.831
Liver fibrosis[a,c]	3 (0–6)	2 (0–5)	0.425
Presence of hepatosteatosis[b]	22 (42)	12 (52)	0.429
Percentage of hepatocytes with steatosis[a]	0 (0–60)	4 (0–50)	0.293
Presence of steatohepatitis[b]	2 (3.8)	4 (17.4)	0.046

[a]Median (minimum–maximum)
[b]Number of cases (%)
[c]Ishak scoring system
HBV: hepatitis B virus; HBeAg: hepatitis B virus e antigen

DISCUSSION

In our study, the rate of NAFLD in patients with chronic HBV infection (52.4%) was higher than that reported in the literature (13.6-44.4%) (3-8,10). When we consider the studies that defined NAFLD as ≥5% fatty involvement of hepatocytes, 13.5-33.7% of patients with chronic HBV infection had NAFLD (4,11,14,20). For papers that considered NAFLD as ≥1% fatty involvement of hepatocytes, the rate was 29.9-44.4%, which is comparable to our study (2,3,5,10).

In epidemiological surveys, NAFLD has been reported to be related to host factors much more than viral factors (3-6,8,10-12). No significant difference was detected between patients with and without NAFLD in the aforementioned studies. However, after the detection of predictors of NAFLD in those studies, it is clear that further subgroup analyses were not performed. Our cohort included patients with a high BMI in general and NAFLD was predominant in older patients. However, when only younger patients and those with a BMI below a certain level were investigated, serum HBV DNA levels were found to be lower in patients with NAFLD. Few articles report viral suppression in cases with NAFLD in the medical literature (6,7,14,16,17).

In a meta-analysis, serum HBV DNA levels were claimed to be lower in patients with NAFLD (7). In a HBV transgenic rat model, when NAFLD developed decreases in serum HBV DNA, HBsAg, and HBeAg levels were reported (21). In the same study, NAFLD was thought to suppress viral replication (21). In another study, HBsAg-positive staining in liver biopsy specimens was reported to be less in cases with NAFLD compared with those without (14). When patients with chronic HBV infection who experienced HBsAg seroconversion were investigated, the age at which HBsAg seroconversion occurred was reported to be younger in cases with NAFLD (17). Furthermore, the prevalence of NAFLD was found to be higher in patients who experienced HBsAg seroconversion than in those who did not (16). An increase in the number of Fas receptors on the surface of hepatocytes has been thought to facilitate apoptosis of those cells,

which resulted in increased viral clearance in patients with NAFLD (22).

Besides papers that claimed suppression of viral replication by NAFLD, there are also studies that reported an increase in the rate of NAFLD with HBV (21,23-26). HBV X protein (HBx) has been shown to increase the transcription of sterol regulatory element-binding protein-1c (SREBP-1c) and peroxisome proliferator-activated receptor (PPAR) (23-26). An increase in SREBP-1c levels has been claimed to promote NAFLD by means of stimulating the synthesis of acetyl-CoA carboxylase 1 and fatty acid synthase (23,26). A decrease in the gene expression of the enzymes responsible for lipid degradation such as cyp4A and an increase in the gene expression of the enzymes responsible for lipid synthesis have been detected in a study (26). In another study, an increase in serum HBV DNA levels has been reported to result in increased SREBP-1c levels and NAFLD (9). Considering all these studies, one may consider that HBV infection may have the potential to cause NAFLD; however, this potential has been less than that of host factors such as metabolic syndrome. Moreover, NAFLD itself appears to suppress viral replication.

Only one study has evaluated the effect of NAFLD on virologic response to entecavir treatment in the literature (18). In that study, it was concluded that virologic response at 24, 48 and 96 weeks of entecavir treatment was less in patients with NAFLD. This outcome was explained by the effect of both the decreased bioavailability of entecavir in fatty hepatocytes and cytochrome enzyme levels on drug metabolism (27,28). Nevertheless, in that study the presence of hepatosteatosis was disclosed by ultrasonography, which has low sensitivity. It is quite impossible to detect NAFLD by ultrasonography unless it is at an advanced stage. We think that the results of this study depend on the evaluation of NAFLD by ultrasonography alone.

In our study, NAFLD seemed to have no effect on virologic response to either tenofovir or entecavir. Only the presence of steatohepatitis was found to predict a more favorable response to treatment at 6 months in the tenofovir arm. The expression of Fas receptors on the surface of hepatocytes has been reported to increase in patients with NAFLD. This has been claimed to result in increased viral clearance in patients with chronic HBV infection (16,22). The increased virologic response to tenofovir that was observed in our study may depend upon an increased immune response against the virus in patients with steatohepatitis. However, the reason for the favorable impact of steatohepatitis on response to tenofovir might be the inclusion of a limited number of patients with steatohepatitis, which might have caused a type 1 error.

Our study has some important limitations. The main purpose of our study was to evaluate the impact of NAFLD on response to antiviral drugs, not that of steatohepatitis. Therefore, the number of patients with steatohepatitis in our study remained quite low. We think that further studies including more patients with steatohepatitis may shed light on this issue.

The results of this study made us think that viral replication decreases in patients with chronic HBV infection in the presence of NAFLD and that NAFLD had no overall impact on virologic response to entecavir and tenofovir treatment.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul Medipol University.

Informed Consent: Written informed consent was not obtained because our study is retrospective multicenter cohort study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - B.C.; Design - B.C.; Supervision - B.C., M.F., A.M.; Materials - E.P.; Data Collection and/or Processing - F.A., A.B., C.Y., E.F., E.P., M.Y.; Analysis and/or Interpretation - B.C., F.A., M.F., A.M.; Literature Search - B.C., C.Y.; Writing - B.C.; Critical Reviews - B.C., F.A., M.F., M.Y., A.M.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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REFERENCES

1. Başaranoğlu M, Örmeci N. Nonalcoholic fatty liver disease: diagnosis, pathogenesis, and management. Turk J Gastroenterol 2014; 25: 127-32. [CrossRef]
2. Peng D, Han Y, Ding H, Wei L. Hepatic steatosis in chronic hepatitis B patients is associated with metabolic factors more than viral factors. J Gastroenterol Hepatol 2008; 23(7 Pt 1): 1082-8. [CrossRef]
3. Poortahmasebi V, Alavian SM, Keyvani H, Norouzi M, Mahmoodi M, Jazayeri SM. Hepatic steatosis: prevalence and host/viral risk factors in Iranian patients with chronic hepatitis B infection. Asian Pac J Cancer Prev 2014; 15: 3879-84. [CrossRef]
4. Rastogi A, Sakhuja P, Kumar A, Hissar S, Jain A, Gondal R, et al. Steatosis in chronic hepatitis B: prevalence and correlation with biochemical, histologic, viral, and metabolic parameters. Indian J Pathol Microbiol 2011; 54: 454-9. [CrossRef]
5. Lesmana LA, Lesmana CR, Pakasi LS, Krisnuhoni E. Prevalence of hepatic steatosis in chronic hepatitis B patients and its association with disease severity. Acta Medica Indones 2012; 44: 35-9.
6. Zheng R, Xu C, Jiang L, Dou A, Zhou K, Lu L. Predictors of hepatic steatosis in HBeAg-negative chronic hepatitis B patients and their diagnostic values in hepatic fibrosis. Int J Med Sci 2010; 7: 272-7. [CrossRef]
7. Machado MV, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. J Gastroenterol Hepatol 2011; 26: 1361-7. [CrossRef]
8. Shi J, Fan J, Wu R, Gao X, Zhang L, Wang H. [Prevalence and risk factors of hepatic steatosis in patients with chronic hepatitis B]. Zhonghua Gan Zang Bing Za Zhi 2008; 16: 519-23.
9. Jiang C-Y, Zeng W-Q, Chen Y-X, Dai F-H, Jiang P. [Effect of HBV on the expression of SREBP in the hepatocyte of chronic hepatitis B patients combined with hepatic fatty change]. Zhonghua Gan Zang Bing Za Zhi 2011; 19: 608-13.
10. 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. [CrossRef]

11. 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. **[CrossRef]**
12. Wong VW, Wong GL, Chu WC, et al. Hepatitis B virus infection and fatty liver in the general population. J Hepatol 2012; 56: 533-40. **[CrossRef]**
13. Xu Q, Jie Y, Shu X, Chen L, Cao H, Li G. [Relationship of fatty liver with HBV infection, hyperlipidemia and abnormal alanine aminotransferase]. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 2009; 23: 141-3.
14. Wang MM, Wang GS, Shen F, Chen GY, Pan Q, Fan JG. Hepatic steatosis is highly prevalent in hepatitis B patients and negatively associated with virological factors. Dig Dis Sci 2014; 59: 2571-9. **[CrossRef]**
15. Wang CC, Hsu CS, Liu CJ, Kao JH, Chen DS. Association of chronic hepatitis B virus infection with insulin resistance and hepatic steatosis. J Gastroenterol Hepatol 2008; 23: 779-82. **[CrossRef]**
16. Chu CM, Lin DY, Liaw YF. Does increased body mass index with hepatic steatosis contribute to seroclearance of hepatitis B virus (HBV) surface antigen in chronic HBV infection? Int J Obes 2007; 31: 871-5.
17. Chu CM, Lin DY, Liaw YF. Clinical and virological characteristics post HBsAg seroclearance in hepatitis B virus carriers with hepatic steatosis versus those without. Dig Dis Sci 2013; 58: 275-81. **[CrossRef]**
18. Jin X, Chen Y, Yang Y, Li Y, Zheng L, Xu C. Association between hepatic steatosis and entecavir treatment failure in Chinese patients with chronic hepatitis B. PloS One 2012; 7: e34198. **[CrossRef]**
19. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999; 94: 2467-74. **[CrossRef]**
20. Joven J, Espinel E, Rull A, et al. Serum fatty acid synthase concentration is increased in patients with hepatitis viral infection and may assist in the prediction of liver steatosis. J Clin Virol 2011; 51: 199-201. **[CrossRef]**
21. Zhang Z, Pan Q, Duan X-Y, et al. Fatty liver reduces hepatitis B virus replication in a genotype B hepatitis B virus transgenic mice model. J Gastroenterol Hepatol 2012; 27: 1858-64. **[CrossRef]**
22. Feldstein AE, Canbay A, Angulo P, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003; 125: 437-43. **[CrossRef]**
23. Brown AJ. Viral hepatitis and fatty liver disease: how an unwelcome guest makes pâté of the host. Biochem J 2008; 416: e15-7. **[CrossRef]**
24. Kim K, Kim KH, Kim HH, Cheong J. Hepatitis B virus X protein induces lipogenic transcription factor SREBP1 and fatty acid synthase through the activation of nuclear receptor LXRalpha. Biochem J 2008; 416: 219-30. **[CrossRef]**
25. Na TY, Shin YK, Roh KJ, et al. Liver X receptor mediates hepatitis B virus X protein-induced lipogenesis in hepatitis B virus-associated hepatocellular carcinoma. Hepatology 2009; 49: 1122-31. **[CrossRef]**
26. Hajjou M, Norel R, Carver R, et al. cDNA microarray analysis of HBV transgenic mouse liver identifies genes in lipid biosynthetic and growth control pathways affected by HBV. J Med Virol 2005; 77: 57-65. **[CrossRef]**
27. Taliani G, Duca F, Lecce R, Livoli D, Pasquazzi C, De Bac C. Hepatic lidocaine metabolism in chronic hepatitis C virus hepatitis with or without steatosis. Hepatology 1995; 21: 1760-1. **[CrossRef]**
28. Leclercq I, Horsmans Y, Desager JP, Delzenne N, Geubel AP. Reduction in hepatic cytochrome P-450 is correlated to the degree of liver fat content in animal models of steatosis in the absence of inflammation. J Hepatol 1998; 28: 410-6. **[CrossRef]**