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

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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.
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 Istanbul 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients without NAFLD</th>
<th>Patients with NAFLD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33 (18–63)</td>
<td>46 (21–73)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>42 (60.9)</td>
<td>48 (63.2)</td>
<td>0.777</td>
</tr>
<tr>
<td>Body mass index</td>
<td>23 (15–37)</td>
<td>29 (15–40)</td>
<td>0.0001</td>
</tr>
<tr>
<td>HBeAg-positive status</td>
<td>26 (37.7)</td>
<td>23 (30.3)</td>
<td>0.346</td>
</tr>
<tr>
<td>Histologic activity index on liver biopsy</td>
<td>8 (2–14)</td>
<td>9 (3–16)</td>
<td>0.566</td>
</tr>
<tr>
<td>Fibrosis on liver biopsy</td>
<td>3 (0–5)</td>
<td>3 (0–6)</td>
<td>0.161</td>
</tr>
<tr>
<td>Serum cholesterol level (mg/dL)</td>
<td>168±35</td>
<td>186±34</td>
<td>0.002</td>
</tr>
<tr>
<td>Serum triglyceride level (mg/dL)</td>
<td>100 (36–160)</td>
<td>114 (39–377)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Serum high-density lipoprotein level (mg/dL)</td>
<td>47 (3–96)</td>
<td>47 (17–71)</td>
<td>0.497</td>
</tr>
<tr>
<td>Serum alanine aminotransferase level (mg/dL)</td>
<td>81 (38–389)</td>
<td>94 (32–424)</td>
<td>0.106</td>
</tr>
<tr>
<td>Serum alanine aminotransferase level (mg/dL)</td>
<td>106 (14–985)</td>
<td>81 (22–360)</td>
<td>0.146</td>
</tr>
<tr>
<td>Serum HBV DNA level (x10^6 copies/mL)</td>
<td>263±650</td>
<td>67±512</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Med (minimum–maximum)

HBeAg: hepatitis B virus e antigen; NAFLD: non-alcoholic fatty liver disease; HBV: hepatitis B virus
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 serum 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).

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).

**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, and NAFLD was predominant in older patients. How-ever, 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.

### 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

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum high-density lipoprotein level</td>
<td>0.864</td>
<td>0.061-0.980</td>
<td>0.023</td>
</tr>
<tr>
<td>Serum HBV DNA level</td>
<td>0.995</td>
<td>0.990-0.999</td>
<td>0.025</td>
</tr>
<tr>
<td>HBV: hepatitis B virus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Non-responder to tenofovir treatment (n=52, 69.3%)</th>
<th>Responder to tenofovir treatment (n=23, 30.7%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 (18–66)</td>
<td>42 (20–67)</td>
<td>0.434</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>34 (65)</td>
<td>12 (52)</td>
<td>0.279</td>
</tr>
<tr>
<td>Body mass indexa</td>
<td>25 (15–35)</td>
<td>27 (20–40)</td>
<td>0.091</td>
</tr>
<tr>
<td>HBV DNA level (x10^6 copies/mLa)</td>
<td>271.06</td>
<td>82.64</td>
<td>0.381</td>
</tr>
<tr>
<td>SBV-positive statusb</td>
<td>22 (42.5)</td>
<td>6 (26.5)</td>
<td>0.181</td>
</tr>
<tr>
<td>Serum alanine aminotransferase level (IU/mL)a</td>
<td>96 (16–515)</td>
<td>84 (27–985)</td>
<td>0.459</td>
</tr>
<tr>
<td>Histologic activity indexc</td>
<td>8 (2–15)</td>
<td>8 (3–15)</td>
<td>0.831</td>
</tr>
<tr>
<td>Liver fibrosisd</td>
<td>3 (0–6)</td>
<td>2 (0–5)</td>
<td>0.425</td>
</tr>
<tr>
<td>Presence of hepatosteatosisb</td>
<td>22 (42)</td>
<td>12 (52)</td>
<td>0.429</td>
</tr>
<tr>
<td>Percentage of hepatocytes with steatosisb</td>
<td>0 (0–60)</td>
<td>4 (0–50)</td>
<td>0.293</td>
</tr>
<tr>
<td>Presence of steatohepatitisb</td>
<td>2 (3.8)</td>
<td>4 (17.4)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

*aMedian (minimum–maximum)

bNumber of cases (%)

cIshak scoring system

HBV: hepatitis B virus; HBeAg: hepatitis B virus e antigen

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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 I 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.

**REFERENCES**