**Relationship between hepatosteatosis and histopathological and biochemical parameters in patients with noncirrhotic chronic hepatitis B**

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**Abstract**

**Aim:** Fatty liver is a common liver disease in the community. Mitochondrial dysfunction and oxidative process cause insulin resistance, causing fatty liver. Fatty liver diseases are higher in patients infected with chronic hepatitis B virus (HBV) than the normal population. We aimed to investigate the relationship between hepatosteatosis and biochemical and histopathological parameters in chronic HBV patients.

**Materials and Methods:** Patients with follow-up between September/2017-November/2019 with chronic HBV diagnosis were included. Demographic and histopathological data of all patients were documented. Comorbid diseases, medications and previous operations were questioned. The presence of steatosis was determined by ultrasonography (USG) to all patients. Biochemical and histopathological data were compared with the presence of steatosis.

**Results:** A total of 94 patients, 50(53.1%) women were included in the study. As a result of liver biopsy; while 20(21.2%) patients had no fibrosis (stage 0), 74 (78.8%) patients had fibrosis (stage 1-3). In USG, 45(47.8%) patients were found to have hepatosteatosis and 49(52.2%) patients were not. In the comparison between the groups; there was no significant relationship between sex, age, liver function tests, HBV-DNA level, hepatic activity index and hepatosteatosis (p>0.05). A significant relation was found between the presence of fibrosis and hepatosteatosis (p=0.021).

**Conclusions:** Chronic HBV is an inflammatory process that ends with cirrhosis and hepatocellular carcinoma, and in this process, fatty liver may increase disease and cause disease progression. In this context, it is important to prevent the development of steatosis in chronic HBV patients. However, this should be supported in prospective studies in larger populations.

**Keywords:** Chronic hepatitis B infection; fibrosis; hepatosteatosis

**INTRODUCTION**

Hepatitis B virus (HBV) is an important health problem worldwide, and it is known that nearly 400 million people are infected. HBV causes a process in the liver tissue that begins with chronic inflammation and ends with fibrosis. HBV is the most common cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma in Turkey (1-5).

Fatty liver is a common liver disease in the community. It is a process that develops due to mitochondrial changes and results in fat accumulation in hepatocytes. Mitochondrial dysfunction and oxidative process cause fatty liver by causing insulin resistance (6). Non alcoholic fatty liver disease (NAFLD) is mostly associated with obesity, type 2 diabetes mellitus and dyslipidemia and is the liver-related outcome of metabolic syndrome (7).

In some recent studies, chronic hepatitis C infection has been evaluated as a risk factor for steatosis. In the same studies, viral load was closely associated with steatosis in hepatitis C patients (7,8). In patients who have been successfully treated for hepatitis C, it has been observed that steatosis also regresses with improved liver function (9). However, the relationship between chronic HBV infection and fatty liver has not been clarified. Although fatty liver is higher in chronic HBV patients compared to normal population, this rate is lower compared to chronic HCV patients (7). In our study, we aimed to investigate the presence of hepatosteatosis and its relationship with biochemical parameters, HBV-DNA level, histological activity index (HAI) and fibrosis in patients with chronic HBV.

**MATERIALS and METHODS**

**Study Design**

94 patients with chronic HBV diagnosis from the gastroenterology and hepatology outpatient clinic of Van University Education and Research Hospital were included in the study. The presence of steatosis was determined by ultrasonography (USG) to all patients. Biochemical and histopathological data were compared with the presence of steatosis.
Van Training and Research Hospital between September 2017 and November 2019 were included in the study. Our study was designed retrospectively. Patients who had HBsAg positivity, and / or liver function test levels with a high course of six months and HBV-DNA positive in the serum sample studied with polymerase chain reaction (Roboscreen, Germany) were evaluated as chronic HBV.

**Inclusion Criteria**

Patients with chronic HBV age 18 and older were included in the study.

**Exclusion Criteria**

The patients who use anti-inflammatory and antibiotic drugs, who use cigarettes and alcohol, who diagnosed with chronic HBV and have advanced fibrosis and cirrhosis as a result of liver biopsy, who have coinfection such as human immunodeficiency virus (HIV), hepatitis D virus (HDV) and hepatitis C virus (HCV), who had liver or kidney transplantation, liver surgery, malignancy, decompensated liver disease, patients with etiology other than HBV that can lead to chronic liver disease, patients with chronic hematological, renal, respiratory, endocrine and cardiovascular problems, who had blood transfusions and pregnant women were not included in the study.

**Data Evaluation**

Demographic data (age, sex), serum biochemical values and HBV DNA levels of all patients were analyzed. Histopathological data of the patients were documented. Patients' liver tissue was examined by ultrasonography (USG) and evaluated for hepatosteatosis. Comorbid diseases, medications and previous operations were questioned. The relationship between hepatosteatosis and biochemical markers, fibrosis and inflammation level was questioned in chronic HBV patients.

**Biochemical and Hematological Measurements**

Biochemical parameters were measured from antecubital venous blood samples taken in the morning hours after 8 hours of fasting. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transeptidase (GGT), alkaline phosphatase (ALP), total protein, albumin, total bilirubin, direct bilirubin levels were examined. HBV DNA measurement was done with real time PCR (Roche). AmpliPrep / COBAS Taq-Man, which is very sensitive in serum HBV-DNA measurement, was used. HBV-DNA levels are expressed as IU / mL.

**Radiological Evaluation**

The patients were examined by three different radiology physicians without any clinical information and evaluated by comparing the echogenicity of liver tissue with kidney by USG. As a result of the evaluation, patients with high echogenicity were considered as hepatosteatosis.

**Liver Biopsy and Histopathological Evaluation**

Liver biopsy was performed using ultrasonography guided 16G biopsy needle. The liver biopsies taken were sent to the pathology laboratory in 10% formaldehyde. After routine tissue follow-up, tissue samples embedded in paraffin were cut into 5 micron thickness and stained with routine Hematoxylin-eosin (H-E) and Masson trichrome and evaluated under a light microscope. Samples with liver biopsy material length below 1.5 cm and the number of portal areas insufficient for evaluation were excluded from the study. The materials were evaluated by three experienced pathologists without clinical information. Knodell histological activity score was used to perform grade and staging (10). The presence of fibrosis in liver biopsies was made according to the Scheuer score (11). Those with a fibrosis score of 0 were considered as group 1 and those with a fibrosis score of 1-3 were considered as group 2. According to the inflammation levels, those with HAI 1-6 were evaluated as group 1, those with 7 and above were considered as group 2.

**Statistical Analysis**

The results of our study were analyzed with the program "The Statistical Package for the Social Sciences 19.0 (SPSS Armonk, NY: IBM Corp."). Data that received continuous values were given as mean (± standard deviation), and categorical data as frequency and percentage (n,%). The data were tested for compliance with the normal distribution using the kolmogorov- simirnov test, histogram and ± sd. Parametric data of the groups were compared using the one-anova test and comparisons between the binary groups were made using the post-hoc test. Chi-square test was used to test categorical data. Cases with p <0.05 were considered statistically significant.

**RESULTS**

A total of 94 patients, 50 (53.1%) women and 44 (46.9%) men, were included in the study. As a result of liver biopsy; while 20 (21.2%) patients had no fibrosis (steage 0), 74 (78.8%) patients had fibrosis (stage 1-3).

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. (% of total)</th>
<th>No. (% in group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5 (11.2%)</td>
<td>30 (61.2%)</td>
</tr>
<tr>
<td>1-2</td>
<td>5 (11.2%)</td>
<td>34 (69.3%)</td>
</tr>
</tbody>
</table>

Table 1. Comparison of hepatosteatosis with biochemical and histopathological parameters in chronic HBV patients
As a result of USG, 45 (47.8%) patients had hepatosteatosis and 49 (52.2%) patients did not have hepatosteatosis. In the demographic comparison between two separate groups made according to the presence of hepatosteatosis; There was no significant difference in terms of gender and age (p: 0.698, p = 0.138 respectively). In the comparison of liver function tests and bilirubin; there was no significant relationship between hepatosteatosis and biochemical markers (p > 0.05). There was no significant relationship between HBV-DNA level, HAI and hepatosteatosis (p > 0.05). A statistically significant relationship was detected between the presence of fibrosis and hepatosteatosis. The presence of fibrosis was significantly higher in those with hepatosteatosis (p = 0.021) (Table 1).

**DISCUSSION**

HBV causes inflammation in the liver tissue, causing tissue damage. Progressive inflammation causes irreversible damage to tissue, fibrosis, and subsequent liver cirrhosis and HCC develop (1-5). In studies conducted, hepatosteatosis with HCV can increase tissue damage and cause the disease to progress more seriously (9). However, the relationship between disease course and hepatosteatosis in HBV is not clear. It is thought that accompanying diseases such as obesity, dyslipidemia, insulin resistance, diabetes mellitus is important in increasing the damage in the liver (6).

In the publications related to the frequency of hepatosteatosis in hepatitis B patients, different rates have been reported between 18-76% (6,12-18). In a study by Gordon et al, 76% hepatosteatosis was observed in HBV patients. But, this study does not reflect the reality, since patients using alcohol were also included (15). Turkey has been identified as 39% frequency in an article that has been written on this subject (16). In our study, steatosis was observed in 45 (47.8%) of our patients.

Peleg et al, stated in their single-center retrospective study that the presence of HBV and steatosis increased the risk of malignancy in hepatic and extrahepatic tissues. In addition, they stated that inflammation caused by HBV inflammation and steatosis, increases the damage in the liver tissue with synergistic effect, accelerates the course of the disease and increases the complications. However, no correlation has been reported between hepatosteatosis and viral load (17). Similarly, in our study, we could not detect the relationship of viral load with hepatosteatosis. In a study by Wang et al, they stated that steatosis in HBV had no effect on fibrosis (6). In parallel, Minakari et al. reached similar results (18). But, in these studies, it is seen that some demographic data such as age and gender that may affect the development of fibrosis in groups with and without steatosis are not in similar rates. Likewise Wang et al. in their study, they reported that the steatosis was low in HBV patients over the age of 40 and that there was a higher rate of steatosis in women (6). While we were designing our study, we took care to distribute the factors that may affect the development of fibrosis in both groups equally. In our study, no difference was found between the groups with and without steatosis in terms of age and gender. When the relationship between steatosis and fibrosis is analyzed; we found that the incidence of fibrosis was higher in patients with hepatosteatosis. Similar to our study, Seto et al. found a significant relationship between hepatosteatosis and the development of fibrosis in a study. In which 1606 chronic HBV patients participated in the Chinese population, factors such as age gender and body mass index, which may affect the development of fibrosis among groups with and without fibrosis, were taken equally. It was observed that the rate of development of fibrosis was 3.6 times higher in the patient group with hepatosteatosis (19).

The HBV is a partial double-stranded DNA virus that encodes four major HBV proteins, protein S, C, X and P. Of these, X protein is mostly present in the cytoplasm and nucleus and has a multifunctional regulatory role. Protein X is responsible for viral replication and cell apoptosis in the cell it enters. It has been shown in studies that the protein X increases with steatosis in hepatocytes, its relationship with the steatosis mechanism has been explained by causing mitochondrial dysfunction. It has been reported to inhibit activation by binding to mitochondrial respiratory chain complex subunits. In this way, glycolysis is prevented and causes an increase in lipogenesis (20,21).

**LIMITATIONS**

Our study has its strengths and limitations. The strengths of our study are laboratory-based, radiological and pathological findings, therefore being more objective, patients’ follow-up and examinations performed in a single center, and the data is analyzed thoroughly and processed objectively. The limitations of our study were that it was designed retrospectively and that it was performed on a small number of patients.

**CONCLUSION**

In conclusion, chronic HBV is an inflammatory process that ends with cirrhosis and HCC. In this process, fatty liver can increase disease and cause disease progression. Regardless of biochemical markers, viral replication and HAI, steatosis can be shown to increase fibrosis in the HBV course. In this context, it is important to prevent the development of steatosis in chronic HBV patients. However, this should be supported in prospective studies with wider participation.

**Competing Interests:** The authors declare that they have no competing interest.

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**Ethical Approval:** Van Education and Research Hospital, Date:26.11.2020.

**Decision number:** 2020/23.

**REFERENCES**


