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The Correlation between Histopathological Stages and Viral Markers of Chronic Hepatitis B infection in Ankara, Turkey

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Abstract- Introduction: The correlation between the Hepatitis B virus (HBV) viral load and liver fibrosis is still researched. Objective: This study aimed to investigate the correlation of viral parameters and the different levels and stages of liver disease in treatment-naïve patients with chronic HBV. Methodology: A total of 128 treatment-naïve chronic HBV patients who underwent liver biopsy were assessed in this retrospective and cross-sectional study. Simultaneously with biopsy, HBsAg (S/Co) was measured with HBeAg (S/Co) chemiluminescent immune assay (Abbott Diagnostics, Germany) and HBV viral load was measured with PCR (Bosphore HBV Quantification Kit v2, Turkey) test. The assessments of liver fibrosis were based on the ISHAK staging system. Results: Out of 128 patients who were included in the study and whose ages ranged from 18 to 73, 100 (78%) were male. A negative but significant correlation was found between HBsAg S/Co and HBV-DNA viral load levels in HBeAg negative and positive patients (r= -0.277, p= 0.01 and r= -0.475, p= 0.001 respectively). Sensitivity, specificity and the area under the curve (AUC) were 51.8%, 57.8% and 0.551 respectively at the viral load value of 5.1 (log) IU/ml for the prediction of fibrosis score ≥ 2. Sensitivity, specificity and AUC were 72.7%, 41.9% and 0.545 respectively at the viral load value of 6.1 (log) IU/ml for the prediction of fibrosis score ≥ 4. Conclusion: Different HBV-DNA levels in treatment-naïve patients with chronic HBV can have effects on the formation of fibrosis in liver tissues and predict the severity of fibrosis. When the indirect serum biomarkers and serum HBV-DNA levels are used together in chronic HBV they may be more helpful in predicting of fibrosis.

Keywords:- *Chronic hepatitis B, viral load, liver fibrosis, liver inflammation*

I. INTRODUCTION

Hepatitis B virus (HBV) is a global health problem. About 292 million people worldwide are chronically infected with HBV and the prevalence of global HBV was reported as 3.9% in 2015 (1).

Based on the serological and biochemical parameters, natural course of chronic HBV infection has 4 phases: HBeAg-positive HBV infection and hepatitis phase and HBeAg-negative HBV infection and hepatitis phase (2).

Liver biopsy keeps its importance in current guidelines for the decision of treatment plan and in the assessment of prognosis (3-5). Due to the risk of complications, subjective assessment, small size of biopsy, and heterogenic distribution of fibrosis in the liver, this technique has caused the search of alternative non-invasive methods(6,7).Various imaging methods (transient elastography, ultrasonography and magnetic resonance imaging) and a series of biochemical methods have been recommended to stage the liver fibrosis (8–10). While the indirect markers that are trying to determine the fibrosis and inflammatory activity level equivalent to the information obtained with liver biopsy are helpful for the diagnosis or exclusion of cirrhosis they clinically have a limited accuracy for the diagnosis of significant fibrosis (11–13).

The assessment of quantitative HBsAg level reflects the transcriptional activity amount of cccDNA and integrated DNA in hepatocytes, one of the main serological markers in the chronic HBV infection. It makes the disease progress and prognosis to be accurately followed up as well as the response to the antiviral treatment (14,15). A correlation between the quantitative HBsAg and liver fibrosis has been observed in different studies (16–18). Serum HBV-DNA levels are a strong prognostic marker of the HBV replication level for chronic HBV infection. The increasing HBV-DNA levels are associated with the rate of progress to cirrhosis and incidence of hepatocellular carcinoma. However, high HBV-DNA levels do not always show significant hepatitis (19). Whether there was a correlation between HBV viral markers and liver histopathology was assessed in this study. Ease of Use.

II. METHODOLOGY

Study Design

A total of 128 HBeAg positive/negative patients who had serum and liver biopsy results between October 2016 and March 2019 were included in this retrospective study. The treatment-naïve patients whose ages ranged from 18 to 73, who had been HBsAg positive for at least 6 months and whose serum HBV-DNA level was ≥10,000 copies/ml (2000 IU/ml) were included in the study. The patients with unqualified or unclear liver pathology results and those with a coinfection (HAV, HCV, HDV, and HIV) were excluded from the analysis.

The ethical approval for the study was obtained from the Non-interventional Research Ethics Committee of Gulhane Training and Research Hospital at the University of Health Sciences (Reference number: 2019/19/352).

Serological tests

HBsAg, HBeAg, anti-HAV, anti-HCV, and anti-HIV tests were qualitatively analyzed in the serum samples sent to the Microbiology Virology Laboratory between October 2016 and March 2019 by using the Architect HBsAg, HBeAg, anti-HAV, anti-HCV, and anti-HIV reactive kits (Abbott Diagnostics, Germany) with the chemiluminescent enzyme immunoassay (CMIA) technique on the Architect i2000SR system (Architect, Abbott Diagnostics, Germany) according to the instructions of the manufacturer. The results of the Architect HBsAg test are assessed with S/Co ratio and S/Co value is accepted as non-reactive in the value of <1.0 and reactive in the value of ≥1. All the samples determined as an intermediate value between 0.80 and 0.99 were reanalyzed according to the instructions of the manufacturer. Only a single result of the same patient was included in the study.

Molecular analysis:

Isolation device (Magnesia 2448 Anatolia Geneworks, Turkey) and HBV-DNA isolation kit (Viral DNA isolation kit, Anatolia Geneworks, Turkey) were used in the detection of HBV-DNA. The PCR mixture prepared with the real-time PCR kit (Bosphore HBV Quantification Kit v2, Turkey) was amplified on the real-time PCR device (Montania 4896 Anatolia Geneworks, Turkey). The kit detects all the HBV genotypes of HBV-DNA between A and H and determines the amount in the human serum or plasma samples. Analytic sensitivity of the kit was 10 IU/ml and linear interval was $1x10^1$-$1x10^9$ IU/ml.

Liver Histology

All the liver biopsy samples were stained with hematoxylin-eosin and Masson trichrome for histological assessment and analyzed by a pathologist. ISHAK staging system was used in detecting the activity level of chronic hepatitis and fibrosis level. Activity level of hepatitis ranged from 0 to 18 and fibrosis level ranged from 0 to 6. Significant fibrosis was defined as the stage 2 fibrosis.

Statistical Analysis

The data were analyzed using SPSS 25 (SPSS Inc., Chicago, IL, USA) software program. The data were expressed as median (interquartile range). The differences between the groups were compared with the non-parametric Mann-Whitney U test or Kruskal-Wallis test for continuous variables and Pearson's Chi-Square or Fisher's exact tests for categorical variables. The correlation between HBsAg and HBeAg and HBV-DNA levels was analyzed with the Spearman's rank correlation analysis. The diagnostic performance cut-off values of HBsAg, HBeAg and HBV-DNA in the prediction of increased fibrosis was assessed using the receiver operating characteristics (ROC) curve and the results were expressed with 95% confidence interval (95% CI). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to determine the optimal cut-off value. $P<0.05$ was accepted as the statistically significant value.

III. RESULTS AND DISCUSSION

Out of 128 patients who were included in the study and whose ages ranged from 18 to 73, 100 (78%) were male and 28 (22%) were female. Out of 128 treatment-naïve patients with chronic HBV, 50 (39%) were HBeAg positive. Demographic, virological and biochemical characteristics of the HBeAg negative and positive patients with chronic HBV were showed in Table 1 and the correlation of the demographic and laboratory findings of the study group with the inflammation and fibrosis score was showed in Table 2.

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HBsAg S/Co (log) and viral load (log, IU/ml) level exhibited a negative but significant correlation in HBeAg negative and positive patients (Spearman's rho=-0.277, p=0.01 and Spearman's rho=-0.475, p=0.001 respectively).

According to the histological assessment results of the liver biopsy, 50.8% (65/128) of the patients had fibrosis (28 patients were stage 1 fibrosis; 16 were stage 2; 10 were stage 3; 7 were stage 4; and 4 were stage 5). The inflammation score ranged from 1 to 18 in 95.3% (67/128) of the patients (55 were grade <4 and 67 were grade ≥4-18).

The performance of viral load (log) in predicting the presence of fibrosis was assessed with ROC analysis. Sensitivity was 51.7%, specificity was 57.8%, area under the curve (AUC) was 0.551 (log), and p=0.37 in the value of 5.1 (log) IU/ml with the highest sum of sensitivity and specificity for the prediction of fibrosis score ≥2 (95% Cl: 0.435-0.666). Sensitivity was 72.7%, specificity was 41.9%, AUC was 0.545 (95% Cl: 0.367-0.723), and p=0.62 in the value of 6.1 (log) IU/ml for the fibrosis score ≥4 (Table 3).

TABLE I. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE PATIENTS WITH CHRONIC HBV

	All patients	**HBeAg negative**	**HbeAg positive**	**P value**
Patients n (%)	128 (100)	78 (61)	50 (39)	
Male	100 (100)	53 (53)	47 (47)	0.001
Female	28 (100)	25 (89)	3 (11)	
Gender (male/female)	3.6/1	2.1/1	16/1	
Age groups n (%)				
<40	78 (61)	37 (47)	42 (53.2)	<0.001
≥40	50 (39)	41 (53)	8 (16.3)	
Age* (year)	29 (21-49)	43 (23-53)	23 (21-28)	<0.001
Male	24 (21-44)	34 (21-52)	22 (21-27)	0.005
Female	45 (37-57)	45 (38-61)	42 (39-47)	0.55
AST*	34 (23-50)	30 (22-41)	38 (27-52)	0.09
ALT*	46 (4-86)	36 (24-73)	58 (29-102)	0.12
Platelet*	220(186-265)	220 (184-240)	220 (196-274)	0.45
HBsAg* (log, S/Co)	3.5 (3.3-3.7)	3.6 (3.5-3.7)	3.3 (2.9-3.5)	<0.001
HBV-DNA* (log; IU/ml)	5.7 (4.1-7.7)	4.6 (3.7-5.8)	7.8 (6.3-8.4)	<0.001
Inflammationscore*	4 (2-6)	4(2-7)	4 (3-6)	0.65
Fibrosis stage*	1 (0-2)	0.5(0-2)	1(0-2)	0.67

*Median, interquartile range; AST, aspartate aminotransferase; ALT, alanine aminotransferase

When all the patients were assessed serum HBV-DNA level (median) was found as 5.7 log (IU/ml). As expected, serum HBV-DNA levels (log, IU/ml) were high in HBeAg-positive chronic HBV (7.8 log) and lower in HBeAg-negative chronic HBV (4.6 log). High HBV viremia levels in HBeAg positive patients were associated with the specific immunosuppressive effect of HBeAg (20). As a result, it reveals that HBV replication is still active in both HBeAg positive and negative chronic HBV. HBV replication continues during the whole chronic HBV infection (21).

In our study, serum HBV-DNA level was significantly higher in HBeAg positive patients compared with the HBeAg negative patients. This result is consistent with the findings of the other studies (20,22–24).

In our study, HBeAg negative patients were older than the HBeAg positive patients, which is consistent with the findings in literature (20,23,25). The young patients with chronic HBV had HBeAg positivity and high viral loads compared with the elderly patients, which is consistent with the findings in the other studies. HBeAg positivity decreases by age (23). Although the innate and acquired immune response are still held responsible for the seroconversion of HBeAg in HBV the increase of HBeAg-negative mutant virions in the serum levels is presented as an alternative hypothesis (26).

A negative but significant correlation was found between the median values of HBsAg S/Co (log) and median values of HBV-DNA viral load (log, IU/ml) in both HBeAg negative and positive patients. The use of quantitative HBsAg titers in the prediction of HBV-DNA level as a reliable marker is still unclear. A positive correlation between the serum HBsAg and HBV-DNA has been revealed in some studies (27,28). Wiegand et al. reported that there was no significant correlation between the HBsAg or HBeAg and HBV-DNA level in chronic HBV patients who had been

treated or who had not been treated (29). The difference of our study from the other studies is that the correlation of HBV-DNA (log, IU/ml) levels and the HBsAg S/Co (log) levels was characterized, not the HBsAg titers. We could not find any similar studies in literature review.

In this study, necroinflammation (95.3%) was observed in most of the patients with chronic HBV. Fibrosis was found in 50.8% of the patients. No strong correlation was found between HBV-DNA viral load and liver inflammation.

Higher viral load was found in the patients with lower fibrosis (F < 2). In a similar study, Demir et al. reported that the patients with non-progressed fibrosis had higher HBV-DNA levels compared with those with progressed fibrosis (30).

There are also other studies reporting no correlation between the high HBV-DNA load and the severity of liver damage (31-35). HBV-DNA load can be affected by the host factors such as immune response of the host and alcohol consumption and viral factors such as genotype and pre-core/core mutations and in addition, fluctuating HBV-DNA levels and immune-mediated damage due to cytokines in the liver can explain this result (36,37).

For the prediction of fibrosis in HBV-DNA viral load value, the sensitivity was 51.4% and the specificity was 57.8% at the level of HBV-DNA ≥ 5.1 (log, IU/ml) in predicting significant liver fibrosis (F≥2) for the liver biopsy indication. Gao et al. found the sensitivity as 71.1% and specificity as 73.4% for the serum HBV-DNA cut-off value of 7.13 (log, IU/ml) in the prediction of fibrosis in HBeAg positive patients (38). Liu et al. revealed that sensitivity, specificity and AUC value were 64.3%, 78.94% and 0.818 respectively for the HBV-DNA cut-off value > 6.68 (log, IU/ml) in the prediction of mild liver fibrosis in the HBeAg positive group (39).

In conclusion, different HBV-DNA levels in treatment-naïve patients with chronic HBV can have effects in the formation of fibrosis in the liver tissues and predict the severity of fibrosis. When the indirect serum biomarkers and serum viral load levels in chronic HBV are used together they may be more helpful in predicting fibrosis.

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