The Association Between Biochemical Parameters and Extent of Disease in Patients with Chronic Hepatitis B

Kronik Hepatit B Enfeksiyonu Olan Hastalarda İleri Evre ile İlişkili Biyokimyasal Parametreler

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ABSTRACT Objective: The aim of the present study was to determine the relationship between serum levels of biochemical parameters and histological evaluation of liver biopsy specimens in patients with chronic hepatitis B infection. **Material and Methods:** This retrospective study was carried out in 21 female and 55 male patients and the mean age was 38 ± 10 years. Liver biopsy was obtained from patients with HBV DNA >105 copies/mL (for HBeAg positive patients) or >104 copies/mL (for HBeAg negative patients) for histological analysis and 24 patients with stage parameters <2 were included in group I and 52 cases with ≥2 in group II. The patients were grouped according to the scoring system for chronic hepatitis modified from Scheuer. **Results:** The HBV DNA levels were 834183 ± 16821275 copies/mL in group I and 93072001 ± 348168594 copies/mL in group II (p= 0.241). The mean level of the serum liver enzyme ALT in patients with stage ≥2 in group II (153.36 ± 227.63 U/L) was statistically higher than the mean ALT level of patients with grade less than 2 in group I (46.58 ± 31.59 U/L) (p= 0.025). While the higher total protein and albumin/globulin ratio in group I was statistically significant (p= 0.005; p= 0.004), the difference in serum albumin levels was not (p= 0.839). The mean α-fetoprotein level (3.13 ± 1.76 IU/mL) of group I patients was less than the level in group II (3.63 ± 3.73 IU/mL); this difference was not statistically significant (p= 0.531). There was also no statistically significant difference in blood platelet counts between group I and II (214041 ± 52410.98 K/uL and 212846 ± 54743.03 K/uL respectively; (p= 0.928)). The international normalized ratio (INR) of patients in group I was 13.4 ± 0.67 and 13.31 ± 0.70 in group II; this difference was not statistically significant (p= 0.623). **Conclusion:** There appears to be an association between serum levels of ALT and albumin/globulin ratio and the extent of hepatic fibrosis in patients with chronic HBV infection. Currently available non-invasive markers of disease activity do not seem to be good predictors for disease progression.

Key Words: Hepatitis B, chronic; pathology

ÖZET Amaç: Çalışmanın amacı kronik hepatit B'li hastalarda serum biyokimyasal parametrelerinin, karaciğer biyopsilerinin histolojik değerlendirilmesi ile ilişkisinin belirlenmesidir. **Gereç ve Yöntemler:** En az 6 aydır, bilinen HBsAg pozitifliği olan, toplam 76 hasta çalışma grubuna alınmıştır. Yaş ortalamaları 38 ± 10 yıl olan 21 kadın ve 55 erkek hasta retrospektif olarak değerlendirilmiştir. Hepatit B virüsü DNA (HBV DNA) kopya sayısı; HBeAg pozitif hastalar için >105 kopya/mL ve HBeAg negatif için >104 kopya/mL olanlardan karaciğer biyopsisi yapılmış evre <2 olan 24 hasta grup I ve evre ≥2 olan 52 hasta grup II olarak alınmıştır. Histolojik evrelendirme modifiye Scheuer sistemine göre yapılmıştır. **Bulgular:** HBV DNA düzeyleri grup I'de 8834183 ± 16821275 kopya/mL ve grup II'de 93072001 ± 348168594 kopya/mL olarak saptanmıştır (p= 0.241). Serum karaciğer enzimlerinin ortalamasına bakıldığında; ALT düzeyleri grup II'de, 153.36 ± 227.63 U/L ve grup I'de 46.58 ± 31.59 U/L saptanmış ve aradaki fark istatistiksel olarak anlamlı bulunmuştur (p= 0.025). Hem total protein hem de albumin/globulin oranı grup I'de istatistiksel olarak anlamlı yüksek saptanmıştır (p= 0.005, p= 0.004). Ancak serum albumin düzeyi açısından iki grup arasında anlamlı fark yoktur (p= 0.839). Alfa-fetoprotein düzeyi; grup I'de (3.13 ± 1.76 IU/mL) grup II'ye göre düşüktür (3.63 ± 3.73 IU/mL). Ama fark istatistiki olarak anlamlı değildir (p= 0.531). Trombosit sayısında gruplar arasında anlamlı fark bulunmamıştır (grup I: 214041 ± 52410.98 K/uL ve grup II: 212846 ± 54743.03 K/uL; (p= 0.928)). İnternasyonal normalizasyon oranı (INR) grup I'de 13.4 ± 0.67 ve grup II'de 13.31 ± 0.70'dir. İstatistiksel olarak anlamlı fark yoktur (p= 0.623). **Sonuç:** Serum ALT düzeyleri ve albumin/globulin oranı ile kronik hepatit B hastalarının fibroz oranları arasında ilişki saptanmış, ama invaziv olmayan yöntemlerin fibroz derecesini belirlemede yeterli performansda olmadıkları görülmüştür.

Anahtar Kelimeler: Hepatit B virüsü, kronik; patoloji

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The hepatitis B virus (HBV) is estimated to have infected more than 2 billion people worldwide, contributing to 400 million chronic cases who have an increased risk of liver-related sequels, including cirrhosis, fulminant liver failure, liver transplantation, hepatocellular carcinoma (HCC) and death.[1,2]

Liver fibrosis is the result of chronic injury. The histopathological pathway of progressive liver disease is characterized by the formation and accumulation of fibrosis, leading to increased distortion of the hepatic architecture causing hepatocellular dysfunction and portal hypertension that is the hallmark of evaluation to cirrhosis. The degree of hepatic necroinflammation (grade) and fibrosis (stage) are strongly associated with the natural history and risk of complications in patients with chronic HBV infection.[3,4]

Current guidelines recommend that patients with HBV-DNA >10^5 copies/mL and persistent or intermittent elevation of aminotransferase levels should be evaluated further with liver biopsy, that is the gold standard for the assessment of fibrosis.[4] This procedure provides information on the severity of necroinflammatory activity and on the stage of fibrosis, features that are essential for estimating prognosis and the need for antiviral therapy.[3] However, biopsy is a costly, invasive method associated with many limitations. It has potential complications like bleeding and pain.[5] It has sampling artifact and is prone to intra- and inter-observer variation in scoring, and underestimation of liver fibrosis with small samples since its performance is size-dependent. Furthermore, it is not accepted by some patients due to its invasive nature. Considering these limitations and patient resistance to undergo liver biopsy, a great interest and many studies have been recently dedicated to the development of non-invasive markers to assess liver fibrosis.[6-10]

The need for simple and reliable non-invasive markers such as blood tests and/or liver imaging modalities that accurately correlate with disease activity and stage was previously mentioned in the management of chronic HBV patients. Although ultrasound examination was also suggested for non-invasive disease staging in HBV as a liver imaging modality, it has limited sensitivity for detecting severe fibrosis.[11] Factors associated with an increased rate of progression to cirrhosis include high serum HBV DNA, co-infection with hepatitis C virus (HCV), hepatitis D virus (HDV) or Human Immunodeficiency virus (HIV), recurrent episodes of acute exacerbation, and severe necroinflammation at diagnosis.

In the light of the above-mentioned knowledge, the aim of the present study was to determine the relationship between serum levels of biochemical parameters and histological evaluation of liver biopsy specimens in patients with chronic hepatitis B infection.[12]

MATERIAL AND METHODS

Study Design and Protocol

260 patients with a diagnosis of chronic HBV infection, as defined by positive hepatitis B surface antigen (HBsAg) for at least 6 months, who presented to the Infectious Diseases and Clinical Microbiology Outpatient Clinic of Kocaeli University, Faculty of Medicine, between January 2004 and November 2006 were evaluated retrospectively. Liver biopsy was performed to the patients who had persistent or intermittent elevation of transaminase levels and HBV DNA elevations [HBV DNA >10^5 copies/mL (for HBeAg positive patients) or >10^4 copies/mL (for HBeAg negative patients)]. Only 76 patients were enrolled in this study because the other 184 patients did not meet the criteria for liver biopsy (HBV DNA negative or >1000 copies/mL).

Informed consent was obtained from all patients participating in the study that was conducted according to the rules of the Declaration of Helsinki.

The exclusion criteria were any other cause of chronic liver disease, cirrhosis, and coinfection with HCV, HIV or HDV.

Virological assays, HBsAg, hepatitis B e antigen (HBeAg), antibodies to HBsAg (anti-HBs), antibodies to HBeAg (anti-HBe) and hepatitis B core antigen (anti-HBc) were determined using com-

mercial (ELISA) assays. The quantitative HBV DNA polymerase chain reaction (PCR) technique was performed for DNA detection. DNA was extracted from 100 μL serum using the QIAamp DNA Mini kit (Qiagen Inc, Hilden, Germany) according to the manufacturer's recommendations. HBV DNA was measured by in-house real-time quantitative polymerase chain reaction with primers from the X region (Forward primer: 5' TGCACTTCGCTTCACCTCTG 3', reverse primer: 5'AGGTCGGTCGTTGACATTGC 3', probe: 5' CGCATGGAGACCACCGTGAACGCC 3'). Serum HBV DNA was quantified using real-time quantitative PCR (RQ-PCR), iCycler IQ system (BioRad Laboratories), with a detection limit of 200 virions/mL.[13]

The serum biochemical parameters including serum alanine aminotransferase (ALT), alpha-fetoprotein (AFP), total protein, albumin, globulin, prothrombin time (PT), and platelet counts were measured.

Statistical Analysis

All statistical analyses were carried out by using a Graphpad Prism 4.0. The results were expressed as mean ± standard deviation. Unpaired two tailed student-t test was used for the comparison of the data. A p value of less than 0.05 was considered significant.

RESULTS

The study was carried out in 21 female and 55 male patients and the mean age was 38 ± 10 years. Liver biopsy was obtained from patients with HBV DNA $>10^5$ copies/mL (for HBeAg positive patients) or $>10^4$ copies/mL (for HBeAg negative patients) for histological analysis. 24 patients with stage parameters <2 were included in group I and 52 cases with ≥2 in group II (Table 1).

The HBV DNA levels were 834183 ± 16821275 copies/mL in group I and 93072001 ± 348168594 copies/mL in group II. The difference between the two groups was not statistically significant (p= 0.241). The mean level of serum liver enzyme ALT in patients with stage ≥2 in group II (153.36 ± 227.63 U/L) was statistically higher than the mean ALT level of patients with grade less than 2 in group I (46.58 ± 31.59 U/L) (p= 0.025).

The mean total protein level of patients in group I was less than the levels in group II. This difference was statistically significant (p= 0.005). The mean serum albumin levels in group I and II were not different (4.37 ± 0.32 g/dL and 4.36 ± 0.31 g/dL, respectively). However, when the albumin/globulin ratio was analyzed, the ratio was 1.33 ± 0.22 in group I and 1.19 ± 0.17 in group II. The higher albumin/globulin ratio in group I was statistically significant (p= 0.004).

The mean α-fetoprotein level (3.13 ± 1.76 Iu/mL) in group I patients was less than the level in group II (3.63 ± 3.73 IU/mL). This difference was not statistically significant (p= 0.531). There was also no significant difference in blood platelet counts between groups I and II (214041 ± 52410.98 K/uL and 212846 ± 54743.03 K/uL respectively; p= 0.928). The INR of patients was 13.4 ± 0.67 in gro-

TABLE 1: Comparison of biochemical parameters between patients with Grade <2 and Grade ≥2 chronic hepatitis B.

Parameters	Group 1 (Grade <2) (n= 24)	Group 2 (Grade ≥2) (n= 52)	p value
ALT levels (U/L)	46.58 ± 31.59	153.36 ± 227.63	0.025*
INR	13.4 ± 0.67	13.31 ± 0.70	0.623
Serum albumin (g/dL)	4.37 ± 0.32	4.36 ± 0.31	0.839
Total protein (g/dL)	7.63 ± 0.61	8.09 ± 0.64	0.005**
Albumin/globulin	1.33 ± 0.22	1.19 ± 0.17	0.004**
HBV DNA (IU/mL)	8834183 ± 16821275	93072001 ± 348168594	0.241
α-fetoprotein (IU/mL)	3.13 ± 1.76	3.63 ± 3.73	0.531
Platelet (K/uL)	214041 ± 52410.98	212846 ± 54743.03	0.928

ALT: Alanine aminotransferase; INR: International normalized ratio; PT: Prothrombin time; HBV DNA: Hepatitis B virus DNA.

The values are expressed as mean ± standard deviation.* Statistically significant p <0.05.

up I and 13.31 ± 0.70 in group II. The difference was not statistically significant (p= 0.623).

DISCUSSION

The most common definition of chronicity is the presence of serum HBsAg for at least 6 months. The outcome of HBV infection is variable, influenced by age at infection, immune response and environmental factors. The criteria for diagnosis of chronic hepatitis B infection includes HBsAg positivity for >6 months, high levels of serum HBV DNA (>100.000 copies/mL for HBeAg positive patients and >10.000 copies/mL for HBeAg negative patients), persistent or intermittent elevation of ALT/AST levels, and liver biopsy showing chronic hepatic inflammatory injury. The patients in our study were diagnosed with chronic hepatitis B infection and their serum levels of non-invasive markers of liver fibrosis and liver biopsy specimens were compared in two groups as grade 2 and greater (group II) and lower than grade 2 (group I).

Very few studies have investigated the role of non-invasive markers of liver fibrosis in hepatitis B. Several non-invasive markers of liver fibrosis have recently been described in patients with hepatitis B, but their use in clinical practice has not established yet since the diagnostic performance of described non-invasive markers is variable depending on the stage of fibrosis and other patient characteristics.

The latest AASLD guidelines on management of chronic hepatitis B recommend that patients with HBV-DNA >10^5 copies/mL and persistent or intermittent elevation of transaminase levels should be evaluated further with liver biopsy.[4]

The amount of HBV DNA in serum is a measure for the level of viral replication. The National Institutes of Health Workshop on Management of Hepatitis B recommended that treatment should be considered for patients with detectable HBV DNA by nonamplified assays (ie, >10^5 copies/mL, or 20.000 IU/mL).[14] However, some HBeAg-positive patients and many HBeAg negative patients have fluctuating HBV DNA levels that decrease to less than 10^5 copies/mL.[15] Furthermore, the threshold HBV DNA level associated with progressive liver disease is unknown.

In our study, the mean serum HBV-DNA level was greater than 8.000.000 copies/mL in group I and 90.000.000 copies/mL in group II. The higher HBV-DNA level in group II patients was consistent with the higher degree of fibrosis in the disease.

ALT level is used commonly as an assessment of liver disease and is considered significant in defining candidates for therapy because of its value in predicting a serologic response to therapy.[16] All the patients in the present study had increased serum alanine aminotransferase levels. The increased serum ALT level is an indicator of necroinflammatory activity and HBV-infected patients with persistently normal ALT levels generally have milder inflammation on liver biopsy specimens than patients with increased ALT levels. The ALT level of patients in our study was significantly higher in subjects with grade 2 and greater (group II) than the patients with grade less than 2 (group I). However, reliance on increased ALT levels as a predictor of treatment candidacy has some limitations, because of other causes of ALT elevations, like fatty liver etc. The extent of liver cell necrosis and the degree of increased ALT levels do not always correlate and ALT measurements may fail to identify patients with necroinflammatory activity or fibrosis, as in hepatitis C.[17] However, some patients with normal ALT levels and increased HBV DNA levels may have significant inflammation and fibrosis on biopsy examination.[18] In three recent preliminary reports, 12%-43% of patients with chronic HBV infection was shown to have persistently normal ALT levels and had stage 2 fibrosis or greater.[19]

Schmilovitz-Weiss et al reported in their study a strong association between levels of serum globulin and IgG and extent of hepatic fibrosis in patients with chronic HBV infection.[20] The increasing fibrosis and worsening of portal hypertension leads to increased sequestration and destruction of the platelets in the enlarging spleen.[21] In addition, studies in liver transplant patients showed that the prog-

ression of liver fibrosis was associated with decreased production of thrombopoietin by hepatocytes, and hence, reduced platelet production.[22-24]

In the present study, total protein and serum albumin levels of patients with chronic HBV infection were analyzed. These parameters were strongly associated with the extent of hepatic fibrosis recognized as the prognostic factor of survival in patients with decompensated chronic HBV. The biological function of the hepatocytes during fibrogenesis is affected by changes in the composition of the extracellular matrix, which could explain the predictive values of these two parameters.[25-27] We also analyzed the albumin/globulin ratio and found it to be decreased in advanced stage when compared to lesser degree of fibrosis. There was no difference between groups associated with fibrosis severity in terms of INR. We also detected a weak association between necroinflammation severity and serum α-fetoprotein levels.

In conclusion, there appears to be an association between serum levels of ALT and albumin/globulin ratio and extent of hepatic fibrosis in patients with chronic HBV infection. Currently available non-invasive markers of disease do not seem to be predictive for disease progression. Prospective, longitudinal studies are required to determine the utility of non-invasive laboratory and radiological markers in predicting disease progression and/or regression over several years. Therefore, liver biopsy along with routine laboratory parameters and HBV replicate markers play an important role to assess disease activity and severity in chronic HBV and to help identify patients in need of antiviral treatment.

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