Research Article

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The Association Between Hepatitis B Virus (HBV)-DNA Levels and Biochemical Markers

Hepatit B Virüs (HBV)-DNA Seviyeleri ve Biyokimyasal Belirteçler Arasındaki İlişki

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ABSTRACT

Objective: In the present study, we aimed to evaluate the association between HBV-DNA levels and biochemical parameters, age, gender, and hepatitis B virus (HBV) serologic markers.

Materials and Methods: A total of 124 HBsAg (+) serum samples of the patients with chronic hepatitis B, were included in the study. HBV-DNA level, HBV serological markers, alanine transaminase (ALT), aspartat transaminase, gama-glutamyl transferase (GGT), lipase, bilirubin, lactate dehydrogenase, and C-reactive protein levels in the samples were evaluated. HBV-DNA levels were quantitatively evaluated by real-time polymerase chain reaction (PCR) and serological markers were evaluated by ELISA.

Results: With molecular testing, 116 samples were positive for HBV-DNA, of them 36.2% had an HBV-DNA level >2000 IU/mL. The number of samples with HBV-DNA levels >2000 IU/mL were higher in females than those of males. 44.4% of all HBeAg (+) patients with elevated ALT levels had an HBV-DNA level >2000 IU/mL. All HBeAg (+) patients, 24.4% of HBeAg (-) patients, and 25.5% of anti-HBe (+) patients had elevated ALT levels. 99 of the 106 anti-HBe (+) carriers, of whom 27 had elevated ALT levels, were PCR positive. All HBeAg-positive samples were HBV-DNA positive. The rate of samples with elevated biochemical parameters except GGT levels were higher serian patients with HBV-DNA level >2000 IU/mL than in patients with HBV-DNA level <2000 IU/mL. A statistically significant relationship was detected only between lipase and HBV-DNA levels.

Conclusion: It is clear that HBeAg seroconversion is not sufficiently definitive to determine the infectivity and it is crucial to evaluate HBV serological tests, HBV-DNA levels, transaminases levels besides the clinical picture of the patient in the diagnosis of the infection. *(Viral Hepatitis Journal 2014; 20(1): 4-7)*

Key words: Hepatitis B virus DNA, alanine aminotransferase (ALT) levels, lipase

ÖZET

Amaç: Bu çalışmada hepatit B virüsü (HBV)-DNA düzeyleri ile biyokimyasal parametreler, yaş, cinsiyet, HBV serolojik göstergeleri arasındaki ilişki incelenmiştir.

Gereç ve Yöntemler: Çalışmaya, kronik hepatit B hastalarından alınan HBsAg (+) 124 serum örneği dâhil edilmiştir. Örneklerde HBV-DNA düzeyi, HBV serolojisi, alanin transaminaz (ALT), aspartat transaminaz, gama-glutamil transferaz (GGT), lipaz, bilirubin, laktat dehidrogenaz, C-reaktif protein düzeyleri incelenmiştir. HBV-DNA düzeyi gerçek zamanlı polimeraz zincir reaksiyonu (PCR) ile serolojik belirteçler ise ELISA ile değerlendirilmiştir.

Bulgular: Moleküler inceleme sonucunda 116 örnekte HBV-DNA varlığı tespit edilmiş, bunların %36,2'sinin HBV-DNA düzeyinin 2000 IU/mL'nin üzerinde olduğu belirlenmiştir. HBV-DNA düzeyi 2000 IU/mL'nin üzerinde olan örnekler kadınlarda daha yüksek sıklıkta bulunmuştur. ALT düzeyinde artış saptanan HBeAg (+) hastaların %44,4'ünde HBV-DNA düzeyi 2000 IU/mL'nin üzerinde belirlenmiştir. ALT yüksekliği HBeAg (+) tüm hastalarda, HBeAg (-) olanların %24,4'ünde ve anti-HBe (+) hastaların %25,5'inde izlenmiştir. Yirmi yedisinde ALT artışı belirlenen 106 anti-HBe (+) hastasının 99'unda PCR pozitif olarak saptanmıştır. HBeAg pozitifliği izlenen tüm örneklerde HBV-DNA (+) olarak belirlenmiştir. GGT dışındaki tüm biyokimyasal parametrelerde yükseklik, HBV-DNA düzeyi 2000 IU/mL'nin üzerinde olan hastalarda 2000 IU/mL altındaki hastalara göre daha yüksek sıklıkta bulunmuştur. İstatistiksel olarak anlamlı bir ilişki sadece lipaz ile HBV-DNA düzeyi arasında belirlenmiştir.

Sonuç: Enfektivitenin tespitinde HBeAg serokonversiyonu tek başına yeterli değildir, yanı sıra HBV serolojik testleri, HBV-DNA düzeyleri ve transaminaz seviyeleri ile birlikte hastanın klinik durumunun da tanıda değerlendirilmesi gerekmektedir. *(Viral Hepatit Dergisi 2014; 20(1): 4-7)*

Anahtar Kelimeler: Hepatit B virüs DNA, alanin aminotransferaz (ALT) düzeyleri, lipaz

Introduction

Hepatitis B virus (HBV) infection is an important infectious disease with the reported prevalence ranging 0.1% to 20% and approximately 350-400 million people worldwide are chronically infected facing off the high risk for developing cirrhosis, fulminant hepatitis, end-stage liver disease and hepatocellular carcinoma. While 1-2% of chronic carriers become HBsAg-negative, 5-10% become asymptomatic carriers or develop chronic hepatitis (1-12). In low endemic areas such as western countries major transmission route is exposure to infectious blood or body fluids, a result of risky sexual behaviours and injection drug users (1,2,5,13). In endemic countries in Southeast Asia and Africa, perinatal infection is the major route for transmission (1,6,13,14,15).

After exposure, HBsAg is the first detectable antigen followed by HBeAg and high levels of serum HBV-DNA are observed. During the natural course of an infection, anti-HBe will arise immediately after HBeAg is cleared, generally causing a decline in viral replication. If the host is able to clear the infection, HBsAg will

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disappear, anti-HBs and anti-HBc IgG become detectable. Patients who remain HBsAg-positive for at least six months are considered to be hepatitis B carriers (1,2,7). Clinical symptoms, elevated serum alanine-transaminase (ALT) levels and positive test result for anti-HBc are useful for the diagnosis. ALT level is an important marker of hepatocelluler injury, and is routinely used in the follow-up of the treatment. Elevated ALT is considered to be associated with active liver disease on histology while normal level is considered to be associated with inactive histology (12,16).

Nowadays, molecular detection of HBV-DNA is widely used to detect viral replication. Patients should be evaluated for HBV-DNA levels, HBeAg status and if possible liver biopsy and genotype of HBV (12). Recently published guidelines recommend antiviral treatment for patients with HBV-DNA levels of >10 000 copies/mL (>2000 IU/mL), coupled with ALT levels of greater than two times the upper limit of normal (ULN) and significant liver fibrosis. HBV-DNA testing should be repeated at 3-6 months intervals and detection an increase in the levels of ALT and aspartat transaminase (AST) (2,12,17).

In this study, we aimed to evaluate the relationship between HBV-DNA levels and age, gender, biochemical parameters of the patients with chronic hepatitis B infection retrospectively.

Material and Methods

A total of 124 serum sample of the patients with chronic hepatitis B infection admitted to Ahi Evran University Training and Research Hospital, Kırşehir, Turkey, during the study period June-November 2012 were included in the study. HBV-DNA level, HBV serological markers, ALT, AST, gama-glutamyl transferase (GGT), lipase, bilirubine, lactate dehydrogenase (LDH), C-reactive protein (CRP), sedimentation tests were evaluated (Normal ranges of the tests; ALT 7-56 IU/L, AST 5-40 IU/L, LDH 45-90 U/L, CRP 0-5 mg/L, Lipase 21-67 U/L, GGT 0-42 IU/L, bilirubine 0.2-1.2 mg/dL). Hepatitis markers were analyzed by chemiluminesans enzyme immunoassay test method (Roche Modular Analytics, E-170; Roche Diagnostics , USA). HBV-DNA test was performed by real-time polimerase chain reaction (PCR) with automated system (ROCHE/COBAS® TaqMan® System) according to manifacturer's instructions.

Statistical Analysis

Statistical comparisons were performed with SPSS software version 15.0 (SPSS, Inc., Chicago, IL). Associations and comparisons were analyzed using the χ^2 test or the Fisher's exact test. All hypotheses were two-tailed and were considered significant at the $p<0.05$ level.

Results

A total of 124 HBsAg-positive serum samples [82 (66.1%) male and 42 (33.9%) female patients with aged between 14-77 years (median 46.14±13.90 years)] were included in the study. Positivity for HBeAg, anti-HBe and anti-HBs were detected among 9 (7.3%), 106 (85.5%) and 8 (6.5%) samples, respectively. All were negative for anti-HBc IgM but 82 (66.1) were positive for anti-HBc IgG. Eight (6.45%) sample were negative for HBV-DNA, of which all were HBeAg-negative. Of the 116 samples positive for HBV-DNA, 74 (63.8%) were <2000 IU/mL and 42 (36.2%) were >2000 IUmL. Female patients were likely to have more higher HBV-DNA levels of >2000 IU/mL compared to male group (40.5% vs 29.3%, p=0.209). Distribution of the serum HBV-DNA levels by age groups were shown on Table 1.

In the second part of the study, transaminase, GGT, lipase, LDH, bilirubine, CRP levels of the patients sera were evaluated. Serum bilirubine levels were in normal range of all the samples tested. Frequencies of elevated biochemical parameters according to HBV-DNA levels of the patients were shown on Table 2.

Samples with HBV-DNA level >2000 IU/mL were likely to have elevated lipase, LDH, CRP, ALT and AST levels compared to samples with HBV-DNA level <2000 IU/mL. But statistically significant relationship was only detected between lipase and HBV-DNA levels (p=0.001).

High levels of ALT were detected among 44.4% of HBeAg-positive, 24.4% of HBeAg-negative and 25.5% of anti-HBe-positive patients. HBeAg-positive sera with elevated ALT (n=4) has HBV-DNA level >2000 IU/mL. 99 of the 106 anti-HBe positive carriers, 27 of whom had elevated ALT levels, were PCR positive. The remaining sera was negative for HBV-DNA. Higher HBV-DNA >2000 IU/mL were detected among HBeAg-positive samples compared to HBeAg-negative patient sera (66.7% vs 30.4%; p=0.058). Most patients were HBeAg-negative and anti-HBe-positive status (104/124). Distribution of HBV-DNA levels of the samples by ALT levels and HBeAg-anti-HBe status was shown on Figure 1 and Table 3. Out of 124 chronic HBsAg carriers, seven

Table 1. Distribution of HBV-DNA levels of the study group (n=124) by age group

Age group	HBV-DNA (IU/mL)		
	Negative	>2000	>2000
1-20 years (n=5)	-	-	5 (100)
21-30 years (n=14)	-	10 (71.4)	4 (28.6)
31-40 years (n=21)	2 (9.5)	12 (57.1)	7 (33.3)
41-50 years (n=29)	4 (13.8)	15 (51.7)	10 (34.5)
51-60 years (n=39)	1 (2.6)	24 (61.5)	14 (35.9)
61-70 years (n=14)	1 (14.3)	11 (78.6)	2 (14.3)
≥ 71 years (n=2)	-	2 (100)	-
Total	8 (6.5)	74 (59.7)	42 (33.9)

Table 2. Percentages of elevated biochemical parameters by HBV-DNA levels of the patient sera

Elevated parameter	% HBV-DNA Level		p	OR (95% GA)
	<2000 IU/mL	>2000 IU/mL		
Lipase (n=41)	22.9	53.7	0.001	3.90 (1.75-8.67)
LDH (n=116)	91.6	97.6	0.269*	3.68 (0.43-31.00)
CRP (n=6)	2.4	9.8	0.092*	4.37 (0.76-24.97)
ALT (n=32)	21.7	34.1	0.136	1.87 (0.81-4.29)
AST (n=45)	33.7	41.5	0.400	1.39 (0.64-3.00)
GGT (n=32)	27.7	22	0.49	0.73 (0.30-1.77)

HBV: hepatitis B virus, LDH: lactate dehydrogenase, CRP: C-reactive protein, ALT: alanine-transaminase, AST: aspartat transaminase, GGT: gama-glutamyl transferase.

were HBeAg-positive/anti-HBe negative, 104 were 41 HBeAg-negative/anti-HBe positive, two patients were HBeAg-positive/anti-HBe positive and 11 were HBeAg-negative/anti-HBe negative.

Discussion

HBV is the cause of an infectious disease affecting approximately 350-400 million people worldwide. Biochemical assessment of liver function, total and direct bilirubin, ALT, AST, alkaline phosphatase, protrombin time, total protein, albumin, serum globulin, complete blood count tests are used in the diagnosis of hepatitis B infection (9). Introduction of molecular diagnostic methods facilitate the diagnosis and follow-up of the response to the antiviral treatment. In this study, the relationship between biochemical-serological markers and HBV-DNA levels of the patients with chronic hepatitis were evaluated. Serum samples were tested for hepatitis serological markers and all were positive for HBsAg.

HBsAg loss or seroconversion to anti-HBs is the most desirable result of antiviral therapy that may occur spontaneously in 1%-3% of cases per year, usually after several years with persistently undetectable HBV-DNA and shows the cure of the chronic infection (2,5,11,17,18). It is suggested that the HBsAg loss rate was lowest in HBeAg-positive patients (11). In this study HBsAg loss was not observed among our patient group. Seroconversion to anti-HBs was detected in 8 (6.5%) samples. Infection is more common between 21-30 age group. Our study consisted of patients between 14-77 years and higher prevalence (54.8%) was detected between 41-60 years. Eight (6.45%) sample were negative for HBV-DNA. Of the 116 samples positive for HBV-DNA, 74 (63.8%) were below 2000 IU/mL and 42 (36.2%) were over 2000 IU/mL.

Chronic infection may present either as HBeAg-positive or negative form (5,10). HBeAg seropositivity indicates the presence of viral particles, DNA polymerase and HBV-DNA in serum and shows active replication, however, variants of the hepatitis B virus do not produce HbeAg (7,8,11). Carriers with HBeAg-negative status, have very little viral multiplication and may be at little risk of long-term complications or of transmitting infection to others (16). Several determinants for HBeAg seroconversion have been reported, including gender, age, ALT level and more recently HBV genotypes (1). Among HBeAg-negative sera low level of HBV-DNA was detected compared to positive samples (2). Older carriers and female patients are more likely to clear HbeAg (1,19). HBeAg seroconversion ranges from 8 to 15% in children or adults with elevated ALT (1,17,18). In this study, out of 124 patients, 9 (7.3%) were HBeAg-positive and 115 (92.7%) were negative. HBeAg-positive patients were found to have high levels of HBV-DNA levels than in HBeAg-negative patients, 100% and 93%, respectively (p=1.000). Most patients (104/124) were HBeAg-negative and anti-HBe-positive. It is reported that HBeAg seroconversion occurs in up to 98% of subjects, and this is not a marker for a cure as it would be in wild type HBV, although it does act as a marker for healing. In our study, anti-HBe seroconversion was detected among 85.5% of the samples. Testing of samples negative for HBsAg with molecular testing methods, showed that some of these has HBV-DNA (20). In this study HBsAg negative sample was not included in this study, so we can not evaluate this case in our study group.

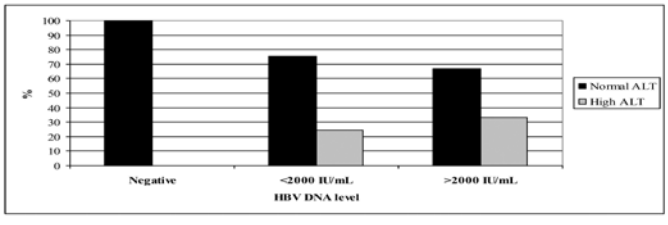

Figure 1. ALT levels of the patient sera by HBV-DNA levels.

HBV carriers should be followed up for life with ALT determinations at least every six months after the first year and periodical measurement of HBV-DNA levels should be performed (1,10,18). It was shown that ALT levels were higher among male patients compared to female (21,22). Overall the sera tested elevated ALT levels were detected in 25.8% of the patients. Similar with the previous studies, ALT levels were higher among male patients compared to female, 29.3% vs 19%, respectively in this study. All sera with HBV-DNA negative have normal ALT levels. Evaluation the relationship between HBV DNA level and ALT levels revealed that, patient sera with >2000 IU/mL were more likely have elevated ALT levels compared to sera with HBV-DNA level <2000 IU/mL, 33.3% v 24.3%, respectively, excluding the patients found to be negative for HBV-DNA. Although ALT level is an important marker for the follow-up of the infection, statistically significant relationship was not detected between HBV-DNA and ALT levels.

GGT levels rise and return to normal levels later than the transaminases levels in the liver diseases. So, the estimation of GGT is of some value in monitoring the progress of acute to chronic hepatitis, when the values persist in high levels. The chronic hepatitis which is caused by the hepatitis B and C viruses is associated with high GGT levels, which can be used as a noninvasive diagnostic marker and as a predictor of fibrosis (23). In this study all sera negative for HBV-DNA have normal range of

Table 3. Distribution of the numbers of the sera by HBV-DNA level, ALT levels and HBeAg-anti-HBe status

Hepatitis marker status		Total number (n)	HBV-DNA	
			<2000 IU/mL	>2000 IU/mL
HBeAg(+)/anti-HBe(-)		7		
	ALT level (normal)	4	2	2
	ALT level (high)	3	-	3
HBeAg(-)/anti-HBe(+)		104		
	ALT level (normal)	78	7	71
	ALT level (high)	26	-	26

HBV: hepatitis B virus, ALT: alanine-transaminase

GGT. Statistically relationship was not detected between HBV-DNA levels and GGT levels and elevation of GGT level was detected in 31.1% and 21.4% of HBV-DNA level <2000 IU/mL and >2000 IU/mL, respectively. The only significant relationship was detected between lipase levels and HBV-DNA levels.

In conclusion, we observed that the highest age distribution of the patients with chronic hepatitis B was 21-40 years, and 41-60 years, mostly among male patient group, higher ALT levels were detected among male patients. It is concluded that HBeAg seroconversion is not definitive to determine the infectivity and it is crucial to evaluate HBV serological tests, HBV-DNA levels, transaminases levels besides the clinical picture of the patient in the management in the diagnosis of infection.

Conflict of interest: None declared.

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