Turk J Gastroenterol 2003; U (3): 157-163

Determination of serum hepatitis B virus DNA in chronic HBsAg carriers: Clinical significance and correlation with serological markers

Kronik HBsAg taşıyıcılarında serum hepatit B virus DNA düzeylerinin klinik önemi ve serolojik işaretler ile ilişkisi

Kendal YALÇIN[1], Halil DEĞERTEKİN[1], M. Nail ALP[2], Selahattin TEKEŞ[2], Ömer SATICI[3], Turgay BUDAK[2]

Dicle University, School of Medicine, Division of Hepatology', Internal Medicine, Medical Biology and Genetics[2], Biostatistics[3], Diyarbakır

Background/aims: *Hepatitis B virus infection is among the most devastating health problems in the world, including Turkey. In this cross-sectional study, we aimed to investigate the correlations between hepatitis B virus genomic load and various measures of the progression of chronic hepatitis B virus infection.* ***Methods:*** *A total of 354 chronic HBsAg carriers [126 inactive HBsAg carriers, 50 asymptomatic replicative carriers (immune tolerant patients), 90 chronic hepatitis B patients and 88 patients with liver cirrhosis] were enrolled into the study. Eligible patients included males and females, 14-62 years of age, with detectable serum HBsAg, HBeAg or anti-HBe in serum at the time of screening and for at least six months before study entry. Serum hepatitis B virus DNA was detected by liquid hybridization, and results under the level of 1 pg/ml were additionally confirmed by polymerase chain reaction.* ***Results:*** *Of 354 patients, 118 (33%) were HBeAg-positive and 236 (67%) HBeAg-negative. Of HBeAg-negative patients, 126 (53%) had normal alanine aminotransferase, 31 (13%) had elevated alanine aminotransferase (chronic hepatitis B) and 79 (33%) had evidence of cirrhosis; corresponding figures in the HBeAg-positive patients were 50 (42%), 59 (50%) and 9 (8%). There is a significant correlation between transaminase values and histological liver damage, whereas no correlation was found between viral replication and liver damage.* ***Conclusions:*** *Hepatitis B virus DNA is an important and specific marker for ongoing hepatitis B virus related liver disease, but alanine aninotransferase was shown to be the best marker for liver inflammation and not hepatitis B virus viral load. Although these findings are not new, they are of some utility since they prevent unnecessary and cost-intensive viral load determinations in chronic HBsAg carriers.*

Key words: Hepatitis B virus, chronic hepatitis B virus infection, HBV DNA, ALT, HAI, HBeAg, anti-HBe

Amaç: *Hepatit B virus infeksiyonu Türkiye dahil tüm Dünya'da en önemli sağlık problemlerinden biridir. Bu çalışmada, HBV genomik yükü ile kronik HBV infeksiyonunun progresyonunu gösteren çeşitli parametereler arasındaki korelasyonun incelenmesi amaçlandı.* ***Yöntem:*** *Çalışmaya 126 inaktif HBsAg taşıyıcısı, 50 asemptomatik replikatif taşıyıcı (immüntoleran hasta), 90 kronik hepatit B ve 88 karaciğer sirozlu vaka olmak üzere toplanı 354 kronik HBsAg taşıyıcısı alındı. Çalışma hastaları en az 6 aylık izlem süresince kalıcı olarak serumda HBsAg, HBeAg ve anti-HBe pozitifliği saptanan, yaşları 14-62 arasında değişen, kadın ve erkeklerden oluşmaktaydı. Serum HBV DNA düzeyi sıvı hibridizasyonla saptandı ve sıvı hibridizasyon değeri l pglml olan hastalarda ayrıca ek olarak PZR incelemesi yapıldı.* ***Bulgular:*** *Toplam 354 hastanın 118'i (%33) HBeAg-pozitif ve 236'sı (%67) HBeAg-negatif hastalardan oluşmaktaydı. HBeAg-negatif hastaların, 126'sı (%53) normal ALT düzeyine sahipken 31'inde (%13) yüksek ALT düzeyi (kronik hepatit B), ve 79'unda ise (%33) siroz saptanırken bu oranlar HBeAg-pozitif hastalarda sırasıyla 50 (%42), 59 (%50) ve 9 (%8) idi. Transaminaz değerleri ile histolojik karaciğer hasarı arasında anlamlı bir korelasyon mevcutken viral replikasyon düzeyi ile karaciğer hasarı arasında anlamlı bir korelasyon bulunmadı.* ***Sonuç:*** *HBV'ye bağlı karaciğer hastalığında HBV DNA önemli ve spesifik bir işaretleyici olmasına karşın bu çalışmada ALT'nin karaciğer inflamasyonunu göstermede en iyi işaretleyici olduğu bulunmuştur. Bu bulgular, yeni olmamasına karşın, kronik HBsAg taşıyıcılarında gereksiz ve pahalı viral yük tayinlerinin yapılmaması konusunda ek bilgiler sağlamıştır.*

Anahtar kelimeler: Hepatit B virüsü, kronik hepatit B virüs infeksiyonu, HBV DNA, ALT, HAI, HBeAg, anti-HBe

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major causes of chronic liver diseases in the world and also in Turkey. HBV causes a broad spectrum of liver diseases ranging from acute self-limited hepatitis to fulminant hepatitis, chronic hepatitis, and asymptomatic infection. It is also one of the main causes of liver cirrhosis and hepatocellular carcinoma (HCC) (1-4). Serum HBV DNA is a specific and active marker for HBV replication (5-8) in the management of these different clinical entities, and hepatitis B e antigen (HBeAg) and antibody to e antigen (anti-HBe) are well known chronological markers for the duration of infection. It is well established that persistence of HBV repli-

Address for correspondence: Dr. Kendal YALÇIN
Dicle Üniversitesi Tıp Fakültesi, İç Hastalıkları Anabilim Dalı, Hepatoloji Birimi, 21280, Diyarbakır, Turkey
Phone: +90 412 248 85 20
E-mail: kendaly@dicle.edu.tr

Manuscript received: 25.2.2003 **Accepted:** 11.6.2003

cation is the key to progression of the disease. But the prognostic value of persistence, and relative presence of replication markers, in the sera of hepatitis B surface antigen (HBsAg) positive carrier individuals are still not completely defined.

In this cross-sectional study, we aimed to investigate the relationships between serum HBV-DNA levels and age, sex, alanine aminotransferase (ALT) activity, histological necroinflammatory activity, and HBeAg status in patients from the southeastern part of Turkey with chronic HBV infection, including inactive carriers, asymptomatic-replicative carriers (immune tolerant patients), and in chronic hepatitis B (CHB) and in liver cirrhosis patients.

MATERIALS AND METHODS

The subjects of this prospective study were 354 consecutive chronic HBsAg carrier individuals (232 males, 122 females), seen in the Hepatology Division of Dicle University Hospital between 1997-2001. The correlation and relative predictive value of hepatitis markers (ALT, histology, serology and HBV DNA) were investigated in a cross-sectional study. Chronic HBsAg carriers were defined as those with steady positivity for HBsAg in their serum for more than six months. Study groups consisted of 126 inactive carriers (Group A), 50 asymptomatic replicative HBsAg carriers (Group B), 90 patients with HCB (Group C) and 88 with liver cirrhosis (Group D). In all patients HBV DNA was measured before the classification.

Chronic asymptomatic HBsAg carriers were classified into the inactive and asymptomatic replicative carrier groups according to the presence of HBeAg, ALT activity, serum HBV DNA levels, and histological activity in the screening period of six months before the study entry. Inactive HBsAg carrier state was defined as persistent HBV infection of the liver without significant, ongoing necroinflammatory disease. Patients who had high levels of HBV replication with presence of HBeAg in serum, but no evidence of active liver disease, characterized by normal ALT levels and minimal or absent histological activity, were considered to be in the immune tolerant phase of infection. CHB was diagnosed by HBsAg positivity and persistent increased levels of ALT for longer than six months, and the presence of histological activity in liver biopsy (9). The diagnosis of liver cirrhosis was done presumably by clinical (presence of splenomegaly, ascites and other peripheral signs of liver failure), radiological (small and atrophic liver, enlarged portal and splenic vein) and endoscopic (presence of esophageal varices) examinations. In any suspicion of diagnosis of patients with liver cirrhosis, liver biopsy was performed, whereas it was not routinely performed due to the risks of the procedure in patients with advanced liver disease.

Patients were excluded at screening if they had been treated previously with interferon or had received antiviral medications; were co-infected with hepatitis C, hepatitis D (HDV) or human immunodeficiency virus (HIV); had consumption of alcohol more than 40 mg/day; and had evidence of liver disease of other etiology (autoimmune, metabolic, and toxic).

Serological markers (HBeAg, anti-HBe, HBsAg and antibodies to hepatitis B surface antigen (anti-HBs) were assessed by qualitative micro-particle enzyme immunoassay (Organon Teknika BV, Boxtel, The Netherlands). Serum HBV DNA levels were detected by liquid hybridization (Digene Hybride Capture System, Beltsville, USA), with a lower limit of approximately 1 pg/ml, and by polymerase chain reaction (PCR) lowest detectability level of 103-104 copies/ml (Techne Cambridge, Duxford, UK). Biochemical and hematological evaluations were done in Central Hospital Laboratory using Abbott Aeroset Auto-analyzer. Liver biopsies were performed in 10 cases of inactive carriers, 20 cases of asymptomatic replicative carriers, all 90 chronic hepatitis B patients and in 10 patients with liver cirrhosis. The biopsy specimens were scored according to the original criteria of Knodell histological activity index (10).

Statistics

The results obtained were analyzed by the Student's t-test. Comparison of two means for independent samples and differences between two percentages were evaluated by Student's t-test. Two-sample Kolmogorov-Smirnov test was used for categorical variables in analyzing the values of necroinflammation and fibrosis. A p value of <0.05 was accepted as significant (11).

RESULTS

The baseline characteristics of the patients are shown in (Table 1). Patients who had serological markers for hepatitis C virus (HCV) and HDV infection were excluded from the study. No patient was found to be seropositive for HIV infection. In 126 cases of Group A, mean age was 30.03+8.95

Table 1. Baseline characteristics of 354 chronic HBsAg carriers

Groups	Age Mean±SD	Sex %, male	ALT (IU/L) Median (range)	NIA Median(range)	HBV DNA (pg/ml) Median (range)
Group A (n=126)	30.03±8.95	57% 72/54	28 (10-89)	1 (0-2)	-
Group B (n=50)	25.58±9.25	60% 30/20	33 (14-50)	1.5 (0-4)	4069 (7-9281)
Group C (n=90)	27.98±9.98	77% 69/21	139 (29-395)	9 (4-15)	2300 (5-6674)
Group D (n=88)	44.28±14.17	70% 62/26	55 (7-354)	-	129 (7-716)

Group A: Inactive HBsAg carriers, Group B: Asymptomatic-replicative carriers, Group C: Chronic hepatitis B, Group D: Liver cirrhosis, NIA: Necroinflammatory activity score, ALT: Alanine aminotransferase

Compared-groups	Age	Sex	ALT	NIA	HBV DNA
A-B	***	NS	*	***	NS
A-C	NS	**	***	***	NS
A-D	***	*	**	NS	NS
B-C	NS	*	**	***	**
B-D	***	NS	**	NS	***
C-D	***	NS	**	NS	***

*: p<0.05, **: p<0.01, ***: p<0.001

years, median ALT level was 28 IU/L (10-89) and median necroinflammatory activity score 1.0 (0-2). In Group A, only four patients had detectable level of serum HBV DNA by PCR (3.17%). In Group B, mean age was 25.58±9.25 years, median ALT level was 33 IU/L (14-50), median necroinflammatory activity score was 1.5 (0-4), and median HBV DNA level 4069 pg/ml (7-9281). In 90 chronic hepatitis B patients (Group C) mean age was found to be 27.98±9.98 years, median ALT level 139 IU/L (29-395), median necroinflammatory activity score 9.0 (4-15), and median HBV DNA level 2300 pg/ml (5-6674). In Group D, mean age was 44.28±14.17 years, median ALT level was 55 IU/L (7-354) and median HBV DNA level was 129 pg/ml (7-716).

Table 2. The rates of serum HBV DNA positivity according to HBeAg status in 354 chronic HBsAg carriers

Groups	No.	HBeAg+ No (%)	Anti HBe + No (%)
Group A	126	0	126 (100%)
HBV DNA positivity	0	4 (3.17%)	
Group B	50	50 (100%)	0
HBV DNA positivity	50 (100%)	0	
Group C	90	59 (66%)	31 (34%)
HBV DNA positivity	59 (100%)	31 (100%)	
Group D	88	9 (10%)	79 (90%)
HBV DNA positivity	9 (100%)	4 (5%)	
Total	354	118 (31%)	236 (69%)

Group A: Inactive HBsAg carriers, Group B: Asymptomatic-replicative carriers, Group C: Chronic hepatitis B, Group D: Liver cirrhosis

Of 354 patients, 118 (33%) were HBeAg-positive and 236 (67%) HBeAg-negative. Of HBeAg-negative patients, 126 (53%) had normal ALT, 31 (13%) had elevated ALT and 79 (33%) had evidence of cirrhosis; corresponding figures in the HBeAg-positive patients were 50 (42%), 59 (50%) and 9 (8%). HBV DNA was positive in 39 of 236 (17%) HBeAg-negative patients tested. Of these 39 patients, 4 (10%) had normal ALT, 31 (80%) had elevated ALT and 4 (10%) had cirrhosis. Thus, 58% of HBeAg-positive and 47% of HBeAg-negative patients had elevated ALT and/or cirrhosis. Among the latter group, 90% of HBV DNA-positive patients had elevated ALT and/or cirrhosis. Overall, 11% of HBsAg-positive patients had HBeAg-negative, HBV DNA-positive liver disease.

All inactive HBsAg carrier patients were HBeAg-negative, while all patients of Group B were HBeAg positive. Serum HBV DNA levels and HBeAg status of all groups are shown in Table 2. The rate of HBeAg-positive patients in Groups A and D were significantly lower than in Groups B and C. In the same way, the majority of patients in Groups A and D were anti-HBe-positive, and the rate of HBV DNA positivity in these patients was found significantly lower than in Group C. The great majority of the patients in Groups B and C were HBeAg-positive.

None of the patients in Group A had a level of HBV DNA above 1 pg/ml by liquid hybridization. The rate of HBV DNA positivity in anti-HBe-posi-

tive patients was found to be significantly lower in Group A compared to Groups C and D. There were significant differences between Groups B, C, and D regarding HBV DNA levels. Patients in Group B and C had significantly higher levels of HBV DNA than the other two groups. According to these results, the highest levels of HBV DNA were detected in replicative carriers and the second highest in CHB patients. HBV DNA levels decreased once liver cirrhosis developed.

The mean age of the patients in Group D was significantly higher than in the other three groups ($p<0.05$ in all comparisons). There was also a significant difference between the mean ages of Groups A and B ($p<0.05$). The proportion of females to males was 1:2 in asymptomatic carriers and 1: 3 in patients with CHB and liver cirrhosis. Statistically significant differences were found between the patients in Group A and Groups C and D according to the sex distribution, and also between Groups B and C.

Significant differences were observed in ALT levels between Group C and the other three groups ($p<0.05$ in all comparisons), whereas there was no significant difference between Groups A and B. The patients in Groups C and D had significantly higher ALT levels than these two groups. There was no significant difference in ALT levels between HBeAg-positive and negative patients in Groups C and D, but a statistical difference was found in histological activity and serum HBV DNA levels. In 109 HBeAg-positive patients, no correlation was seen between HBV DNA levels and liver damage. All patients in Groups A and B with ALT < 1.0 IU/L had necroinflammatory activity<3.

There was a significant difference between HBeAg-positive and negative chronic hepatitis B patients in histological inflammation scores ($p=0.048$), but no difference in fibrosis scores ($p=0.151$) (Table 3). Differences in histological activity were only found to be significant between the patients in Group C and Groups A and B ($p<0.05$ in all comparisons). Fibrosis and complete nodule formation was seen in specimens of cirrhotic patients in a limited number of patients without necroinflammatory activity. The majority of patients with liver cirrhosis had decompensated liver disease. Median necroinflammatory scores were 1.0 and 1.5 in the limited number of patients in Group A and B, respectively, so it was decided not to perform routine liver biopsies in these patients.

DISCUSSION

Hepatitis B virus infection is an important cause of acute and chronic liver disease in our country. In Turkey, epidemiological features of the HBV infection are similar to Mediterranean and Middle East countries. The most common transmission route of infection in our region is the horizontal transmission seen during the early childhood period and adolescence. The prevalence of HbsAg has been observed to be increased during these periods (12). Within the adult population, depending on regions, HBsAg carrier rate differs from 5-8%, seropositivity of anti-HBs 30-50%, and past infection 40-56% in our country (13,14). Early acquisition of

Table 3. Comparisons of HBeAg- and anti-HBe-positive patients

Groups	No	ALT (IU/L) Median (range)	NIA Median (range)	HBV DNA (pg/ml) Median (range)
Group A	126			
HBeAg+	0	-	-	-
Anti-HBe+	126	28 (10-89)	1.0 (0-2)	*
Group B	50			
HBeAg+	50	33 (14-50)	1.5 (0-4)	4069 (7-9281)
Anti-HBe+	0	-	-	-
Group C	90			
HBeAg+	59	140 (30-356)	7 (3-15)	2862.50 (22-6674)
Anti-HBe+	31	136 (29-395)	10 (5-15)	417 (5-4157)
p value		p>0.05	p<0.05	p<0.001
Group D	88			
HBeAg+	9	77 (36-134)	*	192 (7-716)
Anti-HBe+	79	51.5 (7-354)	*	59(47-102)
p value		p>0.05		p<0.05

Group A: Inactive HBsAg carriers, Group B: Asymptomatic replicative carriers, Group C: Chronic hepatitis B, Group D: Liver cirrhosis, *: Not available in numerical scores, ALT: Alanine aminotransferase, NIA: Necroinflammatory activity score.

infection results in wide spectrum of disease ranging from an asymptomatic carrier state to chronic liver disease. Thus, substantial proportions of patients with asymptomatic carrier status, chronic hepatitis B, and liver cirrhosis have been encountered in our population at young ages. The present study aimed to clarify how viremia levels reflect the clinical stages of chronic HBV infection and, in particular, whether 'healthy carriers' can be identified by analyzing HBV DNA levels.

The mean ages of asymptomatic carriers and CHB patients were similar and significantly higher for the patients with liver cirrhosis compared to the other three groups. As the risk of acquisition of HBV is the same range of age for each group in our patient population, similar mean age of asymptomatic carriers and the chronic hepatitis B patients shows that the risk of chronic hepatitis B state in asymptomatic HBsAg carriers has been low and that the asymptomatic carriers do not pose a particular risk, exhibiting a more benign course of liver disease (15, 16). The mean age observed in patients with liver cirrhosis was as expected, and liver cirrhosis most likely developed in chronic hepatitis B patients, in the normal course of disease over time.

In anti-HBe-positive asymptomatic carriers, the ALT level remains within normal range for a long time (15, 16), except in episodes of acute severe exacerbations in rare cases (17). Detectable level of virus in anti-HBe-positive asymptomatic carriers is of clinical value due to risk of acute exacerbations. We found a very low rate of virus replication (3%) in these patients compared to Ljunggren (30) (30%) and Fujiwara (16) (26%) studies. But, patients who were HBeAg-negative/anti-HBe-positive require further study to determine if they were carrying 'precore' or 'e-minus' mutants. Such mutants are known to prevail in HBV carriers from the Mediterranean. It will be difficult to make precise decisions to correlate HBV viral load with e antigen status only, without further stratification according to whether or not they carry precore mutants. But briefly, it can be said that in HBeAg-negative carriers with HBV DNA$<10^5$ copies/ml or ALT<1.0, mild inflammation is indicated, while $>2.8x10^5$ copies/ml of serum HBV DNA may justify further investigations.

Patients in Group B possibly have an early immune-tolerant phase characterized by minimal liver damage despite a high level of hepatitis B virus replication. These patients are asymptomatic, have normal levels of serum ALT and are positive for HbeAg, with a high level of hepatitis B DNA in serum, and are seen in other parts of the world, including the Eastern Mediterranean region and the Middle East, except Asia. This increase may be due to the method of transmission of the virus. These non-Asian patients have the same disease specifics and the same treatment difficulties as the Asian patients. Highly active replication in asymptomatic replicative carriers with low necroinflammatory scores points to ALT determination as a more reliable marker of disease activity.

Despite the low level of HBV DNA, a significantly higher inflammation score was found in anti-HBe-positive CHB patients compared to HBeAg-positive ones. These findings seem to confirm that immune tolerant and CHB patients have different biologic processes; viral replication is always concordant in the former, but is sometimes discordant in the latter with HBeAg/anti-HBe status (18, 19). Detectable HBV DNA is associated with more severe liver damage in HBeAg-negative patients (20, 21), and increased HBV DNA level was associated with significantly higher inflammation scores and higher ALT levels in these patients. Moreover, necroinflammatory activity in the liver did not always parallel viral replication in CHB.

It was demonstrated that the levels of HBV DNA in the HBeAg positive phase were generally higher than those in the anti-HBe positive phase (16-24). The present study reconfirmed that the level of HBV viremia in anti HBe positive asymptomatic carriers was significantly lower than that in HBeAg-positive asymptomatic carriers. In our study 66% of CHB patients had detectable HBeAg in their serum. Thus, in our region, HbeAg-positive patients were seen more frequently than negative patients in CHB. High incidence of HbeAg-positive patients found in our study contrasts results observed in the western part of our country, probably due to the early acquisition of infection. Cirrhosis was more frequent in the anti-HBe-positive group, but the higher occurrence of cirrhosis in this group may be related to a longer duration of the disease (22), because these patients were the oldest ones in the study.

Severe inflammation and necrosis of hepatic tissue are observed in patients with anti-HBe-positive CHB. Although HBV DNA is positively correlated to HBeAg, HBV DNA can be detected in some anti-Hbe-positive patients. These findings indicate that in CHB the severity of liver disease is not

directly related to levels of virus replication, thus suggesting a predominant role of host immune mechanisms.

The positivity rate of HBV DNA was 100% in CHB patients with biopsy-proven liver disease, whereas no active liver disease was observed in asymptomatic carriers with or without HBeAg. Presence of serum HBV DNA with HBeAg positivity was observed in 33.3% of all cases (118 of 354 patients). However, the presence of HBV DNA in sera of anti-Hbe-positive individuals was very low in asymptomatic carriers with normal ALT levels (3.17%). Higher rates were found in anti HBe positive chronic HBsAg carriers (9.8%) with elevated levels of ALT (35 of 354 patients). These data would indicate that HBV DNA is a marker which is closely related to liver disease in anti-HBe-positive patients. Additionally, HBeAg-negative CHB is not an uncommon or benign entity, and chronic liver disease develops in a significant proportion of such patients (25).

In summary, our study showed that there is a significant correlation between ALT activity and inflammation scores, and that the viral load is not a major determinant of the severity of liver damage in patients with chronic HBV infection. Taken together, these results indicate that viral load does not correlate with the severity of liver damage assessed either by enzymatic or histological activity, or with HBeAg status. Thus, monitoring of ALT is of great value for the assessment of hepatocellular damage in patients with chronic HBV infection, and determination of serum ALT concentrations seemss to be a reliable marker of disease activity (26-31). But, there is much to be studied to understand the function of serum HBV DNA in the follow-up of HBsAg carriers, and liver biopsy should be used routinely in the follow-up of asymptomatic carriers when they have high transaminase values. These results are important in the follow-up, diagnosis and treatment of patients with chronic HBV infection.

REFERENCES

1. Lee WM. Hepatitis B virus infection. N Engl J Med 1997; 337: 1733-45.
2. Di Marco V, Lo lacono O, Gamma C, et al. The long-term course of chronic hepatitis B. Hepatology 1999; 30: 257-64.
3. Mc Mahon BJ, Hoick P, Bulkow L, Snowball MM. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. Ann Intern Med 2001; 135: 759-68.
4. Chu CM, Karayiannis P, Fowler MJ, et al. Natural history of chronic hepatitis B virus infection in Taiwan: studies of hepatitis B virus DNA in serum. Hepatology 1985; 5: 431-34.
5. Alberti A, Pontisso P, Fattovich G, et al. Changes in serum hepatitis B virus (HBV) DNA positivity in chronic HBV infection: results of a long-term follow-up study of 138 patients. J Infect Dis 1986; 154: 562-69.
6. Hadziyannis SJ, Lieberman HM, Karvountzis GG, Shafritz DA. Analysis of liver disease, nuclear HBeAg, viral replication, and hepatitis B virus DNA in liver and serum of HBeAg vs anti-HBe positive carriers of hepatitis B virus. Hepatology 1983; 3: 656-62.
7. Bonino F, Hoyer B, Nelson J, et al. Hepatitis B virus DNA in the sera of HBsAg carriers: a marker of active hepatitis B virus replication in the liver. Hepatology 1981; 1: 386-91.
8. Jardi R, Buti M, Rodriguez-Frias F, et al. The value of quantitative detection of HBV-DNA amplified by PCR in the study of hepatitis B infection. J Hepatol 1996; 24: 680-85.
9. Lok AS, Heathcote J, Hoofhagle J. Management of hepatitis B: 2000-summary of a workshop. Gastroenterology 2001; 120: 1828-53. 10. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; *I:* 431-35.
11. Armitage P, Berry G. Statistical methods in medical research. Oxford: Blackwell Scientific, 1994.
12. Değertekin H, Tuzcu A, Yalçın K. Horizontal transmission of HBV infection among students in Turkey. Public Health 2000; 114: 411-12.
13. Değertekin H, Kastellioğlu F. The prevalence of HBsAg in healthy people and several liver diseases in Turkey. Asian Med J 1986; 29: 125-27.
14. Yalçın K, Değertekin H, Alp MN, et al. Serum HBV DNA levels in chronic hepatitis B patients in south-eastern Turkey. Turk J Gastroenterol 2000; 1: S90 (Abstract).
15. Berris B, Sampliner RE, Sooknanan R, Feinman SV. Hepatitis B virus DNA in asymptomatic HBsAg carriers: comparison with HBeAg/anti-HBe status. J Med Virol 1987; 23: 233-39.
16. Fujiwara K, Yokosuka O, Ehata T, et al. The two different states of hepatitis B virus DNA in asymptomatic carriers: HB-e-antigen positive versus anti-HBe positive asymptomatic carriers. Dig Dis Sci 1998; 43: 368-76.
17. Davis GL, Hoofhagle J. Reactivation of chronic type B hepatitis presenting as acute viral hepatitis. Ann Intern Med 1985; 102: 762-65.
18. Burrell CJ, Gowans EJ, Rowland R, et al. Correlation between liver histology and markers of hepatitis B virus replication in infected patients: a study by in situ hybridization. Hepatology 1984; 4: 20-24.
19. Suzuki K, Uchida T, Shikata T. Histopathological analysis of chronic hepatitis B virus (HBV) infection in relation to HBV replication. Liver 1987; 7: 260-70.
20. Lindh M, Horal P, Dhillon AP, Norkrans G. Hepatitis B virus DNA levels, precore mutations, genotypes and histological activity in chronic hepatitis B. J Viral Hepat 2000; 7: 258-67.

21. Chan HL, Tsang SW, Liew CT, et al. Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HBeAg-negative chronic hepatitis B virus infection. Am J Gastroenterol 2002; 97: 406-12.
22. Zarski JP, Marcellin P, Cohard M, et al. Comparison of anti-HBe-positive and HBe-antigen-positive chronic hepatitis B in France. French Multicentre Group. J Hepatol 1994; 20: 636-40.
23. Lieberman HM, LaBrecque DR, Kew MC, et al. Detection of hepatitis B virus DNA directly in human serum by a simplified molecular hybridisation test: comparison to HBeAg/anti-HBe status in HBsAg carriers. Hepatology 1983; 3: 285-91.
24. Stroffolini T, Sagnelli E, Rapicetta M, et al. Hepatitis B virus DNA in chronic HBsAg carriers: correlation with HBeAg/anti-HBe status, anti-HD and liver histology. Hepatogastroenterology 1992; 39: 62-65.
25. Amarapurkar DN, Baijal R, Kulshrestha PP, et al. Profile of hepatitis B e antigen-negative chronic hepatitis B. Indian J Gastroenterol 2002; 21: 99-101.
26. Rapicetta M, di Nardo V, Rozera C, et al. HBV-DNA, HBeAg/anti-HBe serological status in hepatitis B chronic individuals from central Italy. Epidemiol Infect 1990; 104: 511-17.
27. Sakugawa H, Nakasone H, Nakayoshi T, et al. Correlation between serum transaminase activity and virus load among patients with chronic liver disease type B. Hepatol Res 2001; 21: 159-68.
28. Zavaglia C, Mondazzi L, Maggi G, et al. Are alanine aminotransferase, hepatitis B virus DNA or IgM antibody to hepatitis B. core antigen serum levels predictors of histological grading in chronic hepatitis B Liver 1997; 17: 83-87.
29. Borg F, ten Kate FJW, Cuypers HTM, et al. Relation between laboratory test results and histological hepatitis activity in individuals positive for hepatitis B surface antigen and antibodies to hepatitis B e antigen. Lancet 1998; 351; 1914-18.
30. Ljunggren KK, Nordenfelt E, Kidd A. Correlation of HBeAg/Anti-HBe, ALT levels, and HBV DNA PCR result in HBsAg-positive patients. J Med Virol 1993; 39: 297-302.
31. Chun YK, Kim JY, Woo HJ, et al. No significant correlation exists between core promoter mutations, viral replication, and liver damage in chronic hepatitis B infection. Hepatology 2000; 32: 1154-62.