

Determination of serum hepatitis B virus DNA in HBV endemic region: Clinical significance and correlation with serological markers, ALT and AST

HBV endemik bölgede serum HBV DNA düzeylerinin klinik önemi ve serolojik işaretler, ALT, AST ile ilişkisi

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ABSTRACT

Objective: The aim of this study is to investigate, demographics and the relationship between serologic markers, liver enzymes and HBV DNA levels from the patients whose samples were sent to microbiology laboratory for HBV DNA polymerase chain reaction (PCR) test, in one of the greatest cities of Southeast Anatolia, where HBV infection is extremely common.

Method: Between October 2009 and October 2010, blood samples of 1662 patients were sent to our laboratory for HBV DNA PCR testing as part of a routine examination for viral hepatitis in Şanlıurfa Training and Research Hospital in Turkey. Hepatitis markers (HBsAg, HBeAg, anti-HBs, anti-HBc IgM) and liver function enzymes (ALT, AST) are tested simultaneously in these samples. HBsAg, HBeAg, anti-HBs and anti-HBc IgM levels were assessed with chemiluminescent microparticle immunoassay method (Architect-i2000 SR, Abbott, Ireland). ALT and AST levels were measured with commercial kits in Roche Modular-P and Roche-Integra autoanalysator (Roche Diagnostics Mannheim, Germany). HBV DNA PCR: Isolation of HBV DNA from serum samples was done with M24sp automated extraction device (Abbott, USA). Purified DNA was amplified with Real-Time PCR method with the help of Abbott-m2000 sample preparation system (Abbott, USA).

ÖZET

Amaç: Bu çalışmanın amacı, HBV enfeksiyonunun son derece yaygın olduğu Güneydoğu bölgesindeki büyük şehirlerden birinde HBV DNA polimeraz zincir reaksiyonu (PCR) testi için mikrobiyoloji laboratuvarına gönderilen hasta örneklerinde, demografik özellikler ve serolojik belirteçler, karaciğer enzimleri ve HBV DNA seviyeleri arasındaki ilişkileri incelemektir.

Yöntem: Ekim 2009-Ekim 2010 tarihleri arasında Şanlıurfa Eğitim ve Araştırma Hastanesinde viral hepatitin rutin testlerinin bir parçası olan HBV DNA PCR testi için 1662 hastanın kan örneği laboratuvarımıza gönderildi. Bu örneklerde eş zamanlı olarak hepatit belirteçleri (HBsAg, HBeAg, anti-HBs ve anti-HBc IgM) ve karaciğer fonksiyon enzimleri (ALT, AST) çalışıldı. HBsAg, HBeAg, anti-HBs ve anti-HBc IgM seviyeleri kemiluminesan mikropartikül immunoassay yöntemi ile (Architect i2000 SR, Abbott, Ireland) çalışıldı. ALT ve AST seviyeleri Roche Modular P ve Roche Integra otoanalizörde (Roche Diagnostics Mannheim, Germany) ticari kitle ile ölçüldü. HBV DNA PCR: Serum örneklerinde HBV DNA'nın izolasyonu M24sp otomatize ekstraksiyon cihazı (Abbott, USA) ile yapıldı. Saflaştırılmış DNA Abbott m2000 sample preparation system (Abbott, USA) yardımı ile ve Real-Time PCR yöntemi ile DNA amplifikasyonu yapıldı.

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Results: The mean age was 33.7 (Min-Max: 0-89) years old and the majority of the patients were male (Male/Female: 1103/559) in the study. HBsAg was positive in 96.2% of the patients and the median ALT level of these patients were 94 u/l (Min-Max: 4-2390 u/l), while median AST level was 74 u/l (Min-Max: 7-2505 u/l). HBV DNA was positive in 94.6% of HBsAg positive patients and the median HBV DNA level was 3.9×10^4 copies/ml (Min-Max: $35-3.4 \times 10^9$ copies/ml). HBeAg was positive in 17.1% of HBsAg positive patients. Median levels of ALT and AST in HBsAg negative patients (3.8%) were 32 u/l (Min-Max: 7-497 u/l) and 33 u/l (Min-Max: 13-280 u/l) respectively. HBV DNA was positive in only 7.9% of the patients with median HBV DNA level of 155 copies/ml (Min-Max: 34-624 copies/ml). HBeAg was found negative in 71.4% of patients with negative HBsAg and positive HBV DNA. In 26 of 1662 patients (1.6%), both HBsAg and Anti-HBs were negative (N/N). Only two of the N/N patients (7.7%) were found anti-HBc positive. Mean ALT and AST levels of two anti-HBc positive patients were 29.5 u/l and 30.5 u/l, respectively. HBV DNA was negative in one of these patients, and the other was found positive with a level of 3×10^2 copies/ml.

Conclusion: To acquire more reliable results on the diagnosis and follow-up of the treatment of Hepatitis B, quantitative HBV DNA tests should be used in together with more frequently used serologic and biochemical methods.

Key Words: HBV DNA, HBsAg, HBeAg, Anti-HBs, Anti-HBc, ALT, AST

Bulgular: Çalışmamızda ortalama yaş 33,7 (Min-Maks: 0-89) ve örneklerin çoğunluğu erkek cinsiyettedir (Erkek/Kadın: 1103/559). Hastaların %96.2'sinin HBsAg'si pozitif ve bu hastaların ortalama ALT seviyesi 94 u/l (Min-Maks: 4-2390 u/l) iken, ortalama AST seviyesi 74 u/l (Min-Maks: 7-2505 u/l)'dir. HBsAg pozitif hastaların %94.6'sında HBV DNA pozitif ve ortalama HBV DNA düzeyi 3.9×10^4 kopya/ml (Min-Maks: $35-3.4 \times 10^9$ kopya/ml)'dir. HBsAg pozitif hastaların %17.1'inde HBeAg pozitif idi. HBsAg negatif hastaların (%3.8) ortalama ALT ve AST düzeyleri sırasıyla 32 u/l (Min-Maks: 7-497 u/l) ve 33 u/l (Min-Maks: 13-280 u/l)'dir. HBV DNA hastaların sadece %7.9'unda pozitif ve ortalama HBV DNA seviyesi 155 kopya/ml (Min-Maks: 34-624 kopya/ml) idi. HBsAg negatif ve HBV DNA pozitif hastaların %71.4'ünde HBeAg negatif bulundu. 1662 hastanın 26 (%1.6)'sında HBsAg ve Anti-HBs' nin her ikisi de negatif (N/N) idi. Bu N/N hastalarının sadece ikisi (%7.7) anti-HBc pozitif bulundu. İki anti-HBc pozitif hastanın ortalama ALT ve AST seviyeleri sırasıyla 29.5 u/l ve 30.5 u/l idi. Bu hastaların birinin HBV DNA'sı negatif idi ve diğeri 3×10^2 kopya/ml ile pozitif bulundu.

Sonuç: Hepatit B infeksiyonlarının tanısında ve tedavisinin takibinde daha güvenilir sonuçlar elde edebilmek için daha sık kullanılan serolojik ve biyokimyasal testler ile birlikte kantitatif HBV DNA testleri kullanılmalıdır.

Anahtar Kelimeler: HBV DNA, HBsAg, HBeAg, Anti-HBs, Anti-HBc, ALT, AST

INTRODUCTION

Hepatitis B virus (HBV) is a noncytopathic, hepatotropic virus of the Hepadnaviridae family that causes variable degrees of liver disease in humans. HBV carriers are at risk of developing life threatening cirrhosis (30%) and later on hepatic carcinoma (6-17.5%). Despite the availability of a prophylactic vaccine, HBV is estimated to infect around 400

million people worldwide and it is endemic in Asia and Africa (1). It will increase by nearly 50 million people a year with nearly two million deaths related to acute and chronic complications of HBV. World Health Organization reported that the virus causes hepatic carcinoma over 300.000 patients annually. HBV carriers are the most important factor in the

dissemination of HBV infection. Turkey is among the moderate endemic regions with a carrier rate of 3.9-12.5%. Various studies revealed even higher rates in Eastern and Southeastern regions of Turkey with remarkable rates of childhood transmission (2).

Viral antigens and antibodies that emerge against them, are commonly used in diagnosis and for determination of the prognosis of HBV infection (3). Serological tests can provide accurate information about acute, chronic or previous HBV infection. Routine serological tests that are used in the diagnosis of HBV are; Hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B core antibody (anti-HBc), hepatitis B envelope antigen (HBeAg) and HBe antibody (anti-HBe) (4).

HBsAg is the most important marker for the diagnosis of acute and chronic hepatitis B virus and indicates potential infectiousness. It is one of the first serum markers to appear during the course of HBV infection. It is also useful as a follow-up marker, since declining concentrations are observed in resolving hepatitis B. HBsAg usually becomes undetectable after 4-6 months. If HBsAg persists for more than 6 months, the infected individual is considered as a chronic HBV carrier. Anti-HBs antibodies become detectable late in convalescence. Hepatitis B e antigen (HBeAg) protein appears shortly after the appearance of HBsAg and disappears within several weeks as acute hepatitis resolves. Its presence in the serum correlates with presence of viral replication in the liver. Antibody to hepatitis B core antigen (Anti-HBc) IgM is detectable at the outset of clinical disease and as the infection evolves, it gradually decline and become undetectable within six months. Anti-HBc IgG predominates and remains for a long time at detectable levels (5).

Lately, detection of HBV DNA in the serum and viral quantitation have an increasingly important role in diagnosis of the disease, designing the treatment and determination of the efficacy of

antiviral therapy. (6). The correlation between the HBsAg and the number of HBV particles is a key point, since this marker is used with a wide range of HBV particles in blood, depending on the infections state (7). Clinical liver function biomarkers, used for the diagnosis of liver damage and/or evaluation of treatment includes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (8). Due to serious clinical problems that the infection may cause and the potential for obstinacy, accurate use and interpretation of laboratory tests have a crucial role in the diagnosis of the infection (4).

In this study, patient demographics and the relationship between serologic markers, liver enzymes and HBV DNA levels are investigated in patient samples that were sent to our microbiology laboratory for HBV DNA polymerase chain reaction (PCR) test, in one of the developed cities of Southeastern region of Turkey, where HBV infection is extremely common.

MATERIAL and METHOD

This study was carried out between 01 October 2009 and 31 October 2010. Blood samples of 1662 patients were sent to our laboratory for HBV DNA PCR testing as part of a routine examination for viral hepatitis in Şanlıurfa Training and Research Hospital, Turkey. Hepatitis markers (HBsAg, HBeAg, anti-HBs and anti-HBc IgM) and liver function enzymes (ALT, AST) are tested simultaneously in these samples.

HBsAg, HBeAg, anti-HBs and anti-HBc IgM levels were assessed with chemiluminescent microparticle immunoassay method with Architect i2000 SR (Abbott, Ireland) system.

ALT and AST levels were measured with commercial kits in Roche Modular P and Roche Integra autoanalyser (Roche Diagnostics Mannheim, Germany). The normal concentrations in the blood were between 5 to 41 U/L for AST and 5-38 u/l for ALT.

Isolation of HBV DNA from serum samples was done with M24sp automated extraction device (Abbott,

USA). Purified DNA was amplified with Real-Time PCR method using Abbott m2000 sample preparation system (Abbott, USA). The target sequence for the Abbott RealTime HBV assay is in the surface gene in the HBV genome. This region is specific for HBV and is highly conserved. The primers are designed to hybridize to this region with the fewest possible mismatches among HBV genotypes A through H. The lower limit of this commercial test was 34.1 copy/ml where upper limit was 3.41×10^9 copy/ml. Three controls (negative control, low positive control and high positive control) were included in to the study for determination of contamination and evaluation of the results.

RESULTS

One thousand one hundred and three of the subjects were male and 559 were female, with a mean age of 33.7 years (Min-Max: 0-89). Patient demographics and test results according to age groups are summarized in Table 1.

Median ALT level of HBsAg positive patients, which consisted 96.2% of all patients, was 94 u/l (Min-Max: 4-2390 u/l), while median AST level was 74 u/l (Min-Max: 7-2505 u/l). Of these HBsAg positive patients, HBV DNA was positive in 94.6% and median HBV DNA level was 3.9×10^4 copies/ml (Min-Max: $35-3.4 \times 10^9$ copies/ml). Median ALT/AST levels of patients with HBV DNA levels $\leq 10^3$ copies/ml, 10^3-10^5 copies/ml and $\geq 10^5$ copies/ml were 50 (Min-Max: 6-1158 u/l)/39 (Min-Max: 9-1636 u/l) u/l, 54 (Min-Max: 4-2053 U/L)/43 (Min-Max: 7-2505 U/L) U/L and 86 (Min-Max: 9-2390 U/L)/68 (Min-Max: 15-1967 U/L) U/L respectively. Median ALT/AST levels of patients with negative HBV DNA were 35 (Min-Max: 10-156 U/L)/32 (Min-Max: 13-95 U/L) U/L. HBeAg was positive in 17.1% of HBsAg positive patients. HBeAg positivity rates of patients with HBV DNA $\leq 10^3$ copies/ml, 10^3-10^5 copies/ml, $\geq 10^5$ copies/ml were 18.4%, 15.4% and 65.1%, respectively. HBeAg was positive only in 1.1% of HBsAg positive patients with negative HBV DNA. HBeAg, HBV DNA and ALT/AST levels of

Table 1. Demographic characteristics and serological test results of 1662 subjects, Şanlıurfa, October 2009-2010

	NO.	M/F	HBsAg		HBsAg/Anti-HBs			
			P	N	P/P	P/N	N/P	N/N
Sex								
Male	559 (33.6%)		538 (32.4%)	21 (1.3%)	89 (5.4%)	449 (27.0%)	10 (0.6%)	11 (0.7%)
Female	1103 (66.4%)		1061 (63.8%)	42 (2.5%)	158 (9.5%)	903 (54.3%)	27 (1.6%)	15 (0.9%)
Age (years)								
0-14	110 (6.6%)	63/47	108 (96.2%)	2 (0.1%)	23 (1.4%)	85 (5.1%)	2 (0.1%)	0
15-40	1046 (62.9%)	714/332	1015 (61.1%)	31 (1.9%)	126 (7.6%)	889 (53.5%)	15 (0.9%)	16 (1.0%)
41-60	444 (26.7%)	294/150	419 (25.2%)	25 (1.5%)	89 (5.4%)	329 (19.8%)	16 (1.0%)	9 (0.5%)
≥ 61	62 (3.7%)	32/30	57 (3.4%)	5 (0.3%)	9 (0.5%)	48 (2.9%)	4 (0.2%)	1 (0.1%)
Total	1662	1103/559	1599 (96.2%)	63 (3.8%)	247 (14.9%)	1352 (81.3%)	37 (2.2%)	26 (1.6%)

M: male, F: female, P: positive, N: negative

HBsAg positive patients are summarized in Table 2.

Median ALT level of HBsAg negative patients (3.8%) was 32 U/L (Min-Max: 7-497 U/L) and median AST level was 33 U/L (Min-Max: 13-280 U/L). HBV DNA was negative in 92.1% of these patients where it was positive in 7.9%, with a median HBV DNA level of 155 copies/ml (Min-Max: 34-624 copies/ml). HBeAg was negative in 71.4% of patients with negative HBsAg and positive HBV DNA. Anti-HBc and anti-HBs was positive in these patients (0.3% of total)

In 26 of 1662 patients (1.6%), both HBsAg and anti-HBs were negative (N,N). Among these N/N individuals, 24 (92.3%) were anti-HBc negative and 2 (7.7%) were anti-HBc positive. The incidence of being N/N was higher in women (2%) than in men (1.4 %). The prevalence of being N/N was highest in the group aged 41-60 years (2.0%), where it was 1.6% in the age group over 61 years, and lowest (1.4%) in the age group under 40 years (Table 3). The prevalence did not decrease according to age. Mean ALT level of the two anti-HBc positive patients was 29.5 U/L, and mean AST level was 30.5 U/L. HBV DNA was negative in one of these patients, and the other had a HBV DNA level of 3×10^2 copies/ml.

DISCUSSION

HBV viral load measuring is a very important tool for monitoring HBV infected patients. The most direct and reliable measurement of viral replication is HBV DNA quantification, which can replace other indirect methods to assess the efficacy of antiviral therapy used to treat HBV infected patients, such as serologic markers or measurement of liver enzyme functions. Monitoring the HBV viral load can predict the evolution to cirrhosis and hepatocellular carcinoma (9), as well as a rapid and sustained response to treatment as a predictive factor for a favorable treatment outcome. It can also provide an early detection of treatment failure that may be related to poor adherence to therapy or selection of a resistant virus. The likelihood of resistance to nucleotide analogues is very low when HBV DNA level is undetectable during therapy and increases proportionally to the HBV DNA level (10).

Turkey is an intermediate endemic area (2-8%) for HBV infection. However, the east and southeast region of Turkey is known as an endemic area for HBV infection in Turkey (11) and this study is performed in one of the largest cities of Southeastern Anatolia. In our study, mean age (33.7) of HBsAg positive patients

Table 2. The relationship between HBV DNA, HBeAg positivity and ALT/AST levels in HBsAg positive patients, Şanlıurfa, October 2009-2010

			ALT/AST				Total
			N/N	N/H	H/N	H/H	
HBsAg + (96.2%)	HBeAg + (17.1%)	HBV DNA +	69	34	45	122	270
		HBV DNA -	3	0	0	0	3
	HBeAg - (83.0%)	HBV DNA +	612	258	266	107	1243
		HBV DNA -	46	13	6	18	83
Total			730	305	317	247	1599

N: Normal level, H: High level

Table 1. Prevalence of isolated anti-HBc presence in patients who were N/N (Negative/Negative) for HBsAg/anti-HBs, Şanlıurfa, October 2009-2010

	N/N	N/N		Isolated anti-HBc (% of total)
		Anti-HBc -	Anti-HBc +	
Sex				
Male (N:1103)	15 (1.4%)		0	0
Female (N:559)	11 (2%)		2 (10.0%)	0.4%
Age (years)				
0-14 (N:110)	0	0	0	0
15-40 (N:1046)	16 (1.4%)	16 (100%)	0	0
41-60 (N:444)	9 (2.0%)	8 (88.9%)	1 (11.1%)	0.2%
≥61 (N:62)	1 (1.6%)	0	1 (100%)	1.6%
Total (N:1662)	26 (1.6%)	24 (92.3%)	2 (7.7%)	0.1%

M: male, F: female, P: positive, N: negative

was consistent with other studies around the world and Turkey (12-14). A multicentric study found that average age of patients infected with Hepatitis B is lower in Southeastern Anatolia region (26.2 ± 11.3) when compared to other regions (15). Toy et al investigated 339 studies about HBsAg prevalence in Turkey between the years 1999-2009, and found out that the prevalence in East and Southeast Anatolia is highest in the age group of 15-24 years (%12.51) (12). HBsAg positivity was highest among the ages 15-40 in our study. High prevalence in younger age groups may be associated with factors like lower living standards, low socioeconomic status, close contact and a high rate of vertical and horizontal transmission (11), which are also the causes of higher HBV infection rates.

Studies showed that, HBV DNA could be demonstrated either in circulation or in liver tissue in every patient that HBsAg is detected (16). We found a very high rate of HBV DNA positivity (94.6%)

in HBsAg positive patients' blood samples in our study.

We also found HBV DNA in 7.9% of HBsAg negative patients. Similarly, even higher rates in chronic hepatitis patients, were reported in literature (17). This HBV DNA positivity in seronegative patients could be associated with mutant strains (18). These results suggest that, determining HBV replication only by detection of HBV marker is not enough and real-time PCR should be used simultaneously for more reliable results (19).

HBeAg positivity in HBsAg positive patients has reported as 8-13% in Turkey. (15,20-22). In our study this rate was 17.1%, which also indicates high HBV prevalence in our region.

HBeAg is detected during active viral replication, usually in patients with positive serum HBV DNA (4). In our study, HBV DNA was positive in 98.9% of HBsAg and HBeAg positive patients. Presence of

HBV DNA in HBeAg positive patients was found to be 80.5% by Ozbilge et al, 81.6% by Pekbay et al and 61% by Heper et al. (18,23,24). As these results suggest HBV DNA can be found negative in some HBeAg positive patients, which may be related to regional variability of mutant strains. Furthermore, it is very important to know whether the patient has been treated or not when HBV DNA and HBeAg serological tests are interpreted. It should be kept in mind that in some situations, especially when HBeAg is positive, HBV DNA may be negative or low positive while the patient is receiving antiviral treatment. HBV DNA could not be isolated in three HBeAg positive patients in present study. This condition has been reported in similar studies. (23,25,26). Lack of the information about treatment states of these patients is a disadvantage of our study.

HBV DNA was positive in 98.9% of HBsAg and HBeAg positive patients and serum ALT levels were elevated in 61.9% of them while it was normal in 38.1%. These results suggest immune tolerant phase (presence of HBeAg, high positivity of HBV DNA and normal ALT levels) and immune clearance phases (presence of HBeAg, high positivity of HBV DNA and elevated ALT levels), respectively. To precisely determine the phase of chronic hepatitis in patients, these findings have to be evaluated together with liver biopsy results (27). To find out the phase of the illness is especially important in prediction of prognosis and management of disease (28).

The anti-core antibody can induce anti-HBc responses without T-cell activation. This antibody can be found in almost every patient with a previous contact with HBV, even in HBV carriers without other responses. This serological pattern is called 'anti-HBc alone', and might reflect an occult HBV infection (OBI). Anti-HBc is not an ideal marker, the Taormina group recommended its use as a surrogate marker whenever an HBV DNA test is not available to identify potential seropositive OBI individuals such as in cases of blood, tissue or organ donation, or

in cases of patients undergoing immunosuppressive therapy (7). Numerous studies have been performed to identify contamination from HBsAg negative persons, in other words HBV presence in HBsAg negative patients, and various results (between 1/46000 and 1/630000) have been found in different countries (29). Studies in Turkey revealed isolated anti-HBc positivity between 0.011% and 6.4% (29-31). The results of our study showed that 1.6% of individuals were N/N based on routine serological tests for HBV (HBsAg/anti-HBs) and 7.7% of them were isolated anti-HBc positive individuals (2 patients, 0.1% of total subjects) and one of these was low positive for HBV DNA while other was negative. However, real prevalence of isolated anti-HBc positivity is still not known due to different patient numbers of the studies and variations in the sensitivities of the tests that are used in these studies. Because of that, highly sensitive and specific PCR tests are very important for determination of real prevalence (32). Furthermore, it is known that, HBV infection rates in the study region are also an important factor in prevalence of isolated anti-HBc positivity. The geographical factors are found to be highly associated with isolated anti-HBc prevalence, there by endemic HBV infection (33). Our higher prevalence rates were probably due to lower number of HBsAg positive patients in our study group and high HBV prevalence of the region. We suggested that it was necessary, larger studies with accurate and highly sensitive techniques and histopathological examinations to determine the real prevalence of isolated anti-HBc positivity in patients. Generally, infection is considered to be ended when antigens disappear and anti-HBs and anti-HBc antibodies turn up in an individual. However, we found HBV DNA in 0.3% (5/1662) of patients even though antigens were negative and antibodies were positive. This situation supports the idea of positive anti-HBs do not necessarily mean recovery (34, 35). The cause of anti-HBs presence in HBV DNA positive patients has not clarified yet, but it may be attributed to

insufficient neutralization of HBV or deficiency of determining the precise state of HBV infection with routine serologic tests.

When body tissue or an organ such as liver or heart is diseased or damaged, additional AST and ALT releases into the bloodstream, causing elevated levels of the enzymes. Therefore, the amount of AST and ALT in the blood is directly related to the extent of the tissue damage (36). It was found that median ALT and AST levels of HBsAg positive patients were elevated. This elevation in ALT and AST levels may show liver damage due to HBV replication. ALT and AST levels are important markers in diagnosis and management of acute Hepatitis B infection and acute fulminant hepatitis, as well as in diagnosis and follow-ups of inactive HBsAg carriers, according to the latest guidelines (37). It must be kept in mind that liver enzymes are important parameters in management of the patients in addition to molecular

techniques that are frequently used lately.

Studies revealed that HBeAg positive patients have higher replication levels than HBeAg negative ones. Togo et al and Saglık et al showed higher ALT and HBV DNA levels in HBeAg positive patients similar to our study (3). ALT levels elevated in 61.2% of HBeAg positive patients while this rate was only 28.1% in HBeAg negative individuals in our study.

Serological markers and serum transaminase levels are not always adequate in evaluation of HBV infection. HBV DNA detection in serum sample is the best way in finding out the infectivity of the Hepatitis B infection, determining the necessity of antiviral treatment and efficiency of the treatment and evaluating the prognosis of the disease. As a result, quantitative HBV DNA tests should be used together with serological and biochemical test in the management of HBV infections.

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