Original Article

Seroepidemiology of hepatitis B virus (HBV) and relationship to serum transaminase levels in Indian population

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Abstract

Background: Hepatitis B virus (HBV) infection is a serious public health issue that must be addressed. **Aim:** The goal of this study was to investigate the correlation between serological status for hepatitis Be antigen (HBeAg)/anti-HBe, serum transaminase levels, and serum HBV-DNA in patients with chronic HBV infection. **Methods:** A retrospective observational study with 620 patients with persistent HBV infection (mean age, 36.35 years; 506 men) was conducted. All patients tested positive for hepatitis B surface antigen (HBsAg). Liver profile, HBeAg, and anti-HBe antibody tests were conducted for all patients. Additionally, serum HBV DNA was examined using a DNA assay in these individuals. **Results:** Of 620 patients, 114 (18.39%) were HBeAg-positive and 506 (81.61%) HBeAg-negative. A detectable level of HBV DNA was found in 89.79% of HBeAg-positive/anti-HBe negative patients compared to HBeAg-negative/anti-HBe positive carriers 33.69% (*P* value <0.0001). The median viral load was significantly higher in HBeAg-positive cases (4.72 log10 copies/mL) than in HBeAg-negative individuals (4.23 log10 copies/mL; *P* = 0.997). Additionally, a higher proportion of HBeAg-positive samples (*P* = 0.0001) had HBV-DNA levels above 10,000 copies/mL.

Keywords: Alanine aminotransferase (ALT) levels, Hepatitis B, Hepatitis B virus DNA, Hepatitis Be antigens, serologic tests

Introduction

Over a million people die each year from liver cirrhosis or hepatocellular carcinoma as a result of infection with the hepatitis B virus (HBV), which affects close to 350 million individuals worldwide.[1,2] Globally, almost 45% of the inhabitants reside in regions with high chronic HBV prevalence rates. Asia, the Pacific, and sub-Saharan Africa are among the regions that are affected, HBV infection is widespread, and a majority of people contract it during infancy. However, in Western nations, the illness is relatively uncommon and is mostly acquired during adulthood.[3] About 2%–5% of the general population in the Middle East and the Indian subcontinent is infected with chronic HBV.[4]

It is estimated that 7% to 30% of the 350 million HBV carriers have HBV variations that display little or no hepatitis Be (HBeAg) antigen.[5] Hepatitis B infection usually follows a pattern of progression marked by an initial HBeAg-positive phase, where HBV DNA levels in the blood are elevated. Subsequently, patients enter a seroconversion phase, during which HBeAg is cleared from the system, and antibodies against HBeAg (anti-HBe) begin to form. A restoration of aminotransferase to normal levels and a reduction in HBV

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DNA to undetectable levels by unamplified tests are often indicated by this. HBeAg has two clinical forms during the progression of HBV disease. There are two distinct conditions: One is referred to as "chronic inactive," characterized by low levels of persistent aminotransferase and HBV DNA (100,000 copies/ml). The other condition lacks HBeAg, exhibits high alanine aminotransferase (ALT) levels, and has HBV DNA present at the same level (100,000 copies/ml).[6,7] In regions with high endemicity, most individuals who test positive for HBsAg acquire HBV either at birth or throughout the first ten years of life, infection. However, in nations with HBV transmission mainly happens in adults due to low endemicity levels, unprotected sex, or sharing needles with HBsAg carriers.[8]

The presence of HBsAg in the blood is a characteristic of chronic hepatitis B (CHB) infection, persisting for a minimum of six months, whether accompanied by HBeAg or not. This extended duration of HBsAg presence serves as a definitive hallmark for diagnosing chronic hepatitis B. The main indicator of risk for chronic liver illness and liver cancer (hepatocellular carcinoma) later in life is the persistence of HBsAg. During the initial stages of hepatitis B viral infection, HBeAg can be detected either after the appearance of hepatitis B surface antigen (HBsAg) or regularly during or after a burst in viral replication. In patients undergoing antiviral treatment, the seroconversion from HBeAg to antibodies against HBeAg (anti-HBe) indicates a higher likelihood of long-term clearance of HBV and suggests reduced HBV levels, leading to decreased infectivity.[9] A negative HBeAg result might suggest an early acute infection, occurring either before the peak of viral replication or during the early recovery phase when HBeAg levels have decreased below detectable levels. The detection of anti-HBe assists in distinguishing between these two stages. In CHB patients, despite the absence of detectable HBeAg in their serum, the presence of anti-HBe indicates that hepatitis B viral DNA can still be detected in their serum.[7]

Based on recently issued guidelines, Antiviral therapy should be considered for individuals with HBV-DNA levels exceeding 10,000 copies per milliliter (>2000 IU/mL), ALT levels higher than twice the upper limit of normal (ULN), and significant liver fibrosis. Furthermore, when ALT and aspartate transaminase (AST) values increase, HBV-DNA testing should be conducted every 3 to 6 months.[10,11] HBV DNA in serum and HBeAg are significant HBV infection indicators that show active viral replication, increasing the likelihood of infection transmission. The beginning of the treatment method may encounter a problem in the developing world due to a lack of laboratory evaluation of viremia load or a failure to detect the existence of HBV DNA. Serum HBeAg and serum transaminases in HBsAg-positive individuals may be used in this situation as indicators of active viral replication, necessitating the immediate start of antiviral therapy. The recent study aimed to enhance our understanding of the condition by examining the biochemical, serological, and underlying risk factors in individuals who tested positive for HBV.

Materials and Methods

Patient selection

This study was an observational retrospective investigation conducted from January 2017 to December 2022. Participants were recruited after meeting specific inclusion criteria, which required a history of being positive for HBsAg for at least six months and being chronic hepatitis B patients receiving care in the outpatient department. Patients having HIV, autoimmune disorders, alcohol abuse, and chronic hepatitis C or all other immunodeficiency conditions were excluded. Therefore, 620 patients in total were recruited. Before enrolling patients in the study, they were informed and taken a signed consent form.

Serological markers

Freshly collected serum samples were used for HBsAg assay using third generation HBsAg kit (Med Source^{O3}, ELISA, reference no. HBSC050). Serum Anti-HBe and HBeAg levels were measured using ARCHITECT (Abbott, USA, reference no. 65801, 45280) as per the manufacturer's instructions.

Virological markers

Real-time polymerase chain reaction (PCR) was used to measure the amount of HBV DNA (HBV Real-^{TM} Artus kit, Qiagen Reference no. 4506265). The data on demographics, medical history, serum transaminases, and alkaline phosphatase were recorded. The normal ranges for the biochemical tests ALT and AST were 7 to 56 IU/L and 5 to 40 IU/L, respectively. High ALT was defined as a value equal to or greater than 2 times the upper limit of the normal range, which corresponds to 112 IU/L. All tests were conducted following institutional ethics and guidelines.

Statistical analysis

Statistical analyzes were conducted using the Statistical Program for Social Sciences (SPSS). The Chi-square test was employed to assess categorical data in the study. To compare parametric quantitative data, the student *t*-test was utilized, while nonparametric data were compared using the Wilcoxon rank-sum (Mann-Whitney) test. It was decided whether to analyze qualitative data using Fisher's exact or Pearson Chi-square tests. $P < 0.05$ was considered to be significant.

Results

A total of 506 males and 114 females were enrolled in the study, with a male-to-female ratio of (4.43:1). The participants' mean age was 36.35 ± 14.76 years, with an age range spanning from 2.5 to 82 years. The patients were categorized based on age and we found only 95 patients from the younger age group (<21 years), 34 (5.48%) patients from the older age group (>61 years) while the predominant population (303 out of 620) 48.87% came under the sexually active category (i.e., 21-40 years). [Table 1] A total of 114 (18.39%) HBeAg-positive individuals were detected, and the majority of them (44 cases) fell into the 21- to 40-year-old age range. Only five patients fell into the >61-year-old age group.

However, a total of 479 individuals (77.26%) were found to be anti-HBe positive, with the majority of these patients (255 cases) falling into the 21–40 year age range and the remaining 29 falling into the >61 year age range.

HBeAg positivity in the population of HBsAg-positive individuals accounted for 18.39% (95% CI: 15.41%–21.67%). HBeAg positivity among male subjects was 15.48% (95% CI: 12.73%–18.58%) and among female subjects was 2.90% (95% CI: 1.73%-4.55%), which was non-significant ($P = 0.504$). The presence of HBeAg positivity showed a significant association with sex, with 15.43% of males and 2.90% of females being HBeAg-positive ($P = 0.0001$). Among the 98 patients who were anti-HBe negative and HBeAg-positive, 88 patients (89.79%) had detectable levels of HBV DNA, while only ten patients (10.20%) had undetectable levels of HBV DNA. About 156 (33.69%) of the 463 individuals with anti-HBe and HBeAg positivity also had detectable HBV DNA levels and the remaining 307 (66.31%) patients lacked detectable HBV DNA levels. [Table 2]. One hundred and fifty six infections among 506 HBeAg-negative infections had HBV DNA (30.83%). Only 26 infections or 6.91% of the 376 HBV DNA-negative infections had HBeAg.

Out of the 620 samples subjected to HBV DNA load testing, 376 samples (60.64%) showed no detectable HBV DNA, while 244 samples (39.35%) exhibited varying degrees of detectable HBV DNA, with median HBV DNA levels of 4.43 log10 copies/mL (ranging from 2.67 to 8 log10 copies/ml). A statistically significant difference in HBV DNA positivity between these two groups was observed ($P = 0.0001$). The viral load ranged from zero (in patients with undetected HBV DNA) to one billion times 108. The frequency and proportions of various HBV DNA level ranges are shown. According to the study findings, HBeAg-positive cases had a mean viral load ranging from 7.50 to 7.63 log10 copies/mL, while HBeAg-negative individuals had a mean viral load ranging from 6.78 to 7.10 log10 copies/mL. However, there was no statistically significant difference in viral load between the two groups ($P = 0.335$).

Regarding age, the group with HBV DNA copy count greater than $10^{\wedge}7$ showed a substantially lower mean age compared to the other groups, and this difference was found to be statistically significant ($P < 0.001$). [Table 3] HBeAg-positive samples showed a higher prevalence of HBV-DNA >10000 copies/mL (49.12%) compared to HBeAg-negative patient sera (18.77%), and this difference was statistically significant ($P = 0.0001$). HBeAg-negative patients had lower levels of ALT and AST compared to HBeAg-positive patients ($P < 0.0001$). However, no significant differences were observed between the two groups concerning sex and age ratio and HBV DNA levels. Table 4 displays the laboratory data and clinical information of the patients. One hundred and thirty seven patients had normal ALT while 483 patients were found to have raised or high ALT while 316 patients had normal AST while 304 patients were found to have raised or high AST. Among the HBeAg-positive patients, 57 out of 114 (50%) had high levels of ALT. For HBeAg-negative patients, 200 out of 506 (39.53%) exhibited high ALT levels, while among the anti-HBe positive patients, 178 out of 479 (37.16%) showed high ALT levels.

The correlation between HBV DNA levels (<10000 copies/mL and >10000 copies/mL) and ALT levels existed regardless of whether ALT levels were normal or raised. Samples with HBV-DNA levels >10000 copies/mL were more likely to have raised or high ALT and AST levels (135 and 73, respectively) compared to samples with HBV-DNA levels <10000 copies/mL (80 and 71, respectively). This difference was statistically significant ($P = 0.029$). In the current investigation, 81.37% of HBeAg-positive infections with high ALT levels, and detectable circulating levels of HBV DNA were observed. In the comparison, HBV DNA was merely found in 41.66% of HBeAg-positive infections with normal ALT levels.

Table 1: Age distribution of study population with number of HBeAg, anti-HBe positive and HBV-DNA levels

Age group (years)	Total no.	HBeAg-positive	Anti-HBe positive	HBV DNA (copies/ml)		
				Negative	<10000	>10000
<21	95	24 (25.26)	53 (55.79)	39 (41.05)	19 (19.51)	29 (30.53)
21-40	303	44 (14.52)	255 (84.16)	191 (63.04)	45 (15.19)	75 (24.75)
41-60	188	41 (21.81)	142 (75.53)	124 (65.96)	2 (13.83)	38 (20.21)
>61	34	5 (14.71)	29 (85.29)	22 (64.71)	3 (8.82)	9 (26.47)
Total	620	114	479	376 (60.65)	93 (15.0)	151 (24.35)

Table 2:Detectable HBV DNA level in chronic HBV carriers

Patient	HBV DNA			Total
	Not detected	<10000 copies/mL	>10000 copies/mL	
HBeAg-positive/anti-HBe positive	16	…	…	16
HBeAg-positive/anti-HBe negative	10	32	56	98
HBeAg-negative/anti-HBe positive	307	61	95	463
HBeAg-negative/anti-HBe negative	43	…	…	43
Total	376	93	151	620

Discussion

In this study of chronic hepatitis B carriers, a significant association was found between circulating HBV-DNA levels and the presence of HBeAg/anti-HBe status. The patients with greater anti-HBe positivity rates (77.26%) were probably long-term carriers. Only 33.69% of 463 individuals who were anti-HBe positive but HBeAg-negative had detectable HBV DNA levels. The pre-core mutant virus was believed to be present in these cases.[12-14]

The HBV DNA burden in the current study was 244/620 (39.35%) cases, which was comparable to earlier studies where 35%–60% of HBV carriers tested positive for HBV DNA by Real-time PCR.[15,16]

The mean viral load in HBeAg-positive cases was 7.50 ± 7.63 $\log_{10}$ copies/mL, as compared to 6.78 ± 7.10 $\log_{10}$ copies/mL in HBeAg-negative individuals ($P = 0.335$). This is in contrast to some other studies where this comparison was found to be significant.[17,18]

The percentage of HBeAg positivity in this study was 114 (18.39%), which is comparable to other studies.[18,19] The presence of viral replication in the liver is correlated with the presence of HBeAg in serum. Few recent studies that have shown the high prevalence of HBeAg-negative CHB infections in the United States, Asia, Northern Europe, and the Mediterranean emphasized the rising prevalence of HBV infections.[20-22] Increased sensitivity of the testing method and changes in the pre-C region of the virus were observed in the study[9,22] and/or an effective National Expanded Immunization Program on Immunization in India are some potential causes of the rise in HBeAg-negative infections in the current study. Since the production of HBeAg in human and animal models is not necessary for viral replication, it is now believed that HBeAg functions as a tolerogen and modifies the host immune response. A chronic inactive carrier status with HBV DNA levels below 10,000 copies per milliliter and normal liver histopathology may also be present in people with HBV infection who do not have HBeAg. It is essential to differentiate the HBV mutant variety resulting from mutations in the pre-core or core promoter region of the viral genome, which leads to HBeAg-negative CHB, from the inactive carrier state and chronic hepatitis.[23]

In the most recent investigation, we discovered an unremarkable pattern in the positivity of HBeAg in groups of various ages ($P > 0.05$). However, this study demonstrated a significant relationship between HBeAg-positive and sex (males: 15.43% vs. females: 2.90%; $P = 0.0001$), which is consistent with other findings.[24] However, it may be related to different pathogenesis-related mechanisms involving the sex hormones, smoking, drinking, and sexual activity.

The current study proved that as HBV DNA levels grow, HBeAg positivity rises as well. HBeAg-positive samples showed significantly higher levels of HBV-DNA (>10000 copies/mL) observed in comparison to HBeAg-negative patient sera (49.12% vs. 18.77%; $P = 0.0001$). These findings suggest that both HBV DNA and HBeAg are accurate indicators for determining the levels of virus replication. One hundred and fifty six infections with HBV DNA positivity (30.83%) were among the 506 HBeAg-negative infections. However, only 26 infections (or 6.91%) out of 376 HBV DNA-negative infections had HBeAg. These findings suggest that HBV DNA testing may provide a more accurate diagnosis of HBV and a more accurate measure of viral DNA replication than serum HBeAg. The HBeAg-negative group showed persistently high HBV DNA levels, which could be attributed to two potential reasons. First, some individuals in this group may have undiagnosed hepatitis B infections, leading to sustained elevation of HBV DNA. Second, it is possible that HBeAg titers in these individuals were below the detection threshold, but they still had a functional impact on viral replication, resulting in elevated HBV DNA levels. Furthermore, studies have demonstrated the HBV pre-C gene mutation has been shown to inhibit HBeAg expression without affecting HBV DNA replication. This mutation accounts for the presence of high HBV DNA levels in certain individuals despite the absence of detectable HBeAg.[9] Thus, HBeAg levels along with HBV DNA should be used for assessing HBV replication and antiviral treatment.

Table 3: Correlation between HBeAg and HBV-DNA

HBV DNA copies/mL	Patients	HBeAg		
		Age Mean±SD	Positive, %	Negative, %
$<10^3$	20	36.2±10.42	5 (25)	15 (75)
10^3–10^5	147	34.24±14.34	44 (29.93)	103 (70.07)
10^5–10^7	36	37.22±14.32	10 (27.78)	26 (72.22)
$>10^7$	41	31.99±19.16	29 (70.73)	12 (29.27)

Table 4: Comparison of demographic, clinical, and virological data between HBeAg-positive and negative patients mean±SD

	HBeAg-positive patients	HBeAg-negative patients	*P*
Male:Female	96:18	410:96	0.504
Age	35.01±15.86	36.65±14.49	0.284
Serum ALT (IU/L)	158.75±139.34	112.24±95.01	0.0001
Serum AST (IU/L)	96.11±76.57	63.59±78.43	0.0001
No. of positive patients with >10000 copies/mL HBV-DNA level	56/114	95/506	0.0001
HBV-DNA levels, mean $\log_{10}$ copies/mL	7.50±7.63	6.78±7.10	0.335
HBV-DNA levels, mean >4$\log_{10}$ copies/mL (>10000 copies/mL)	7.69±7.7	6.99±7.42	0.366

Previously many studies have suggested that serum ALT levels should be assessed with HBV DNA viral load utilized to ascertain HBV status and prognosis.[10,25] In this study, we observed a correlation between circulating HBV DNA levels, ALT levels, and HBeAg status. Notably, measurable amounts of circulating HBV DNA were found in 81.37% of HBeAg-positive infections were found in individuals with elevated ALT levels. Additionally, HBV DNA was detected in 41.66% of HBeAg-positive infections with normal ALT levels. These findings indicate a potential relationship between HBV DNA levels, HBeAg status, and ALT levels in individuals with hepatitis B infection ALT levels, and the presence of HBeAg in individuals with hepatitis B infection.

Indeed, based on the findings, it can be inferred that serum ALT levels might serve as a useful marker in HBeAg-positive patients to assess HBV activity and infectiousness. Therefore, a combination of HBeAg, ALT, and HBV DNA measurements could provide valuable insights for the study aimed to comprehensively evaluate hepatitis B viral replication and the host's immune response. By analyzing these markers together, clinicians can gain a better understanding of the disease progression and tailor appropriate management strategies for patients with hepatitis B infection. While interpreting the results few limitations of the study have to be considered. First, the site of HBV genome mutations was not detected. The estimation of the fraction of HBV-infected people who have different mutations is not feasible due to the lack of reported HBeAg-negative patients who had a history of antiviral treatment. There is a complicated and multifaceted link between HBV and serum transaminase levels in the Indian population. When evaluating transaminase results in the context of HBV infection, healthcare providers should take into account the specific patient's clinical history, viral load, and other pertinent parameters. Care for those with HBV in the Indian community requires close observation and a holistic approach to liver health.

In many peripheral hospitals, HBV DNA detection is not commonly performed due to the involvement of expertise and higher costs, whereas HBeAg and ALT detection are relatively straightforward and inexpensive tests. Even though HBeAg serology may detect HBV replication, it might not have enough precision to measure HBV replication by itself. Hence, in situations where HBV DNA PCR is not feasible or accessible for quantitative detection of HBV DNA, combining knowledge of the it is possible to determine HBV activity and infectiousness using the HBeAg marker and serum ALT levels. Nevertheless, for a comprehensive diagnosis of the infection, it is essential to consider HBV serological testing, HBV DNA levels, and transaminase levels in conjunction with the patient's clinical condition.

Conclusion

In comparison, age, sex ratio, and HBV DNA levels do not significantly differ between individuals with HBeAg positivity and those who are HBeAg-negative. However, a notable difference is observed in the number of patients with >10000 copies/mL of HBV-DNA levels between the two groups. To accurately diagnose the infection, it is crucial to take into account HBV serological testing and HBV DNA levels, and transaminase levels, besides the patient's clinical condition. This is because HBeAg seroconversion by itself cannot be used to verify the virus's viability. A comprehensive evaluation of multiple factors is necessary for a thorough understanding of the patient's HBV infection status.

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Conflicts of interest

There are no conflicts of interest.

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