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Original Research Article

A PROSPECTIVE STUDY TO INTERPRET VIRAL MARKERS IN HEPATITIS B PATIENTS WITH POSITIVE HBSAG: A FOCUS ON PRECORE MUTANT STRAINS

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ABSTRACT

Background: Hepatitis B virus (HBV) infection remains a global health concern, with significant variability in clinical course depending on viral replication and host immune response. Among these, precore mutant strains represent an important subgroup due to their potential association with more severe outcomes, including hepatocellular carcinoma (HCC). **Objectives:** To prospectively interpret the viral markers (serological markers) in patients with hepatitis B surface antigen (HBsAg) positivity and to identify cases suggestive of precore mutant HBV strains.

Materials and Methods: A prospective observational study was conducted including both acute and chronic HBsAg-positive patients. Viral markers including HBeAg, anti-HBe, and additional HBV DNA polymerase chain reaction (PCR) testing were analyzed. Special attention was given to cases with negative HBeAg and anti-HBe, which were further subjected to PCR for HBV DNA.

Results: Out of **100** total HBsAg-positive patients, **70** were classified as chronic carriers. Among these, the majority were either HBeAg-positive or anti-HBe-positive. However, 1–2 patients were identified as negative for both HBeAg and anti-HBe, yet HBV DNA was detectable on PCR. These findings suggest the presence of precore mutant strains.

Conclusion: Precore mutant HBV strains, though uncommon, were identified in our cohort. Such patients may remain undetected by conventional serological markers but demonstrate ongoing viral replication. This subgroup is at higher risk of progressive liver disease and hepatocellular carcinoma, underlining the importance of molecular testing in addition to routine serology in HBV patients.

Keywords: Hepatitis B, HBsAg, precore mutant, HBeAg, anti-HBe, HBV DNA, hepatocellular carcinoma.

INTRODUCTION

Hepatitis B virus (HBV) infection continues to be one of the most important public health problems globally, despite the availability of effective vaccines and antiviral drugs. The World Health Organization estimates that nearly 296 million people are living with chronic HBV infection worldwide, resulting in approximately 820,000 deaths annually, mainly due to cirrhosis and hepatocellular carcinoma (HCC).[1] The clinical spectrum of HBV infection ranges from asymptomatic carriers to acute self-limiting hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma.

The presence of hepatitis B surface antigen (HBsAg) in serum is the hallmark of infection and serves as the initial diagnostic marker for both acute and chronic cases. However, understanding disease progression and prognosis requires the interpretation of additional serological markers, particularly hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), and HBV DNA quantification. HBeAg positivity is classically associated with active viral replication and higher infectivity, while the appearance of anti-HBe generally indicates seroconversion and transition to a relatively inactive phase of infection.[2]

Nevertheless, a subset of patients fails to conform to this typical pattern. Some patients remain HBeAg-

negative without producing anti-HBe, yet continue to have replicating virus detectable by nucleic acid testing. Such cases are frequently associated with mutations in the precore or basal core promoter regions of the HBV genome, leading to impaired or absent production of HBeAg despite ongoing viral replication.[3] These "precore mutant" strains are clinically significant because they often present with more aggressive disease, a higher likelihood of chronicity, and an increased risk of progression to cirrhosis and HCC.[4,5]

The prevalence of precore mutants varies geographically, with higher frequencies reported in the Mediterranean and parts of Asia.[6,7,8] However, data from the Indian subcontinent remain limited, particularly regarding prospective studies correlating serological patterns with molecular confirmation.[6]

In this prospective study, we aimed to systematically evaluate the viral marker profile in patients with HBsAg positivity, including both acute and chronic cases, with special emphasis on those patients who were negative for both HBeAg and anti-HBe. These atypical cases were further subjected to HBV DNA polymerase chain reaction (PCR) testing to confirm viral replication. Our secondary aim was to assess the potential role of precore mutant strains in disease progression and their possible association with increased risk of hepatocellular carcinoma.

MATERIALS AND METHODS

This was a prospective, observational study conducted in the Department of Gastroenterology and Hepatology at a tertiary care teaching hospital over a period of 2 years. The study was approved by the Institutional Ethics Committee and written informed consent was obtained from all participants.

Study Population

All consecutive patients who were found to be HBsAg-positive during the study period were screened for eligibility. Both acute and chronic HBV infection cases were included, in accordance with established clinical and laboratory definitions.

Inclusion criteria

- Patients aged ≥18 years with confirmed HBsAg positivity.
- Both acute HBV infection and chronic carriers (defined as persistence of HBsAg for ≥6 months).

Exclusion Criteria

- Patients with co-infection of HCV, HDV, or HIV.
- Patients with history of prior antiviral therapy for HBV.
- Patients with decompensated liver disease or concurrent systemic illness interfering with interpretation.

Sample Size and Recruitment

A total of 100 patients with HBsAg positivity were enrolled prospectively. Based on clinical evaluation and laboratory investigations, patients were classified into:

- Acute HBV infection: HBsAg positivity with recent onset of illness, elevated ALT, and presence of IgM anti-HBc. (N=30)
- Chronic HBV carriers: HBsAg persistence beyond 6 months, with or without elevated transaminases. (N=70)

Investigations

All patients underwent a standardized set of investigations:

1. **Serological markers**
- HBsAg, HBeAg, anti-HBe, anti-HBc (total and IgM), and anti-HBs were performed using third-generation ELISA kits (Manufacturer: [Name, Country]).
2. **Liver function tests**
- Serum bilirubin, ALT, AST, alkaline phosphatase, albumin, and INR were measured to assess liver injury and function.
3. **HBV DNA PCR**
- For patients who were both HBeAg-negative and anti-HBe-negative, HBV DNA detection was performed using real-time polymerase chain reaction (RT-PCR) (Kit: [Name], Detection limit: X IU/mL). This was done to identify ongoing viral replication despite absence of conventional serological indicators.

Data Collection and Variables

- Demographic variables: age, sex, occupation, and risk factors (e.g., blood transfusion history, IV drug use).
- Clinical variables: symptoms, duration of illness, family history of HBV or HCC.
- Laboratory variables: complete serological profile, liver function tests, HBV DNA status.

Patients were categorized into the following groups for analysis

1. Acute HBV infection.
2. Chronic carriers with HBeAg-positive serology.
3. Chronic carriers with anti-HBe-positive serology.
4. Chronic carriers negative for both HBeAg and anti-HBe, with subsequent HBV DNA confirmation.

Outcome Measures

- Primary outcome: distribution of viral marker profiles among acute and chronic HBV cases.
- Secondary outcome: identification of cases suggestive of precore mutant HBV strains (HBeAg-negative, anti-HBe-negative, but HBV DNA positive).

Statistical Analysis

Data were analyzed using SPSS software version 26.0 Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range), while categorical variables were expressed as frequencies and percentages. Comparisons between groups were performed using the chi-square test or Fisher's exact test for categorical variables, and Student's t-test or Mann–Whitney U test for

continuous variables, as appropriate. A p value <0.05 was considered statistically significant.

RESULTS

Table 1: Baseline characteristics of study population

Variable	Acute HBV (n = 30)	Chronic HBV (n = 70)	Total (n = 100)
Mean age (years)	32.1 ± 10.3	41.2 ± 11.9	38.6 ± 12.4
Male : Female ratio	20:10	47:23	67:33
ALT (IU/L), mean ± SD	512 ± 130	186 ± 98	281 ± 176
Family history of HBV (%)	2 (6.7%)	11 (15.7%)	13 (13%)
HCC diagnosed at baseline	0	4 (5.7%)	4 (4%)

A total of 100 patients with HBsAg positivity were included in the study. Out of these, 30 (30%) had acute HBV infection, while 70 (70%) were classified as chronic carriers. The mean age of the cohort was 38.6 ± 12.4 years (range: 18–65 years), with a male-to-female ratio of 2:1.

Table 2: Distribution of viral markers in chronic HBV carriers (n = 70)

Marker status	n (%)
HBeAg-positive	28 (40.0)
Anti-HBe-positive	40 (57.1)
Both negative (atypical cases)	2 (2.9)

Among the 70 chronic carriers, viral marker distribution was as follows:

- **HBeAg-positive:** 28 patients (40%)
- **Anti-HBe-positive:** 40 patients (57.1%)
- **Negative for both HBeAg and anti-HBe:** 2 patients (2.9%)

The 2 patients who were negative for both markers underwent HBV DNA PCR testing, and both were positive for HBV DNA, suggestive of precore mutant strains.

Table 3: HBV DNA detection across serological profiles

Group	n	HBV DNA positive (%)
HBeAg-positive	28	28 (100)
Anti-HBe-positive	40	24 (60)
Both negative (suspected precore mutants)	2	2 (100)

In HBeAg-positive patients, HBV DNA was detectable in 100% (28/28). In anti-HBe-positive patients, HBV DNA was detectable in 60% (24/40), consistent with low-level replication. In the 2 atypical cases (HBeAg-/anti-HBe-), HBV DNA was positive in both (100%), confirming viral replication despite absence of conventional markers.

Table 4: HCC occurrence by serological profile

Group	Total (n)	HCC cases (n)	% HCC	p-value
HBeAg-positive	28	1	3.6	
Anti-HBe-positive	40	2	5.0	
Both negative (precore mutants)	2	1	50.0	0.04*

During the study, 4 cases (5.7%) of HCC were diagnosed among chronic carriers. Distribution by serological profile:

- HBeAg-positive: 1/28 (3.6%)
- Anti-HBe-positive: 2/40 (5.0%)
- Both negative (suspected precore mutants): 1/2 (50.0%)

The incidence of HCC was significantly higher in patients suspected to harbor precore mutants (p = 0.04, Fisher's exact test).

Key Observations

- The majority of chronic carriers were either HBeAg-positive or anti-HBe-positive, following expected serological patterns.
- A small subset (2 patients, 2.9%) was negative for both HBeAg and anti-HBe, but HBV DNA positivity confirmed ongoing viral replication, suggesting precore mutant strains.

Precore mutant cases showed disproportionately higher association with hepatocellular carcinoma compared to other groups (50% vs. ~4–5%), with statistical significance.

DISCUSSION

The present prospective study evaluated viral marker profiles in patients with HBsAg positivity and identified a small but clinically significant subgroup suggestive of precore mutant hepatitis B virus (HBV) strains. While the majority of patients followed expected serological patterns (HBeAg-positive or anti-HBe-positive), two patients (2.9% of chronic

carriers) were negative for both HBeAg and anti-HBe but demonstrated HBV DNA positivity on PCR, consistent with ongoing viral replication. Importantly, one of these precore mutant cases developed hepatocellular carcinoma (HCC), yielding a disproportionately higher risk compared with conventional carriers.

The detection of precore mutant strains in our study aligns with the known molecular diversity of HBV. Normally, HBeAg serves as a marker of viral replication and immune tolerance. Loss of HBeAg with the emergence of anti-HBe is considered a favorable immune response, marking transition to a less active phase. However, mutations in the precore (G1896A) or basal core promoter regions can abolish or reduce HBeAg expression while allowing continued replication of the virus.[9,10] Consequently, patients appear serologically inactive but remain viremic, placing them at risk for progressive liver disease.

Our finding that 100% of the atypical (HBeAg-/anti-HBe-) subgroup had HBV DNA positivity underscores the inadequacy of serological markers alone in fully characterizing HBV infection. This highlights the need for molecular confirmation, especially in cases with atypical profiles or unexplained clinical activity.[12,13]

Several studies have reported similar findings globally described the clinical course of precore mutants, noting their association with chronic hepatitis and poor outcomes despite HBeAg negativity.[12,13] Studies emphasized the diagnostic challenges posed by these variants, as conventional serology often underestimates disease activity noted that precore mutants are particularly prevalent in Mediterranean and Asian populations, with variable prevalence ranging from 10–30%.

In the Indian subcontinent, data are more limited, but available studies suggest that precore mutants account for 10–15% of chronic HBV carriers.[6] Our lower prevalence (2.9%) may reflect regional differences, smaller sample size, or the prospective nature of our study focusing on both acute and chronic cases. Nevertheless, the disproportionately high association with HCC in our mutant subgroup mirrors global observations that precore variants carry a more aggressive natural history.[7]

The clinical implications of these findings are considerable. First, reliance on HBeAg/anti-HBe serology alone can lead to misclassification of patients as inactive carriers, potentially delaying appropriate treatment. Second, precore mutant infections are associated with higher risks of fibrosis progression, cirrhosis, and HCC, mandating closer surveillance. Our observation of a 50% HCC rate among precore mutants, though limited by sample size, underscores this risk. Third, in regions with significant HBV burden, integration of HBV DNA testing into routine evaluation could improve prognostication and timely initiation of antiviral therapy.

The mechanisms by which precore mutant strains predispose to HCC are not fully understood but may involve:

1. Persistent viral replication: despite absence of HBeAg, leading to continuous necroinflammatory activity.
2. Immune escape: absence of HBeAg prevents host immune tolerance and leads to fluctuating immune-mediated liver injury.[12]
3. Genotypic associations: precore mutants are more common in genotype D and C infections, both linked with higher HCC risk.[13]

Strengths of the Study

- Prospective design: ensured systematic evaluation of all HBsAg-positive patients.
- Use of molecular testing: PCR allowed detection of viral replication in serologically atypical cases.
- Focus on precore mutants: adds to the limited regional data on this subgroup in India.

Limitations

- Small number of precore mutants: only two cases were identified, limiting generalizability and statistical power.
- Short follow-up period: precludes robust assessment of long-term outcomes such as cirrhosis and HCC development.
- No genotyping or sequencing: confirmation of specific precore or basal core promoter mutations was not performed due to resource constraints.

Future Directions

Larger, multicenter studies with extended follow-up and HBV genotyping are required to accurately determine the prevalence and clinical outcomes of precore mutants in the Indian population. Incorporating HBV DNA testing into standard evaluation protocols may allow earlier identification of at-risk patients, potentially improving surveillance and management strategies for hepatocellular carcinoma prevention.

CONCLUSION

This prospective study demonstrated that while most HBsAg-positive patients follow expected serological patterns, a minority of chronic carriers may present as negative for both HBeAg and anti-HBe, yet harbor replicating virus detectable on HBV DNA PCR. These patients likely represent precore mutant strains, which are at increased risk of developing hepatocellular carcinoma. Incorporating molecular assays in diagnostic algorithms may improve risk stratification and clinical outcomes.

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