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ORIGINAL RESEARCH

Impact of PC/BCP Mutations on Seroconversion and Relapse in HBeAg-Positive Chronic Hepatitis B Patients Treated with Nucleoside Analogues

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Background: Precore/Basal core promotor (PC/BCP) mutations are critical mechanisms by which hepatitis B virus (HBV) evades host immunity and antiviral therapy. These mutations are prevalent in HBeAg-positive chronic hepatitis B (CHB) patients, potentially leading to suboptimal responses to nucleos(t)ide analogues (NAs) and high relapse risk after treatment discontinuation.

Objective: This study aimed to investigate the impact of PC/BCP mutations on seroconversion and relapse rates and analyze their association with drug resistance mechanisms. The study also evaluated the significance of mutation count (one, two, or three mutations) and specific types of mutations (A1762T, G1764A, G1896A) in relation to the seroconversion and relapse processes.

Methods: From 2016 to 2019, 48 HBeAg-positive CHB patients were collected and divided into mutation (n=37) and non-mutation (n=11) groups based on PC/BCP status. Seroconversion rates after 144 weeks of NA therapy and relapse rates after 48 weeks of treatment discontinuation were analyzed. Baseline viral load (HBV DNA), liver function (ALT), and Precore/ Basal Core Promoter (PC/BCP) mutation status were analyzed for their correlation with clinical outcomes.

Results: Among the 37 patients in the mutation group, 9 exhibited G1896A mutation, 15 exhibited A1762T/G1764A double mutations, 13 exhibited A1762T/G1764A/G1896A triple mutations. The mutation group showed significantly lower seroconversion rates than the non-mutation group (37.8% vs 81.8%, P=0.016). The seroconversion rate was inversely correlated with the number of mutations, with triple mutations (A1762T, G1764A, G1896A) associated with the lowest seroconversion rate. The mutation group exhibited a 100% relapse rate (14/14 cases) with HBeAg reactivation, while no relapses occurred in the non-mutation group (0/9 cases, P=0.0001). PC/BCP mutations (eg, A1762T/G1764A) likely reduce NA sensitivity by enhancing viral replication (upregulating pgRNA transcription) and immune evasion (HBeAg epitope variation), leading to delayed treatment response and viral rebound after discontinuation of therapy.

Conclusion: PC/BCP mutations are independent risk factors for poor NA response and high relapse rates in HBeAg-positive CHB patients. Patients with these mutations should be managed as "occult HBeAg-negative CHB" to avoid premature treatment discontinuation. Routine PC/BCP mutation testing is recommended to guide individualized treatment duration in HBeAg-positive CHB patients.

Keywords: Hepatitis B virus, PC/BCP mutations, nucleos(t)ide analogues, seroconversion, relapse

Introduction

Chronic hepatitis B virus (HBV) infection remains a major global health concern, with approximately 300 million people chronically infected worldwide and over 800,000 deaths annually attributed to HBV-related liver disease progression, including cirrhosis and hepatocellular carcinoma (HCC).[1,2] The burden is particularly high in Asia and sub-Saharan Africa, where perinatal and early childhood transmission predominate.[3] With the widespread clinical application of nucleos(t)ide analogues (NAs), such as entecavir and tenofovir, the risk of progression to end-stage liver disease has been significantly reduced.[4] A key therapeutic milestone in HBeAg-positive chronic hepatitis B (CHB) is the seroconversion

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from HBeAg to anti-HBe, which has long been recognized as a marker of durable immune control and favorable long-term outcomes.[5,6] Patients achieving HBeAg seroconversion with sustained low levels of HBV DNA typically exhibit improved liver histology and reduced risks of cirrhosis and HCC.[7] Accordingly, the American Association for the Study of Liver Diseases (AASLD) recommends that in non-cirrhotic, immune-active HBeAg-positive patients, NA therapy can be discontinued after a consolidation period of at least 12 months following confirmed HBeAg seroconversion.[8]

However, this conventional treatment endpoint is increasingly being challenged by emerging evidence regarding the role of HBV genomic mutations, particularly those occurring in the precore (PC) and basal core promoter (BCP) regions, in shaping both virological and serological responses.[9,10] Among the most well-characterized mutations are G1896A in the PC region, which introduces a premature stop codon that abolishes HBeAg expression, and the A1762T/G1764A double mutation in the BCP region, which suppresses HBeAg production while enhancing pregenomic RNA (pgRNA) transcription and viral replication[10,11.] These mutations disturb the typical correlation between HBeAg status and underlying disease activity, often resulting in discordant serological profiles, such as the presence of active viral replication despite a negative HBeAg status.

Clinically, patients harboring PC/BCP mutations are frequently misclassified as having achieved immune control based on serologic markers alone[12,13.] These patients often exhibit lower HBeAg titers, reduced rates of seroconversion, and markedly higher relapse rates following NA cessation, despite achieving virological suppression during therapy. In fact, studies have shown that the cumulative recurrence rate after NA withdrawal in patients with HBeAg seroconversion can reach up to 80%, rendering current withdrawal criteria unreliable for a substantial proportion of patients.[14] Moreover, for patients with HBeAg-negative CHB driven by PC/BCP mutations, no validated treatment endpoint for NA discontinuation currently exists, highlighting a critical gap in current clinical management.[15]

The clinical significance of PC/BCP mutations extends beyond serological ambiguity. These mutations alter HBV replication efficiency and modulate host immune responses, thereby influencing treatment efficacy and resistance patterns. Despite their known prevalence in both HBeAg-positive and -negative CHB patients, routine genotypic testing for these mutations is not yet standard practice in many regions, even though such testing could provide crucial prognostic information and guide individualized treatment duration, particularly in high-prevalence settings.[16]

One major challenge lies in the inadequate discrimination between wild-type and PC/BCP-mutant infections among HBeAg-positive patients prior to or during antiviral therapy. There are two common but clinically indistinguishable scenarios in current practice: (1) patients initially infected with wild-type HBV who undergo genuine HBeAg seroconversion during therapy, potentially achieving immune control; and (2) patients with either mixed infections or mutant-dominant strains, in whom HBeAg becomes negative not due to immune control, but due to mutation-induced suppression of HBeAg expression. In the latter scenario, although seroconversion is observed, the underlying infection resembles HBeAg-negative CHB, which lacks validated endpoints for NA cessation. These patients may be inadvertently considered eligible for treatment discontinuation, exposing them to high risks of relapse and liver disease progression.

To date, no large-scale studies have systematically explored the frequency, virological patterns, and clinical outcomes of such mixed or variant-dominant infections within the context of current NA therapy. This critical knowledge gap impairs our ability to refine antiviral treatment strategies and optimize discontinuation criteria. Thus, we propose a retrospective analysis of historical data to investigate the prevalence and clinical impact of PC/BCP mutations among HBeAg-positive patients undergoing NA therapy. This study aims to delineate serological and virological characteristics associated with mutant strains, clarify their implications for treatment endpoints, and ultimately guide the development of more precise, mutation-informed antiviral management strategies.

Methods

Study Design, Ethics, and Participants

This retrospective study was conducted at the People's Hospital of Taixing between July 2016 and July 2019. From July 2016 to July 2019, a total of 126 patients with confirmed CHB were screened from outpatient departments. The inclusion criteria were: (1) persistent positivity for HBsAg for more than six months, (2) HBeAg-positive status at baseline, and (3) no prior exposure to any NA therapy. Exclusion criteria included: (1) co-infection with hepatitis C virus

(HCV), hepatitis D virus (HDV), or human immunodeficiency virus (HIV); (2) autoimmune hepatitis, alcoholic liver disease, drug-induced liver injury, or fatty liver disease; (3) pregnancy or lactation; and (4) decompensated cirrhosis or hepatocellular carcinoma at baseline.[17]

After applying these criteria, 48 patients were selected. To assess the clinical relevance of HBV genomic variations, all participants underwent Sanger sequencing to detect mutations in the PC and BCP regions. Based on the presence or absence of these mutations, patients were stratified into two groups: a mutation group (n = 37), defined by one or more mutations in the PC/BCP regions, and a non-mutation group (n = 11), characterized by wild-type sequences at both loci.

The study protocol was reviewed and approved by the Ethics Committee of the People's Hospital of Taixing. All procedures involving the use of human participants' historical data were conducted in accordance with the institutional guidelines and the ethical principles of the Declaration of Helsinki. Given the retrospective nature of the study using de-identified archived data, the requirement for written informed consent was waived by the Ethics Committee.

Antiviral Treatment and Follow-Up Protocol

All patients received oral nucleos(t)ide analogue (NA) therapy according to the AASLD guidelines for the management of chronic hepatitis.[8] Two first-line antiviral agents (both are first-line NAs) were used in this study: entecavir at a dosage of 0.5 mg once daily or tenofovir disoproxil fumarate at a dosage of 300 mg once daily. The selection of the specific agent was based on clinical judgment and patient preference. All patients received monotherapy with a potent NA that has a high genetic barrier to resistance.

The total duration of treatment documented in medical records was 144 weeks and was divided into three distinct phases: baseline evaluation, active antiviral therapy from week 0 to week 144, and a post-treatment follow-up period from week 144 to week 192. Throughout the treatment phase, patients underwent comprehensive clinical and laboratory evaluations at 24-week intervals. These assessments included physical examinations, quantitative HBV DNA testing, serological markers (including HBeAg and anti-HBe), and liver function tests such as alanine aminotransferase (ALT).

Discontinuation of NA therapy was considered only for patients who met all of the following criteria: documented HBeAg seroconversion confirmed by two separate tests at least six months apart, persistent suppression of HBV DNA to levels below 10 IU/mL for a minimum of 12 months, and sustained normalization of ALT levels. Patients who fulfilled these criteria entered a 48-week off-treatment observational period, during which they were closely monitored for signs of virologic or serologic relapse.

Relapse was strictly defined as the reappearance of HBeAg in a patient who had previously achieved seroconversion, accompanied by a virological rebound with HBV DNA levels exceeding 2000 IU/mL. For patients who experienced relapse, antiviral therapy was promptly reinitiated in accordance with routine clinical management guidelines.

Laboratory Assessments

Serological markers of HBV, including HBsAg, HBeAg, and anti-HBe, were measured both qualitatively and quantitatively using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Kehua Bio-Engineering Co., Ltd., Shanghai, China). The assay was based on a double-antibody sandwich ELISA technique, performed in accordance with the manufacturer's instructions. In brief, 100 μL of serum sample was added to microplate wells pre-coated with monoclonal antibodies, followed by washing steps and incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies. The absorbance was read at 450 nm using an ELISA reader (Thermo Multiskan FC), and positivity was determined according to predefined cutoff values established by the manufacturer.

Liver function parameters, including ALT, AST, and serum albumin, were assessed using an automated clinical chemistry analyzer (Roche Cobas c501, Roche Diagnostics, Basel, Switzerland). ALT and AST levels were measured using a kinetic ultraviolet method at 37°C, while albumin concentrations were determined through a bromocresol green (BCG) dye-binding assay.

Quantification of serum HBV DNA was carried out using the COBAS AmpliPrep/COBAS TaqMan HBV Test, Version 2.0 (Roche Molecular Systems, Branchburg, NJ, USA). This real-time polymerase chain reaction (PCR) assay targets a conserved region of the HBV genome and offers a dynamic quantification range from 20 IU/mL to 1.7×10^8 IU/

mL, with a lower limit of detection of 10 IU/mL. Each sample was analyzed in duplicate to ensure analytical precision, and internal quality control procedures were performed as per the manufacturer's recommendations.

PC/BCP Mutation Detection and Sequencing

Serum HBV DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's standard protocol. The BCP and PC regions, spanning nucleotide positions 1601 to 2000, were amplified by nested PCR using primer sets.[18] The outer primers included a forward primer 5'-CTG CAA CTT CTC AGA CTC CCA-3' and a reverse primer 5'-GAG GCC TCC AAA GAA CTC CAC-3', while the inner primers consisted of a forward primer 5'-CAG GAT TCC AGA GTC TTT AAC-3' and a reverse primer 5'-GGA GGC GAG GAA GGA AGA GC-3'. PCR amplification was performed under the following conditions: initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds; followed by a final extension at 72°C for 10 minutes.

The purified PCR products were subjected to bidirectional Sanger sequencing using the ABI Prism 3730XL Genetic Analyzer (Applied Biosystems, USA). The Sanger sequencing method involves enzymatic incorporation of chain-terminating dideoxynucleotides labeled with fluorescent dyes during DNA synthesis, allowing the determination of the nucleotide sequence based on fragment size and fluorescence detection. The resulting sequences were aligned with the wild-type HBV genotype C reference sequence (GenBank accession number X02763) using BioEdit software version 7.2 to identify mutations at key sites. Mutations of interest included A1762T and G1764A in the BCP region and G1896A in the PC region. According to the sequencing results, patients were classified into two groups: a mutation group, defined by the presence of one or more mutations within the PC/BCP regions, and a non-mutation group, characterized by wild-type sequences in these loci.

Statistical Methods

Sample size was calculated using G*Power software. The expected HBeAg seroconversion rates were 80% in the non-mutation group and 40% in the mutation group. Statistical power was set at 80% ($\beta = 0.2$), with a significance level of $\alpha = 0.05$. Calculations indicated that at least 10 patients per group were required. To account for potential dropouts, the sample size was increased to 15 patients per group, totaling 30 patients. Ultimately, 48 patients were collected to ensure the robustness of the study results. Statistical analysis was conducted using SPSS version 17. Missing data were managed using the Intention-to-treat (ITT) approach. Baseline characteristics were summarized with categorical data presented as percentages and continuous data expressed as mean ± standard deviation or median, depending on the distribution. For group comparisons, categorical data were analyzed using χ^2-tests and Fisher's exact test, while continuous data were analyzed using independent samples *t*-tests or Mann–Whitney *U*-tests, based on normality. Spearman correlation analysis was used to assess the relationships between ALT levels, PC/BCP mutations, and HBeAg seroconversion. Logistic regression analysis was performed to evaluate the impact of multiple factors, including age, gender, ALT levels, and HBV DNA levels, on HBeAg seroconversion and relapse rates. Adjusted odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated. The significance level for all hypothesis tests was set at $P < 0.05$.

Results

Baseline Characteristics

The study included 48 patients, with 37 in the mutation group and 11 in the non-mutation group. The baseline characteristics of the two groups are summarized in Table 1. The mean age of patients was 38.5 ± 6.2 years in the mutation group and 37.1 ± 5.8 years in the non-mutation group ($P = 0.450$), indicating no significant difference between the two groups. Gender distribution was also comparable, with 28 males and 9 females in the mutation group, and 8 males and 3 females in the non-mutation group ($P = 0.673$).

Liver function parameters were similar across both groups. The mean ALT levels were 650.0 ± 300.0 U/L in the mutation group and 500.0 ± 250.0 U/L in the non-mutation group ($P = 0.079$). The mean HBV DNA levels were 7.2 ± 0.5 log10 copies/mL in the mutation group and 7.0 ± 0.4 log10 copies/mL in the non-mutation group ($P = 0.212$). The

Table 1 Baseline Characteristics of Patients in the Mutation and Non-Mutation Groups

Characteristic	Mutation Group (n=37)	Non-Mutation Group (n=11)	*P-value*
Age (years)	38.5 ± 6.2	37.1 ± 5.8	0.450
Gender (Male/Female)	28/9	8/3	0.673
ALT (U/L)	650.0 ± 300.0	500.0 ± 250.0	0.079
HBV DNA (log10 copies/mL)	7.2 ± 0.5	7.0 ± 0.4	0.212
HBeAg (IU/mL)	400.0 ± 200.0	350.0 ± 150.0	0.335
HBsAg (IU/mL)	5000.0 ± 1000.0	4500.0 ± 800.0	0.422

Note: Data are presented as mean ± standard deviation. *P*-values were calculated using independent samples t-tests or chi-square tests.

levels of HBeAg were also similar between the two groups, with a mean of 400.0 ± 200.0 IU/mL in the mutation group and 350.0 ± 150.0 IU/mL in the non-mutation group *(P* = 0.335). The mean HBsAg levels were 5000.0 ± 1000.0 IU/mL in the mutation group and 4500.0 ± 800.0 IU/mL in the non-mutation group (*P* = 0.422). These findings confirm the homogeneity of the two groups at baseline, making them suitable for subsequent comparative analysis.

Seroconversion Rates

Seroconversion rates were compared between the mutation and non-mutation groups at several time points, and the results are presented in Table 2. At 24 weeks, the mutation group had a seroconversion rate of 18.9% (7/37), while the non-mutation group had a higher rate of 45.5% (5/11), with no significant difference (*P* = 0.113). Significant differences were seen at all other time points. Notably, at 48 weeks, the mutation group had a seroconversion rate of 29.7% (11/37), compared to 72.7% (8/11) in the non-mutation group (*P* = 0.016). At 72 weeks, the seroconversion rate in the mutation group increased to 35.1% (13/37), while the non-mutation group was still 72.7% (8/11) (*P* = 0.040). At 96 weeks, the mutation group had a seroconversion rate of 37.8% (14/37), significantly lower than the non-mutation group's rate of 81.8% (9/11) (*P* = 0.016). These data indicate that the mutation group had a significantly lower likelihood of achieving seroconversion at each time point compared to the non-mutation group.

Relapse Rate After Treatment Cessation

The relapse rates after 48 weeks of drug withdrawal were significantly different between the two groups, as shown in Table 3. Among the 23 individuals who achieved seroconversion, 14 were in the mutation-positive cohort, and 9 in the mutation-negative cohort. In the mutation-positive group, all 14 patients experienced relapse, resulting in a 100% relapse rate, while none in the mutation-negative group experienced relapse (0%). This stark contrast highlights the substantial impact of PC/BCP mutations on relapse risk after treatment cessation. Statistical analysis revealed a highly significant difference between the two groups (χ^2 = 23, *P* =0.0001).

Correlation Between ALT Levels and Seroconversion Rates

The correlation between ALT levels and seroconversion rates was assessed and is summarized in Table 4. In our study, ALT levels were categorized into three ranges: < 100 U/L, 100–500 U/L, and > 500 U/L. This classification is primarily based on the distribution characteristics of ALT values in HBeAg-positive CHB patients, as the majority of patients in the cohort had ALT

Table 2 Seroconversion Rates at Different Time Points

Time (Weeks)	Mutation (n/N, %)	Non-Mutation (n/N, %)	*P* (Fisher)
24	7/37 (18.9%)	5/11 (45.5%)	**0.113**
48	11/37 (29.7%)	8/11 (72.7%)	**0.016**
72	13/37 (35.1%)	8/11 (72.7%)	**0.040**
96	14/37 (37.8%)	9/11 (81.8%)	**0.016**

Note: Seroconversion rates were compared using Fisher's exact test.

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Table 3 Relapse Rates After 48 Weeks of Drug Withdrawal

Group	Cases	HBeAg Reactivation
Mutant	14	14(100%)
Non-Mutant	9	0(0%)
χ^2		23.00
P		0.0001

Notes: Relapse was defined as the reappearance of HBeAg accompanied by HBV DNA levels > 2000 IU/mL. Of the 48 enrolled patients, 23 achieved seroconversion, including 14 in the mutation group (100% relapse rate) and 9 in the non-mutation group (0% relapse rate). Statistical comparison was performed using Fisher's exact test.

Table 4 Correlation Between ALT Levels and Seroconversion Rates

ALT (U/L)	Seroconversion Rate in Mutation Group (%)	Seroconversion Rate in Non-Mutation Group (%)	*P* (Fisher)
<100	8/20 (40.0)	4/5 (80.0)	0.160
100–500	5/14 (35.7)	3/4 (75.0)	0.275
>500	1/3 (33.3)	2/2 (100.0)	0.400

Note: Seroconversion rates were compared using Fisher's exact test.

levels concentrated within the aforementioned intervals. The grouping aims to objectively reflect the gradation differences in liver inflammation among the study population, which is consistent with the standards used in clinical practice for distinguishing mild, moderate, and severe hepatocellular injury in this specific cohort, facilitating the analysis of the association between different degrees of inflammation and seroconversion rates. In the mutation group, patients with ALT levels below 100 U/L had a seroconversion rate of 40.0% (8/20), while the non-mutation group had a significantly higher rate of 80.0% (4/5)($P = 0.16$). For patients with ALT levels between 100 and 500 U/L, the seroconversion rate in the mutation group was 35.7% (5/14), compared to 75.0% (3/4) in the non-mutation group ($P = 0.27$). When ALT levels exceeded 500 U/L, the mutation group had a seroconversion rate of 33.3% (1/3), while the non-mutation group had a rate of 100.0% (2/2) ($P = 0.40$).

Correlation Between HBV DNA Levels and Seroconversion Rates

The relationship between HBV DNA levels and seroconversion rates was also analyzed, and the results are presented in Table 5. In the mutation group, patients with HBV DNA levels below 6.0 log10 copies/mL had a seroconversion rate of 45.4% (5/11), compared to 85.7% (6/7) in the non-mutation group ($P = 0.15$). For patients with HBV DNA levels between 6.0 and 7.0 log10 copies/mL, the seroconversion rate was 35.3% (6/17) in the mutation group and 75.0% (3/4) in the non-mutation group ($P = 0.27$). In patients with HBV DNA levels greater than 7.0 log10 copies/mL, the seroconversion rate in the mutation group dropped to 33.3% (3/9). These results indicate a negative correlation between HBV DNA levels and seroconversion in the mutation group, suggesting that higher viral loads may impair the likelihood of seroconversion in patients with PC/BCP mutations.

Table 5 Correlation Between HBV DNA Levels and Seroconversion Rates

HBV DNA (log10 copies/mL)	Seroconversion Rate in Mutation Group (%)	Seroconversion Rate in Non-Mutation Group (%)	*P-value*
< 6.0	5/11 (45.4)	6/7 (85.7)	0.151
6.0–7.0	6/17 (35.3)	3/4 (75.0)	0.272
> 7.0	3/9 (33.3)	0/0 (NA)	-

Notes: Seroconversion rates were compared using Fisher's exact test. HBV DNA levels were categorized into < 6.0, 6.0–7.0, and > 7.0 log10 copies/mL. It was based on viral load distribution in our HBeAg-positive CHB cohort and clinical criteria for assessing viral replication activity in chronic hepatitis B management.

Table 6 Correlation Between PC/BCP Mutation Types and Seroconversion/Relapse Rates

Mutation Type	Number of Cases	Seroconversion Rate (%)	Relapse Rate (%)
G1896A	9	4/9 (44.4)	4/4 (100.0)*
A1762T/G1764A	15	6/15 (40.0)	6/6 (100.0)*
A1762T/G1764A/G1896A	13	4/13 (30.8)	4/4 (100.0)*
Wild-type	11	9/11 (81.8)	0/9 (0.0)

Notes: Relapse cases refer to patients who achieved seroconversion and discontinued treatment in each mutation subgroup (4 cases in the G1896A group, 6 cases in the A1762T/G1764A group, and 4 cases in the A1762T/G1764A/G1896A group). *Statistical analysis was performed using Fisher's exact test, which revealed a statistically significant difference in the recurrence rate between this mutant subgroup and the wild-type group ($P < 0.05$).

Correlation Between PC/BCP Mutation Types and Seroconversion/Relapse Rates

Table 6 shows that among the 48 patients, there were significant differences in seroconversion and relapse between the PC/BCP mutation subgroups and the wild-type group. The wild-type group had the highest seroconversion rate (9/11, 81.8%), while the results in the mutation subgroups were as follows: 44.4% (4/9) in the G1896A group, 40.0% (6/15) in the A1762T/G1764A group, and 30.8% (4/13) in the A1762T/G1764A/G1896A triple mutation group. All mutation subgroups had a 100.00% relapse rate (4/4 in the G1896A group, 6/6 in the A1762T/G1764A group, and 4/4 in the triple mutation group), whereas there was no relapse in the wild-type group (0/9, 0.0%).

Comparison of HBV DNA Levels After Discontinuation of Treatment

At 48 weeks after treatment discontinuation, the HBV DNA levels in the mutation group (14 patients) were all ≥ 2000 IU/mL, which was significantly higher than that in the non-mutation group (9 patients) with HBV DNA < 20 IU/mL (detection limit). The difference was statistically significant ($P < 0.001$). These results suggest that patients with PC/BCP mutations have a large viral rebound and strong replicative activity after treatment discontinuation.

Discussion

CHB remains a global health challenge, affecting millions of individuals worldwide[19]. Despite advancements in antiviral therapies, including NAs, achieving sustained suppression of HBV replication remains a difficult task for a substantial proportion of patients[20]. Particularly, the role of PC/BCP mutations in the response to treatment and the risk of relapse following therapy discontinuation is gaining increasing attention[21,22]. These mutations play a crucial role in modulating the virological and immunological landscape of the disease, influencing the effectiveness of antiviral therapies and the likelihood of relapse after drug withdrawal.

Our study demonstrates that PC/BCP gene mutations significantly influence the seroconversion rate and relapse rate in HBeAg-positive CHB patients undergoing NA antiviral therapy. Specifically, patients in the mutation group exhibited a significantly lower seroconversion rate compared to the non-mutation group, and there was a clear negative correlation between the number of mutations and seroconversion success. Notably, patients carrying the triple mutation A1762T/G1764A/G1896A had the lowest seroconversion rate at 30.8%, while the non-mutation group reached 81.8%. These results suggest that the presence of PC/BCP mutations may significantly inhibit the host immune response, thereby hindering sustained viral suppression. One possible mechanism is that mutations such as A1762T and G1764A significantly upregulate the transcription of pregenomic RNA (pgRNA), enhancing viral replication and leading to prolonged treatment duration and suboptimal efficacy of NA therapy. Previous studies by Yang et al also reported similar findings, confirming the adverse impact of PC/BCP mutations on seroconversion in HBeAg-positive patients.[23]

Additionally, another possible mechanism is that PC/BCP mutations may lead to immune escape, particularly the G1896A mutation, which disrupts the T-cell epitope of HBeAg, hindering effective immune responses[24]. Although HBeAg-negative patients are typically considered to have achieved immune control, patients harboring PC/BCP mutations may exhibit inconsistent serological profiles, such as HBeAg negativity while still exhibiting active viral replication. Globally, HBV infection is estimated to cause between 500,000 to 1 million deaths annually.[25] Suppressing HBV replication is a key strategy in delaying liver disease progression and reducing mortality. Antiviral therapy can effectively

suppress viral replication, maintaining HBV levels at very low concentrations, thus significantly lowering the risk of HCC and liver disease-related death[26,27]. However, despite successes in controlling viral replication, HBsAg clearance remains a major challenge. The high relapse rate after discontinuation of treatment further complicates the management of CHB. Therefore, identifying reliable indicators for treatment response and relapse prediction is crucial for clinical decision-making and improving patient prognosis.

Importantly, our study found that PC/BCP mutations not only affect seroconversion but also significantly impact the relapse rate after discontinuation of nucleotide analog treatment. In this study, the relapse rate in the mutation group reached 100% at 48 weeks after discontinuation, while the relapse rate in the non-mutation group was 0%. This significant difference highlights the key role these mutations play in predicting relapse risk. Patients carrying PC/BCP mutations, due to ongoing viral activity, remain at significantly higher risk of relapse even after achieving HBeAg seroconversion during antiviral therapy. The upregulation of viral replication caused by mutations such as A1762T and G1764A necessitates an extended duration of NA treatment to fully suppress viral activity[23,28]. Consequently, these patients are more prone to viral rebound and HBeAg reactivation after treatment cessation. Similar phenomena have been reported in previous studies, including that by Jiang et al, which found that HBeAg-positive patients with PC/BCP mutations exhibited significantly higher relapse rates after discontinuation of NA treatment.[21,29] Our study further extends these findings by directly associating relapse rates with the mutational burden in the PC/BCP region. Patients with the triple mutation (A1762T/G1764A/G1896A) are particularly prone to relapse, underscoring the need for more refined approaches when defining the criteria for NA treatment cessation.

Furthermore, we observed a negative correlation between seroconversion rate and ALT levels in the mutation group. Specifically, patients with ALT levels below 100 U/L had a seroconversion rate of 40%, while those with ALT levels above 500 U/L had a significantly lower seroconversion rate of 30%. This finding contradicts the traditional view that elevated ALT levels typically reflect hepatocellular damage and are associated with better treatment outcomes[30]. In this study, elevated ALT levels may reflect a stronger immune response against the virus, paradoxically undermining the efficacy of NA therapy. Although ALT elevation usually indicates active viral replication and liver injury, in the context of PC/BCP mutations, this immune response may exacerbate liver inflammation, leading to poor treatment response.[31] Moreover, we observed that in the mutation group, ALT levels were negatively correlated with HBeAg seroconversion, meaning that higher ALT levels were associated with lower seroconversion rates. This suggests that PC/BCP mutations may alter immune regulation mechanisms, reducing hepatocellular injury and maintaining lower ALT levels. This finding challenges the traditional notion of ALT as a simple biomarker for disease progression, highlighting the complex interactions between viral mutations, immune responses, and liver inflammation. In the mutation group, the negative correlation between HBV DNA levels and seroconversion further emphasizes the negative impact of high viral loads and quasi-species diversity on treatment efficacy. These findings suggest that mutant strains with high viral loads may exhibit enhanced drug resistance, complicating treatment efforts.

To date, there is no curative treatment for chronic HBV infection. Long-term use of NAs has been shown to effectively suppress viral replication, thereby reducing the incidence of HCC and liver cirrhosis complications[32–34]. Nonetheless, HBsAg clearance remains an ideal goal of antiviral therapy but is still difficult to achieve. For example, 48 weeks of pegylated interferon therapy can increase HBsAg clearance rates by enhancing immune responses.[35] However, discontinuation of NA treatment is typically associated with high viral relapse rates and elevated ALT levels, potentially increasing the risk of adverse prognoses.[36] According to the Asian Pacific Association for the Study of the Liver (APASL) guidelines, discontinuation of NA antiviral therapy is recommended only after at least 2 years of treatment, provided that HBV DNA is undetectable in three separate tests over a 6-month interval and ALT levels are normal[37]. However, there are significant discrepancies between the cessation criteria and viral and clinical relapse rates, with more than 40% of patients requiring retreatment after discontinuing oral antiviral therapy.[38] Despite these criteria, relapse rates remain as high as 80%, emphasizing the need for improved standards for HBV treatment cessation. Future studies should focus on developing more precise and personalized cessation guidelines, considering factors such as PC/BCP mutations, baseline viral load, and host immune response.

Our findings indicate that the independent association between pre-treatment ALT levels and PC/BCP mutations exists only in HBeAg-positive patients, consistent with a study conducted in the United States[39]. However, our results contradict those of an

international study, which showed an age-related association but found no significant correlation with age.[40] This discrepancy may be due to a smaller sample size. Moreover, there was a positive correlation between pre-treatment ALT levels and HBeAg seroconversion, in agreement with numerous domestic and international studies.[41–43] However, the relationship between ALT and HBeAg seroconversion in this study was relatively weak, suggesting that other factors may play a significant role in determining treatment response. Future research should explore the potential interactions between ALT, PC/BCP mutations, and other clinical and virological factors to better understand their combined impact on HBeAg seroconversion. Additionally, 77.08% of HBeAg-positive individuals carry the PC/BCP mutation in CHB patients. This finding is consistent with many domestic and international studies.[39,44,45] Our research highlights the importance of monitoring PC/BCP mutations in HBeAg-positive CHB patients to optimize the duration of antiviral therapy. Routine mutation monitoring should become standard practice to guide personalized treatment plans, reduce premature treatment interruptions, decrease relapse rates, and improve patient prognosis.

Based on our findings, we recommend incorporating PC/BCP mutation testing into routine monitoring for CHB patients. For HBeAg-positive chronic hepatitis B patients carrying PC/BCP mutations, the same treatment management approach should be applied as for HBeAg-negative patients. Given that these patients have a relapse rate of up to 100% after discontinuation, clinicians should carefully assess the timing of treatment cessation to avoid premature cessation leading to disease relapse and exacerbation. Furthermore, our results contribute to evaluating the risk of disease progression. By detecting PC/BCP mutations, high-risk individuals for disease progression can be identified early, allowing for timely implementation of aggressive interventions such as intensified antiviral therapy and close monitoring of liver pathology to improve long-term patient outcomes.

Although this study provides valuable insights into the impact of PC/BCP mutations on seroconversion and relapse rates, several limitations should be acknowledged. The relatively small sample size and short follow-up period may limit the generalizability of our findings. While this study focused on HBeAg-positive patients, it remains unclear whether similar effects are observed in HBeAg-negative or genotype-specific populations. Future research with larger sample sizes and longer follow-up periods is needed to validate our findings and explore the immune escape and resistance mechanisms induced by these mutations. Furthermore, research on the interactions between PC/BCP mutations, baseline viral load, and host immune responses will provide further insights into the optimal management of CHB patients. Lastly, multi-center studies are necessary to assess the global relevance of PC/BCP mutation testing and refine treatment guidelines for HBeAg-positive CHB patients.

In conclusion, PC/BCP mutations are key factors in predicting treatment failure and relapse in HBeAg-positive CHB patients. Our findings underscore the importance of routine mutation testing in HBeAg-positive patients to guide treatment decisions and improve clinical outcomes. By incorporating mutation monitoring into clinical practice, clinicians can better tailor personalized treatment plans, reduce the risk of relapse, and prevent disease progression. Future research should focus on elucidating how these mutations alter viral replication, immune responses, and treatment outcomes to ultimately achieve more personalized and effective CHB treatment strategies.

Abbreviations

NA, Nucleos(t)ide Analogues; PC/BCP, Precore/Basal Core Promotor; CHB: Chronic Hepatitis B; NA, Nucleoside Analogues; HBeAg, hepatitis B e antigen; ITT, Intention-to-treat; ALT, Alanine Aminotransferase.

Data Sharing Statement

All data and materials supporting the findings of this study are available within the article and from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This retrospective study was approved by the Ethics Committee of Taixing People's Hospital, and the requirement for informed consent was waived (approval number: LS2025045). The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All procedures followed were in compliance with the ethical guidelines for medical research involving human subjects.

Consent for Publication

The authors confirmed that they have obtained consent from the patient for publication of this case report.

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Disclosure

The authors declare no competing interests in this work.

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