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Case Report
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Concurrent HBsAg and Anti-HBs Induced by a PreS Deletion Mutant in a Chronic Hepatitis B Patient

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Abstract

Background: Hepatitis B Virus (HBV) infection is a serious global health problem. This coexistence of HBsAg and anti-HBs is generally considered to be the result of immune-associated escape mutations which include deletion and point mutations within the preS/S region of the genome. This case report reveals another mechanism.

Case Report: This study was designed to analyze the reasons for concurrent HBsAg and anti-HBs in a patient with chronic HBV infection. Serological markers of HBV infection were determined by ELISA. The preS/S gene were analyzed by gene amplification and sequencing. The tests revealed that HBsAg and anti-HBs coexisted in this patient with mixed infections of full-length preS/S virus strain and preS1 183 bp deletion mutant, and the mutant disappeared along with the anti-HBs after one year, which means that the mutant strain was cleared by the detected antibodies.

Conclusion: It is speculated that the production of anti-HBs targeted specifically to the preS1 deletion mutant strain instead of the strain with full-length large S protein. This is quite different from the other immunopathogenetic mechanism for concurrent HBsAg and anti-HBs.

Keywords: Hepatitis B virus; Chronic Hepatitis B; HBsAg; anti-HBs; preS deletion mutant

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Background

Hepatitis B Virus (HBV) infection is a serious global health problem. It can result in a self-limiting acute infection or Chronic Hepatitis B (CHB) depending on the interaction between the host's immune system and the virus [1]. HBV codes for three Hepatitis B surface Antigen (HBsAg) proteins (large, middle, and small). The large multifunctional HBsAg protein surrounding the hepatitis B virion is coded by the preS1/preS2/S region of the genome. The Myristoylated N-terminal portion of the preS1 domain contains a ligand that binds to the Sodium Taurocholate Cotransporting Polypeptide (NTCP) receptor found on human hepatocytes that is necessary for infection. Specific antibody responses targeting HBsAg play an important role in neutralizing HBV infectivity and clearing noninfectious HBsAg particles from peripheral blood. Thus, the presence of antibodies to HBsAg (anti-HBs) indicates viral clearance and is often a sign of recovery from HBV infection. In contrast, once the disease progresses to Chronic Hepatitis B (CHB), anti-HBs generally does not coexist with HBsAg.

In those cases where concurrent HBsAg and anti-HBs occurs in patients with CHB, atypical serological characteristics are observed. This coexistence of HBsAg and anti-HBs is generally considered to be the result of immune-associated escape mutations which include deletion and point mutations within the preS/S region of the genome [2]. However, no significant or identical differences have been reported in the incidence of amino acid substitutions in the small S gene region of the HBV genome from CHB patients with and without anti-HBs [3] which appears to contradict the hypothesis that HBV escape mutants are selected by concurrent anti-HBs.

In this paper that evaluates the full-length large HBsAg, a deletion mutant large HBsAg and anti-HBs coexisted in the serum of a patient with chronic HBV infection. After a year, both anti-HBs and mutant HBsAg disappeared. The correlation between immunity and mutation in this case warrants further investigation.

Case Presentation

Peripheral blood samples originated from a 43-year-old Chinese male patient with genotype C CHB, who had never been vaccinated and was admitted to the 960th Hospital of the PLA Joint Logistics Support Force for regular physical examination. Routine screening discovered that he had concurrent HBsAg and anti-HBs. Peripheral blood samples (5 mL), identified as Sample 1 and Sample 2, were collected on two separate occasions at an interval of one year. This study was approved by the hospital ethics committee.

Serological markers of HBV infection were determined by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Xiamen Xinchuang, Shanghai Kehua, China). Positive and negative controls were included in each test. The tests were carried out according to the instructions of each kit. The virological tests of Sample 1 were reactive for HBsAg, anti-HBs, Hepatitis B e Antigen (HBeAg), and Hepatitis B core antibody (anti-HBc). HBV DNA viral load was 2.16×10^5 IU/mL. The results for Sample 2 revealed reactivity against HBsAg, HBeAg, and anti-HBc, with an HBV DNA viral load of 1.29×10^4 IU/mL, but no anti-HBs was detected.

QIAamp MinElute Virus Spin Kits (Qiagen, Hilden, Germany) were used to extract DNA from the serum samples. DNA was aliquoted and stored at −8°C until use. Commercially available HBV real-time PCR kits (Piji Biotec, Shenzhen, China) in a Light Cycler 2.0 Real-Time PCR system (Roche Diagnostics, Basel, Switzerland) were utilized to quantify the viral load of HBV DNA. The preS/S gene regions were amplified using primers P1 and R1 (Table 1) on a thermal cycler (BIO-RAD MyCycler, BIO-RAD, CA) using PrimeStar HS high-fidelity DNA polymerase (Takara, Dalian, China). The PCR products from Sample 1 and Sample 2 were recovered from agarose gel and purified using Agarose Gel DNA Purification kits (Takara, Dalian, China) prior to being sequenced directly. The products of Sample 2 were further cloned into the pMD18-T Vector system (Takara, Dalian, China) and ten clones were selected for sequencing and analysis.

The PCR product is about 1,410 bp spanning nucleotide positions 2825–1019. Electrophoresis after preS/S PCR amplification of Sample 1 showed two distinguishable bands located at about 1,200 bp, and 1,400 bp, respectively. Sequencing results of the products recovered from the two bands in Sample 1 revealed that one of the bands featured a large deletion mutation in preS1 (Figure 1), compared to a full-length preS/S segment in the other band implying that there were two HBV variants present in the same sample. Comparison of the nucleotide sequence of this mutant with the amino acid (aa) sequence of the wild-type virus showed that the 183 bp deletion in the preS1 region was located at aa 57-117 of the large HBsAg polypeptide.

In contrast to this finding, Sample 2 showed only one band located at about 1,400 bp by electrophoresis. The PCR product and clones were sequenced, and the results showed that only full-length large surface protein was present. No preS1 deletion mutant was found by sequencing.

Discussion and Conclusions

Although concurrent HBsAg and anti-HBs have been reported in patients with chronic HBV infection, the clinical and virological characteristics of these patients are not well described. Two immunopathogenetic mechanisms have been proposed to explain the circumstances leading to the coexistence of HBsAg and anti-HBs.

Figure 1: The nucleotide sequences of the pre-S1 regions obtained from Sample 1 and Sample 2. Sample 1 showed two clearly distinguishable bands located at about 1,200 bp, and 1,400 bp after preS/S PCR amplification, "Sample 1-1" represented deletion mutant located at about 1,200 bp, "Sample 1-2" represented full length strain in pre-S1 region located at about 1,400 bp. Sample 2 showed only one band located at about 1,400 bp by electrophoresis. Genotype C reference sequence (AB014381) is shown at the top. Positions of nucleotide sequences of the pre-S1 region and the start codon of pre-S1 and pre-S2 genes are indicated above the reference sequence. "------" indicates deletion sequences in Sample 1-1 compared with the reference.

First, frequent studies support the hypothesis that concurrent HBsAg and anti-HBs are associated with virus strains with HBsAg mutations that have escaped anti-HBs neutralization [4]. PreS deletions and "a" determinant mutation can lead to antigenic changes in HBsAg which result in the concurrence of HBsAg and anti-HBs in the blood since preS/S mutations may alter HBsAg binding properties, thereby masking antibody recognition of HBsAg [5]. A second hypothesis is that HBV escape mutants are not selected by anti-HBs that is coexisting with HBsAg in CHB patients. Rather, the coexistence of HBsAg and anti-HBs in this situation may be explained as anti-HBs that has unmatched specificities for the HBsAg rendering it unable to bind to the antigen [6].

In this paper, detection of Sample 2 showed that the mutant disappeared along with the anti-HBs, which we propose means that the mutant strain was cleared by the detected antibodies. Thus, we speculate that the state of concurrent HBsAg and anti-HBs in Sample 1 results from the production of anti-HBs targeted specifically to the preS1 deletion mutant strain in deference to the strain with full-length large HBsAg and did not lead to a selection of escape mutants. Correspondingly, this is quite different from the other immunopathogenetic mechanism mentioned above. The virological findings of this case remain interesting. It is possible that the preS1

Table 1: Primers used in this study.

Primer	Sequence (5'-3')	Polarity	Domain	Positions
P1	TTCTTGGGAACAAGAGCTAC	Sense	pre-S1/Pol	2825-2844
R1	GCAAAGCCCAAAAGACCCACAAT	Antisense	HBsAg/Pol	997-1019

deletion strain induced by immune pressure generated anti-HBs of high affinity and specificity for the mutant. Moreover, host-related factors such as immunocompetence, may have contributed to the abnormal response pattern observed in this patient.

In a similar study that did not include HBV DNA sequencing [7], concurrent HBsAg and anti-HBs were detected in 18 of 1462 CHB patients (1.2%) of which 15 (83.3%) were born in Asia/Australia. During a median follow-up of 4 years in 12 subjects, anti-HBs became undetectable in half after approximately 1.9 years. Independently, we have presented HBV DNA sequencing data from the preS region of the S gene in a genotype C CHB patient in which anti-HBs and a preS1 deletion variant strain became undetectable after one year suggesting that the patient was able to generate an HBV mutant-specific immune response. It will be interesting to follow the patient to elucidate whether the ability to generate an HBV-specific immune response positively affects the clinical course, which may have implications for HBV outcome. It also may stimulate additional studies as to the relevance of preS1 deletions in this process since most of the previous investigations have focused on the small polypeptide of the S gene instead of the preS region with its many immune epitopes and functional domains [8].

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References

1. Xiang Y, Yang Y, Chen P, Lai X, Shi S, Li S, et al. Analysis of serum hepatitis B virus RNA levels among HBsAg and HBsAb copositive patients and its correlation with HBV DNA. Medicine (Baltimore). 2021;100(40):e27433.
2. Huang X, Qin Y, Zhang P, Tang G, Shi Q, Xu J, et al. PreS deletion mutations of hepatitis B virus in chronically infected patients with simultaneous seropositivity for hepatitis-B surface antigen and anti-HBs antibodies. J Med Virol. 2010;82(1):23-31.
3. Mesenas SJ, Chow WC, Zhao Y, Lim GK, Oon CJ, Ng HS. Wild-type and 'a' epitope variants in chronic hepatitis B virus carriers positive for hepatitis B surface antigen and antibody. J Gastroenterol Hepatol. 2002;17(2):148-52.
4. Brunetto MR. Chance and necessity of simultaneous HBsAg and anti-HBs detection in the serum of chronic HBsAg carriers. J Hepatol. 2014;60(3):473-5.
5. Huang X, Ma C, Zhang Q, Shi Q, Huang T, Liu C, et al. Impact of "a" determinant mutations on detection of hepatitis B surface antigen (HBsAg) in HBV strains from Chinese patients with occult hepatitis B. J Med Virol. 2017;89(10):1796-803.
6. Zhang JM, Xu Y, Wang XY, Yin YK, Wu XH, Weng XH, et al. Coexistence of Hepatitis B surface Antigen (HBsAg) and heterologous subtype-specific antibodies to HBsAg among patients with chronic hepatitis B virus infection. Clin Infect Dis. 2007;44(9):1161-9.
7. Lee WM, King WC, Schwarz KB, Rule J, Lok ASF; HBRN Investigators. Prevalence and clinical features of patients with concurrent HBsAg and anti-HBs: Evaluation of the hepatitis B research network cohort. J Viral Hepat. 2020;27(9):922-31.
8. Chen B-F. Hepatitis B virus pre-S/S variants in liver diseases. World J Gastroenterol. 2018;24(14):1507-20.