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Short communication

Detection of a premature stop codon in the surface gene of hepatitis B virus from an HBsAg and antiHBc negative blood donor

Sibnarayan Datta [a], Arup Banerjee [a], Partha K. Chandra [a], Subhasis Chakraborty [b], Subir Kumar Basu [b], Runu Chakravarty [a,*]

[a] *ICMR Virus Unit, Kolkata, ID & BG Hospital Campus, Kolkata, West Bengal, India*
[b] *Institute of Blood Transfusion Medicine and Immunohematology (IBTMI), Kolkata, West Bengal, India*

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Abstract

Background: In blood donors, HBV infection is detected by the presence of serum hepatitis B surface antigen (HBsAg). However, some mutations in the surface gene region may result in altered or truncated HBsAg that can escape from immunoassay-based diagnosis. Such diagnostic escape mutants pose a potential risk for blood transfusion services.
Results: In the present study, we report a blood donor seronegative for HBsAg and antiHBc, but positive for antiHBs who was HBV DNA positive by PCR. Sequencing of the HBsAg gene revealed presence of a point mutation (T–A) at 207th nucleotide of the HBsAg ORF, which resulted in a premature stop codon at position 69. This results in a truncated HBsAg gene lacking the entire 'a' determinant region. However, follow-up of the donor after 2 years revealed clearance of HBV DNA from the serum.
Conclusion: The case illustrates an unusual mutation, which causes HBsAg negativity. The finding emphasizes the importance of molecular assays in reducing the possibility of HBV transmission through blood transfusion. However, developing more sensitive serological assays, capable of detecting HBV mutants, is an alternative to expensive and complex amplification-based assays for developing countries.

Keywords: HBsAg; Transfusion; HBV DNA; AntiHBs; PCR

1. Introduction

Hepatitis B surface antigen (HBsAg) is the most important marker for hepatitis B virus (HBV) diagnosis (Weber, 2005a). HBsAg detection is routine for screening blood donors. An HBsAg negative donation is generally considered safe. However, HBsAg diagnostic assays are based on antigen–antibody interaction, and are thus susceptible to mutations (Carman, 1997; Weber, 2005a). Therefore, transmission of HBV from HBsAg negative donors does occur (Brechot et al., 2001). Due to this, additional testing of antiHBc was recommended (Mosley et al., 1995), but subsequently transmission from antiHBc negative donors was also reported (Almeida and Cardosa, 2006; Leperche et al., 2001). Thus, residual risk of HBV transmission from donors with early acute, resolving, occult infection or atypical mutations still exists (Weber, 2005a).

With the advent of sensitive PCR assays, HBV DNA can be detected among HBsAg negative individuals. This serologic-molecular pattern is termed occult HBV infection and has been reported among blood donors (Allain, 2004). Weber, 2005a has recently reviewed the role of S gene variability and its influence on diagnostics assays. Recently, an expert advisory meeting suggested PCR amplification and sequencing of samples with discordant results, to determine if a mutant sequence is present (Gerlich, 2004). According to a recent review (Weber, 2005b) only two cases of diagnostic escape HBsAg mutants in blood donors have been reported until now

* Corresponding author at: ICMR Virus Unit, Kolkata, ID & BG Hospital Campus, GB-4, 1st Floor (East Wing), 57 Dr. Suresh Chandra Banerjee Road, Post Office-Beliaghata, Kolkata 700010, West Bengal, India. Tel.: +91 3323537425; fax: +91 3323537424.
E-mail address: runugc@hotmail.com (R. Chakravarty).

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Table 1
Serological and virological profile of the subject at presentation and follow-up

Assays performed	At presentation (February 2004)		At follow-up (February 2006)	
	Index[a]	Results	Index[a]	Results
HBsAg	0.65[b]	Negative	0.79[b]	Negative
	0.53[c]	Negative	0.13[c]	Negative
AntiHBc	1.76[b]	Negative	3.19[b]	Negative
AntiHBs	35 IU L^{-1} [b]		<10 IU L^{-1} [d]	
HBV DNA	1.2×10^3 copies mL^{-1}		Not detectable	
ALT	46 IU L^{-1} [e]		32 IU L^{-1} [e]	

[a] Index: signal/cut-off.
[b] Hepanostika/BioMerieux, Boxtel, The Netherlands.
[c] Standard Diagnostics Inc., Kyonggi-do, Korea.
[d] DiaSorin, Saluggia, Vercelli, Italy.
[e] Ecoline ALAT, Merck Specialties, Mumbai, India.

(Jongerius et al., 1998; Levicnic-Stezinar, 2004). Whatever be the cause, failure to detect HBsAg poses a potential danger of introducing contaminated blood into the public supply (Levicnic-Stezinar, 2004).

In India, HBsAg testing is done for donor screening. Anti-HBc screening is not routine, as it may result in rejection of one of four potential donors (Vivekanandan et al., 2004). Nandi and Banerjee (1992) reported HBV DNA positivity in 24% of the blood donors who were HBsAg negative. Transfusion associated HBV (TAHB) is estimated at approximately 1.5% in post-surgical recipients and 50% or more in multiple-transfusion associated recipients in India (Chaudhuri et al., 2003).

In this study, we report the detection and quantification of HBV DNA by PCR assay in a blood bank donor with anti-HBs as the only marker, where further investigations revealed an unusual premature stop codon mutation in the S gene sequence.

2. Results

In February 2004, blood from a 24 years old, male, first time donor, was collected from a voluntary blood donation camp, and sent to IBTMI, Kolkata for routine donor screening and processing of the donation. Parallel, as a part of a study on occult HBV and genetic variability of S gene among general population, an aliquot of the blood sample was collected and sent to ICMR Virus Unit, Kolkata, for further tests, including HBV DNA detection and analysis. The donor consented for participation in the study and sample was collected after interview and filling of a detailed questionnaire.

Serum was separated and stored at −20 °C. Sample was screened by commercial ELISA. Initially the sample was weakly reactive for HBsAg; however, on repeat testing, it gave negative result, with two different kits (Table 1). Other markers were subsequently tested and the results are presented in Table 1. The donor was negative for anti-HCV (Ortho-Clinical Diagnostics, NJ, US) and antiHIV (BioMerieux, Boxtel, The Netherlands). Interestingly, examination of the donor questionnaire revealed that he was never vaccinated against HBV. Thus, antiHBs positivity in the case was interesting.

Suspecting an unusual case, HBV DNA was extracted from 200 μL of serum by proteinaseK-phenol/chloroform method and amplified by an in house nested PCR assay (sensitivity 10^2 copies). PCR was carried out using primers amplifying the S gene, distal X/BCP and core gene. Guidelines for performing PCR (Kwok and Higuchi, 1991) were followed strictly and amplification was carried out at least twice to rule out false positivity. HBV DNA was quantified (Applied Biosystems SDS7000, Foster City, USA). PCR products were directly sequenced from both directions and analyzed as described earlier (Datta et al., 2006). Sequence data for the distal X/precore/BCP region, surface and core gene region have been submitted in the Genbank (accession nos. DQ160161, DQ160162 and DQ267972, respectively).

Phylogenetic analysis of the S gene revealed that the HBV isolate was of genotype D, subgenotype D1 subtype *ayw2*. Strikingly presence of a single nucleotide substitution from thymidine (T) to adenosine (A) at 207th nucleotide (nucleotide position 362 from the unique *Eco RI* site) of the HBsAg ORF was noted. Deduced amino acid sequence of the corresponding substitution showed a change from TGT (cysteine) to TGA (stop) at amino acid position 69 of HBsAg. The amino acid sequence deduced from the overlapping polymerase gene showed a substitution of serine to threonine at position 413 in reverse transcriptase domain, due to the above T–A substitution. Sequence of the BCP/precore region revealed no double mutations at nucleotides 1762/1764 positions of basal core promoter or at nucleotide 1896 of the precore region, nucleotide at 1858 was T.

However, on follow-up after 2 years (February 2006), HBsAg was still undetectable (Table 1) and by this time HBV DNA was also not detectable by PCR assay indicating the clearance of the infection.

3. Discussion

Presence of HBV DNA in HBsAg negative blood donors, due to mutations in the S gene has been described in the literature (Jongerius et al., 1998; Levicnic-Stezinar, 2004). Two cases, with serological pattern similar to that of ours have been documented recently (Almeida and Cardosa, 2006; Weber et al., 2005). Although similar mutations have been reported in patients with chronic HBV infection (Weinberger et al., 1999; Yang et al., 2003), to our knowledge, a premature stop codon mutation has not been reported earlier in blood donors.

The present donor showed a serology indicative of resolving infection, with HBsAg eliminated after many years (Weber, 2005a) or has HBsAg, undetectable by diagnostic assays. Interestingly, antiHBc remained undetectable. The non-detection of antiHBc may be due to tolerance to HBV