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Case report

Detection of HBV DNA by nested-PCR in a HBsAg and anti-HBc negative blood bank donor

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Abstract

Background: The criteria and protocol adopted in serological screening of blood bank donors have significantly reduced the possibility of HBV transmission. However, it is possible that, in a very recent phase of HBV infection, HBsAg seronegative donors be able to transmit the virus. This study reports a case of a donor with this serological profile who was involved in the viral transmission to a seronegative receptor.
Case report: A blood donor had her sample tested for HBsAg and anti-HBc, which resulted negative. At the second donation the sample demonstrated to be seropositive for anti-HBc, anti-HBs and seronegative for HBsAg. The first stored sample was tested for the presence of HBV DNA. Two fragments could be identified in the genomic region corresponding to HBV core and precore. Only one individual was involved in the transfusion of hemo-derivatives originating from the processing of this bag, and was seropositive for HBsAg, HBeAg and anti-HBc markers and seronegative for the anti-HBe and anti-HBs markers.
Conclusion: This case illustrates the possibility of the occurrence of HBV transmission from blood bank donors seronegative for HBsAg and anti-HBc. This fact could be associated with the possibility of the donor to be in the pre-seroconversion phase of a recent infection, when the levels of HBsAg present in the circulation are below the limits of detection. The implementation of molecular tests or higher sensitivity HBsAg assays could further reduce the risk of HBV transmission via blood transfusion.

Keywords: HBV DNA; Blood donor; Nested-PCR; HBsAg

1. Introduction

Although serologic profiles of Hepatitis B Virus (HBV) infection markers are well documented, they may not be good indicators of viral infectiousness (Laperche et al., 2001; Prince et al., 2001). It is generally accepted that the diagnosis of infection by Hepatitis B Virus is based on the presence of the surface antigen (HBsAg) in the bloodstream (Hino et al., 2002), since that it can generally be detected while still in the incubation period (Badur and Akgun, 2001).

After the introduction of HBsAg in the screening panel of blood bank donors, in 1971, the incidence of post-transfusional Hepatitis B has been reduced substantially, although post-transfusional HBV-related cases are still observed in 0.3–1.7% of HBsAg negative hemoderivative recipients (Bodhiphala et al., 1999). In addition, it has been admitted that blood bank donor screening does not totally eliminate the risk of HBV infection through blood transfusion (Jongerius et al., 1998), since the absence of this marker in the serum does not exclude the presence of HBV-related DNA (Bréchot et al., 2001; Lai et al., 1989; Shih et al., 1990).

In spite of few reports documenting post-transfusional infection by HBV in receivers of HBsAg negative blood (Larsen et al., 1990; Wang et al., 2002), the determination of the prevalence of HBV/DNA in HBsAg negative samples is of great importance in the prevention and control of the agent

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transmission. With the advent and application of highly sensitive viral nucleic acid molecular amplification techniques, the presence of this serologic-molecular profile has been demonstrated in chronic liver infection carriers (Saito et al., 1999) and in otherwise healthy blood bank donors. This has significant epidemiological and public health interest, with particular relevance for blood banks (Bodhiphala et al., 1999).

In this study we report the detection of the HBV genome by nested-PCR in one HBsAg negative blood bank donor and reemphasize the importance of the introduction of the Nucleic acid amplification test (NAT) for HBV in blood bank donor screening.

2. Case report

In September 2002, a serum sample originating from a first-time blood donation by 60 year-old woman, born in Goiás State, Brazil, was tested for HBV markers according to the adopted protocol for blood bank donor screening. After the serologic evaluation, the sample presented negative by second generation immunoenzymatic assay for HBsAg (Monolisa HBsAg Plus, EIE monoclonal – BIORAD), with an optical density (OD) of 0.033 and a cut off value (CO) of 0.050 (index: 0.66) and was also negative for indirect anti-HBc EIE (Monolisa anti-HBc Plus – BIORAD), with an OD of 0.003 and CO value of 0.288. She was not vaccinated against HBV. Thus, as there wasn't laboratorial or clinical restriction, the hemoderivatives originating from the bag collected were used for transfusional procedures.

In March 2004, the donor came for donation. Serologic analysis of this sample demonstrated a repeatedly positive result for anti-HBc with a mean OD of 1.783 and CO of 0.273 (index: 6.5), which was confirmed after a second competitive immunoenzymatic assay (BioMerièux Hepanostika, anti-HBc, EIE) with an OD of 0.037 and CO de 0.368; negative results for HBsAg, with an OD of 0.004 and CO of 0.043, and positivity for the presence of anti-HBs antibodies (BioMerièux Hepanostika, anti-HBs, EIE) with an OD of 2.718 and CO of 0.253 (index: >10.0).

Face to these results, an aliquot of the stored sample originating from the first donation was tested for the presence of HBV DNA by an "in house" molecular technique. For this procedure, 500 UL of serum were used for DNA extraction which was conducted in duplicate by the DNAzoIBD method (Invitrogen, São Paulo, Brasil), in accordance with the manufacturer's instructions. The sediment obtained was collected and solubilized in 200 UL of sterilized water. Then 1 UL of DNA was added to a reaction mixture containing reaction buffer 1×, 0.2 mM of dNTPs, 3 mM of $MgCl_2$, oligonucleotides (20 pmol) and 1 U of Taq DNA Polymerase. The nested-PCR was performed in the same conditions using 1 UL of the product of the first round PCR. Two pairs of primers, corresponding to the core and precore region of HBV genome were used in the assay: 1763 – 5′ GCT TTG GGG CAT GGA CAT TGA CCC GTA TAA 3′ and 2032 – 5′ CTG ACT ACT AAT TCC CTG GAT GCT GGG TCT 3′, used in the first amplification reaction; 1778 – 5′ GAC GAA TTC CAT TGA CCC GTA TAA AGA ATT 3′ and 2017 – 5′ ATG GGA TCC CTG GAT GCT GGG TCT TCC AAA 3′, used in the second amplification reaction. After denaturation at 95 °C for 5 min, the DNA was amplified in 35 cycles (94 °C for 30 s, 42 °C for 30 s, 72 °C for 2 min) followed by a final elongation at 72 °C for 7 min. The amplification products were fractionated by electrophoresis in 2% agarose gel, where two fragments of 270 and 240 base pairs could be observed and corresponded to core and precore genomic regions of HBV, respectively.

Search of recipients involved in the acquisition of hemoderivatives originating from the donation bag concluded that a single individual had received erythrocytes concentrate originating from the 2002 collection. A sample from the receiver involved, a man aged 64, was collected and submitted for serologic evaluation for the markers HBsAg, anti-HBc, HBeAg, anti-HBe and anti-HBs. The sample repeatedly tested positive for HBsAg (Monolisa HBsAg Plus, EIE, Monoclonal, 2nd generation – BIORAD) OD: >3.000/CO: 0.050 (index: >10.0), and anti-HBc (Monolisa anti-HBc Plus, EIE, indirect – BIORAD) OD: 2.849/CO: 0.274 (index: 10.3). These results were confirmed by a second HBsAg immunoenzymatic assay (Murex Diagnostic HBsAg, version 3.0, EIE) OD: >3.000/CO: 0.149 (index: >10.0) and anti-HBc assay (Hepanostika anti-HBc, EIE, Uni-Form competitive) OD: 0.063/CO: 0.513. The sample was also shown to be reactive for HBeAg (ETI-EBK-2/Diasorin HBeAg) OD: 3.412/CO: 0.090 (index: >10.0) and negative for anti-HBe (ETI-AB/EBK, Diasorin, anti-HBe) OD: 4.035/CO: 0.698 and anti-HBs (Hepanostika anti-HBs) DO: 0.069/CO: 0.199.

3. Discussion

The case reported here is similar to others described in the literature (Bodhiphala et al., 1999; Jongerius et al., 1998; Larsen et al., 1990; Prince et al., 2001; Wang et al., 1991). It emphasizes the serologic and molecular profile of a blood bank donor, which revealed initially negative serology for the viral markers HBsAg and anti-HBc of HBV, but with HBV-related DNA detected by nested-PCR.

Despite continued improvement of selection criteria of blood bank donors and of the applied serologic test protocols in sample evaluation, the transmission of HBV infection by blood transfusion still occurs in a proportion of 1:63.000 cases (Korelitz et al., 1997; Schreiber et al., 1996) even after the transfusion of blood which demonstrates negativity for 3rd generation HBsAg assays (Zervou et al., 2001).

The reasons for the persistent residual risk of transmission includes the existence of chronic HBV carriers who express undetectable levels of HBsAg in the blood circulation (Chemin et al., 2001; Huo et al., 1998); the presence of mutant forms of HBV resulted from simple or multiple alterations in the 'a' determinant of hepatitis B virus surface antigen, as a