Case Report

Anti-HBs positive occult hepatitis B virus carrier blood infectious in two transfusion recipients

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Background/Aims: Occult hepatitis B infection (OBI) in blood donations is not considered infectious when anti-HBs is present.

Methods: Four months after transfusion of eight blood components during coronary arterial bypass surgery, a 59-year-old patient developed acute hepatitis B. A second 71-year-old patient transfused with a red cell concentrate (RCC) from one of these donations had early HBV infection 7 months post-transfusion. Samples were tested for HBV serological markers and HBV DNA was quantified and sequenced.

Results: One implicated donation contained anti-HBc, anti-HBs (12 IU/L) and 180 IU/ml of HBV DNA. Previous and subsequent samples contained 3–10 times lower viral load and slightly variable anti-HBs. Two previous donations did not cause HBV infection. Recipients of the FFP and RCC from the index donation were both HBV infected and carried genotype D strains with sequences identical to the donor strain.

Conclusions: Despite anti-HBs, an OBI carrier transmitted HBV to two immunocompetent transfusion recipients.

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Keywords: HBV; Occult HBV; Infectivity; Blood transfusion

1. Introduction

In Slovenia, approximately 100,000 donations per year are collected. However, in 2005–2007, six cases of HBV transmission by transfusion were reported. Incidence was probably underestimated due to a high frequency of subclinical infection. Since HBsAg serological screening with a sensitive assay is systematically performed, transfusion transmission of HBV can originate from either recent infections in the pre-HBsAg seroconversion window period or occult HBV infection (OBI). OBI is defined as an atypical carrier state characterized by the presence of HBV DNA in plasma without detectable hepatitis B surface antigen (HBsAg) with or without antibodies to hepatitis B core antigen (anti-HBc) and hepatitis B surface antigen (anti-HBs) [1].
It is generally accepted that HBV DNA in blood may carry the risk of transmission, particularly in the pre-HBsAg window phase [2]. However, the transmission risk of OBIs is not well defined, although some cases of OBIs with anti-HBc only infectious by transfusion were described [2,3].

HBV transmission by blood components from a single anti-HBs positive OBI donation to two recipients is presented.

2. Case report

A patient who had been transfused 4 months previously with five units of fresh frozen plasma (FFP) and three units of RCC was suspected of acute hepatitis B. Stored samples from each implicated donation were tested for HBV markers. Seven samples were HBV marker negative. One sample was anti-HBc reactive and contained HBV DNA. The implicated donor was identified and stored samples from eight previous donations and one donation subsequent to the index donation as well as three follow-up samples were tested for HBV markers.

The first recipient of an FFP unit from the index donation was a 59-year-old male who was screened negative for HBV markers 3 days prior to cardiac arterial bypass. He was transfused on 23rd June, 2005. Four months later, clinical and laboratory evidence of acute Hepatitis B was obtained. ALT level was 1821 IU/L, HBsAg and anti-HBc IgM became reactive. No sample was available for HBV DNA testing. In a sample collected 4 months later, HBsAg was undetectable, IgM anti-HBc remained present and HBV DNA was at low level (Table 1).

The second recipient of the index donation was a 71-year-old female who received two units of RCC following orthopedic surgery. No pre-surgical HBV screening was performed and no post-surgical evidence of HBV infection was noted. A blood sample obtained 7 months after transfusion was anti-HBc negative but HBsAg positive and contained a high level of HBV DNA (Table 1). Nine months post-transfusion, ALT level was 566 IU/L. At 14 months post-transfusion the patient had recovered.

2.1. Methods

Routine blood donation screening for HBsAg was performed using Abbott PRISM (Abbott laboratories, Delkenheim, Germany). HBsAg repeat testing, anti-HBc and anti-HBs assays were performed with Abbott AxSYM. Cobas Amplicor HBV Monitor (Roche, Basel, Switzerland) and in-house real-time PCR (QPCR) as previously described were used to detect and quantify HBV DNA [4]. Basic core promoter/pre-core region (BCP/PC), Pre-S/S regions and full HBV genome were amplified, sequenced and phylogenetically analyzed as described [5].

3. Results and discussion

The index donation met the criteria defining ‘occult’ hepatitis B virus carriage since the plasma contained no detectable HBsAg but HBV DNA, anti-HBc and low titer of anti-HBs. This pattern was consistent 7 and 16 months after the index donation. Seven prior donations carried anti-HBc and anti-HBs although HBV DNA ranged between 7 and 63 IU/ml when tested

<table>
<thead>
<tr>
<th>Time from Index donation (m)</th>
<th>HBsAg</th>
<th>Anti-HBc</th>
<th>Anti-HBc IgM</th>
<th>Anti-HBs (IU/L)</th>
<th>HBV DNA (IU/ml)</th>
<th>HBV genotype</th>
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<tr>
<td>Donor 45</td>
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<td>ND</td>
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<td>+16</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>25</td>
<td>Neg 40</td>
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</tr>
</tbody>
</table>

Recipient 1

| –3 days                     | –     | –        | ND           | –               | ND ND           | D            |
| +4                          | +     | +        | +            | –               | ND ND           | D            |
| +8                          | –     | +        | +            | –               | 12 185          | D            |

Recipient 2

| +7                          | +     | –        | –            | –               | 1.1 x 10^6     | D            |
| +14                         | –     | +        | +            | –               | Neg ND          | D            |

–, non-reactive; ND, not done; Neg, negative.
with a sensitive in-house assay but was consistently undetectable by a commercial assay except in the Index sample. This pattern indicates recovery from >5 years past HBV infection (Table 1). Despite being tested with the high sensitivity assay, two of the nine donor samples tested remained HBV DNA negative, suggesting fluctuations of viremia. Prior to the index donation, anti-HBs levels were essentially stable (15–29 IU/L) but increased from 12 to 53 IU/L 3 months later suggesting minimal immune response. There was no clinical evidence that 14 previous donations and one subsequent donation were infectious to recipients. Pre- and post-transfusion samples from recipients of −71 and −13 month-donations showed no serological evidence of HBV infection. The −71 recipient was negative for HBsAg, anti-HBc and anti-HBs pre-transfusion, and 4 months post-transfusion. HBsAg was negative but anti-HBc was not tested. The −13 month recipient did not carry HBsAg, anti-HBc or anti-HBs 42 months after transfusion. In contrast, there is strong evidence that both recipients of the index donation were HBV infected since acute hepatitis B occurred in recipient 1, 4 months after transfusion. In recipient 2, the 7-month post-transfusion sample containing HBsAg and high HBV DNA load without anti-HBc strongly suggested recent acute HBV infection and was followed by serological evidence of recovery (Table 1). A high ALT level 9 months post-infection and was followed by serological evidence of HBV transmission with a sensitive in-house assay but was consistently undetectable by a commercial assay except in the Index sample. This pattern indicates recovery from >5 years past HBV infection (Table 1). Despite being tested with the high sensitivity assay, two of the nine donor samples tested remained HBV DNA negative, suggesting fluctuations of viremia. Prior to the index donation, anti-HBs levels were essentially stable (15–29 IU/L) but increased from 12 to 53 IU/L 3 months later suggesting minimal immune response. There was no clinical evidence that 14 previous donations and one subsequent donation were infectious to recipients. Pre- and post-transfusion samples from recipients of −71 and −13 month-donations showed no serological evidence of HBV infection. The −71 recipient was negative for HBsAg, anti-HBc and anti-HBs pre-transfusion, and 4 months post-transfusion. HBsAg was negative but anti-HBc was not tested. The −13 month recipient did not carry HBsAg, anti-HBc or anti-HBs 42 months after transfusion. In contrast, there is strong evidence that both recipients of the index donation were HBV infected since acute hepatitis B occurred in recipient 1, 4 months after transfusion. In recipient 2, the 7-month post-transfusion sample containing HBsAg and high HBV DNA load without anti-HBc strongly suggested recent acute HBV infection and was followed by serological evidence of recovery (Table 1). A high ALT level 9 months post-transfusion that normalized after 14 months further supported this conclusion. The 4-month and probably 7-month long incubation time observed in recipients 1 and 2, respectively, could be explained by a relatively low infectious dose further decreased by partial anti-HBs neutralization (calculated on the basis of 180 IU/ml of HBV DNA and 200 ml of FFP for recipient 1 at 200,000 copies and 20,000 copies in 20 ml of RCC plasma for recipient 2). Published data indicated that lower infectious dose prolonged HBV incubation time and milder symptoms [6]. Transfusion transmission was further demonstrated by the Pre-S/S sequence identity between the index donation, recipient 1 and recipient 2 strains from follow-up samples. The whole genome sequences of recipient 2 and index donation were identical. Strains were of genotype D. Of note, the deduced amino acid sequence of the S protein was wild-type when compared to the genotype D consensus sequence except for A117T and S133Y, neither of these substitutions being recognized as escape mutants. An escape mutant mechanism explaining the infectivity of the index donation but not of the other donations from the donor was thus excluded. Similar cases of breakthrough HBV infection with wild-type strains have been described [7]. Although suppression of the HBV replication and gene expression is a reported cause of occult HBV [8], no mutation in the parts of the genome implicated in replication was found. Imperfect containment of viral replication by the donor immune system is the most likely cause of low levels of HBV DNA.

The stability of HBV DNA load and anti-HBs in multiple samples preceding the index donation and tested simultaneously contained 6–10 times less viral DNA than the index donation (Table 1). It is therefore speculated that the main factor singling out the index donation was a temporarily higher viral load sufficient to overcome the relatively weak neutralizing capacity of a low anti-HBs level (Table 1). This interpretation is supported by the subsequent increase in anti-HBs level suggesting a weak immune response.

Published data reporting the infectivity of OBIs by transfusion are rare. One case of transmission by a donation carrying anti-HBc without anti-HBs was reported in Japan [2]. Another study reported five donors (4 genotype D, one genotype A2) with OBI also carrying only anti-HBc transmitting to recipients. Of 51 traced recipients, 28 (54.9%) either developed fulminant, fatal, hepatitis B (3 cases) or carried anti-HBc post-transfusion although no pre-transfusion testing was performed [3]. In the Japanese study, 16 donations contained both anti-HBc and anti-HBs and no evidence of HBV transmission was found [2] confirming previous results [9]. The two cases reported here appear to be the first related to an OBI donor with anti-HBs. Data collected in Poland indicated that approximately 50% of OBIs in asymptomatic, apparently healthy, blood donors carry anti-HBs [10] and that levels of DNA and anti-HBs are variable as reported here.

Considering that the recipients at age 59 and 71, respectively, might have presented a mild, age-related, immunodeficiency added to the trauma of major surgery might have played a role in increasing susceptibility to viral infection [11]. The fact that approximately 50% of recipients of blood components in Western Europe present some degree of immunodeficiency related to age, chemotherapy or therapeutic immunosuppression suggests an increased susceptibility to HBV infection [12]. Limited but convincing evidence that OBIs can be infectious and can be detected by HBV DNA screening should be carefully considered by the health authorities of countries where neither anti-HBc nor HBV NAT are implemented.

Despite their apparent uniqueness, our two cases of HBV transmission need to be factored in discussions regarding HBV blood safety policy. They clearly illustrate that the neutralizing capacity of low-level anti-HBs is limited and reinforce the validity of considering anti-HBs below 100 IU/L to be poorly protective from infectivity when HBV DNA is present. However, even in the presence of higher levels of anti-HBs, in a severely immunodeficient recipient, HBV DNA-containing blood might be infectious and the clinical expression severe.
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References


