Evaluation of blood units with isolated anti HBC for the presence of HBV DNA

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Abstract. We screened blood donors in one center in Saudi Arabia for a safety transfusion. We found that among 5043 blood donors negative for HCV and HIV, the incidence of HBsAg positivity was 2.97%. When antiHBc antibody was measured (HBcIg) in HBsAg negative donors, we observed that 21.47% were positive indicating previous exposure to hepatitis B virus. The HBcIg positive blood was further screened for HBsAb and the specimens were found to be reactive in 81.54%. Based on these data blood transfusion was permissible from donors who showed HBsAg negativity, HBcIg positive and HBsAb reactive blood. In order to ensure safety transfusion an aliquot of specimens (n = 80) was further analyzed for HBV DNA by PCR. We found only one specimen positive with incidence of 1.25%. So we recommended restricting transfusion from the previously mentioned donors to emergencies.

Keywords: Blood units, HBsAg, HBcIg, HBV DNA, Saudi

1. Introduction

Hepatitis B viral infection is a major cause of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma, and is one of the 10 most common causes of death worldwide [15]. Most of the acutely infected adults recover spontaneously and completely from acute HBV infection. Only a small fraction (3–5%) of HBV infected adults become chronic carriers of hepatitis B surface antigen, which is a marker for chronic infection. Children born to infected mothers or infected in the first year of life are more susceptible to chronic infection [20]. The number of HBV carriers was also reported to be more than 350 million worldwide [14]. This carrier rate was shown to be higher when there is associating risk factor as schistosomiasis [9].

In Saudi Arabia there were many sporadic studies in different locations with no statistical records but the prevalence rate of HBsAg in high risk groups, such as intravenous drug users, was shown to be 6.1% compared to 0.5% in non intravenous drug users [19]. Another risk factor in blood donations is GB virus C (hepatitis G), which was reported to be prevalent in 2% of healthy Saudi donors as estimated by RT-PCR [1]. A third risk factor is hepatitis C of which the prevalence rate in healthy Saudi donors was estimated from our records to be 0.7% (unpublished data).

HIV, HTLV and sexually transmitted diseases are not considered a major problem in Saudi donors. The risk of transmission is very low and there are no records or reported studies of HIV or HTLV transmitted virus through transfusion.

The above mentioned records demonstrate that HBV infection is a major health problem that deserves more attention, especially in the field of blood transfusion,
as little or no reports to our knowledge are available regarding prevalence in healthy donors or in post-transfusion hepatitis.

The incidence of post-transfusion hepatitis B has been greatly reduced by screening blood donors for HBsAg. However, HBV still accounts for a certain number of post-transfusion hepatitis. Residual risk of post-transfusion HBV infection due to seroconverting donors has been estimated to be 1 per 63,000 units [23]. It was found that the addition of anti-HBc screening helped in preventing some cases of HBV transmission [24].

With the advantage of genomic amplification methods, the potential use of HBV DNA detection was raised but has not yet been implemented in any country for blood screening [3]. It was anticipated that the extreme sensitivity of this technology would potentially be effective in identifying donations capable of transmitting HBV in the absence of detectable HBsAg, either early in infection or following clearance of HBsAg when anti-HBc is present in the absence of high titre of Anti-HBs [2].

Therefore, we attempted to study the potential HBV infectivity of anti-HBc +ve blood donation by the use of genomic amplification of viral DNA, in order to assess the safety of HBsAg -ve/Anti-HBc +ve blood unit for blood donation.

2. Donors and materials

All blood donations were carried out over a period of seven months (October 1999–April 2000). Blood donors were questioned and proved to be apparently healthy by excluding those who had histories of previous operations, tattooing, blood transfusion or drug addiction as potential sources of infections.

HBsAg was measured by Abbott Auszyme monoclonal qualitative third generation enzyme immunoassay kit. Hepatitis BcIg total and HBsAb were determined by Abbott enzyme immunoassay kit. HBV DNA was carried out by qualitative in-house PCR using primers from pre-s regions as described previously [6]. This amplified a highly conserved region of 460 bp that has been shown to increase the precision of detection and quantification of HBV DNA as described previously [8]. All blood specimens were also checked routinely for HCV Ab, HIV Ab, HIV P24, HTLV and RPR.

Table 1

<table>
<thead>
<tr>
<th>HBcIg positivity in HBsAg positive and negative sera of blood donors</th>
<th>HBcIg +ve</th>
<th>HBcIg% positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg +ve n = 150</td>
<td>n = 150</td>
<td>100%</td>
</tr>
<tr>
<td>HBsAg -ve n = 4893</td>
<td>n = 1051</td>
<td>21.47%</td>
</tr>
</tbody>
</table>

Total number of donors = 5043 HBsAg +ve 2.97%.

Table 2

<table>
<thead>
<tr>
<th>HBsAb positivity in HBcIg positive, HBsAg negative donors</th>
<th>HBcIg +ve n = 1051</th>
<th>HBcIg% positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAb (n)</td>
<td>n = 857</td>
<td>81.54%</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>HBV DNA in HBcIg positive and negative sera</th>
<th>HBcIg +ve n = 80</th>
<th>HBcIg -ve n = 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA one (1.25%)</td>
<td>none (zero%)</td>
<td></td>
</tr>
</tbody>
</table>

3. Results

Screening the HBsAg positive blood showed almost 100% positivity for HBc Ig, whereas HBsAg negative sera showed only 21% positivity, indicating previous exposure to the virus (Table 1). These results demonstrate the prevalence rate of carrier state of HBV to be 2.97%.

Table 2 demonstrates that more than 4/5 of the blood donors developed immunity against viral exposure after disappearance of HBsAg and developing anti HBc.

The polymerase chain reaction results for HBV DNA were negative in all random samples except one (Table 3). The positive specimen was HBsAg negative and anti-HBc positive. When the anti HBc negative sera were analyzed for HBV DNA as a control, all specimens (n = 22) were negative.

4. Discussion

Screening of blood donors for HBsAg has greatly reduced the risk of post-transfusion HBV infection. Residual risk for post transfusion (PT) HBV infection due to seroconverting donors, or due to the existence of individuals with immuno-silent HBV infections who have detectable HBV DNA sequences in serum and /or liver still remains as a possibility [3].

The inclusion of anti-HBc as a method of screening of blood donors is not yet settled. Some considered that the use of this additional marker of HBV infection remained useful on two accounts: to eliminate infectious blood units carrying anti-HBc as the
only serologic marker of HBV infection [23], and to serve as an indicator of life style for high risk populations who may carry known or unknown transmissible viruses [4]. However, it has been shown that PT HBV infections originating from blood donors without any HBsAg markers are relatively frequent [18,21]. Such a situation can be related either to donations collected during the serologically silent window-period or to a variant of HBV that induces an antibody response not detected by anti-HBc assays [10], or to exceptional individuals who do not respond to HBV antigens. In none of these situations will anti-HBC screening be efficient in the prevention of transfusion transmission of HBV. HBV DNA screening in individual donations should be able to prevent most window period-related infections and infection by variants, provided that the amplification primers are chosen in a sufficiently conserved region of the genome [2].

It was previously shown that the frequency of HBV DNA in samples with isolated anti-HBc from low risk groups such as blood donors were either negative [17, 25] or with a percentage of positivity ranging between 7% and 30% [11,18].

In our study, HBV DNA was detected in 1.2% of the samples tested for HBV DNA from HBsAg negative/anti HBc positive blood donors. Our results are in agreement with another study which showed that HBV DNA was positive in 3.5% of HBsAg negative blood samples of apparently healthy individuals [5]. Similarly, some recent studies revealed that HBV wasn’t cleared even after the disappearance of HBsAg and that HBV DNA could be detected in a substantial proportion of healthy donors who are anti-HBc positive [16, 28].

HBV apparent clearance as demonstrated by the positive HBV DNA in HBsAg negative specimens may be attributed to the presence of HBsAg mutant forms that were undetected by the available screening assays [13]. The clearance also may be due to the existence of hepatocytes containing non-secreting viral surface proteins (due to S promotor mutants) within the dilated vesicles encountered in chronic hepatitis B [27].

In China a high incidence of HBV DNA was found among blood donors though they were screened for HBsAg [26]. Furthermore, HBV DNA was detected in HBsAg negative/anti-HBc positive blood showing statistical significant relationship between HBV DNA and titer of anti-HBc as HBV DNA was only detected in blood with high titers of anti-HBc [22]. Other studies also showed that in HBsAg negative population the latter becomes immunocomplexed and escapes detection by standard tests. Such population was shown to be positive for HBV DNA by PCR [12]. It was therefore recommended to exclude from transfusion blood from those donors [28].

On the other hand, HBV DNA was not detected at all in anti-HBc positive blood donors in Northwestern Greece [29]. In the study of Zervou and his colleagues, anti-HBc reactivity was found in 282 donors from a total of 6696 donors (4.2%); none tested HBV DNA positive. In addition they denied the occurrence of any case of transfusion associated HBV infection after the transfusion of isolated anti-HBc positive units [29]. In another study no donations with isolated anti-HBc were HBV DNA confirmed positive [2].

Thus, it is clear that the data available about HBV DNA screening of isolated anti-HBc positive samples is still controversial. It remains that each area determines the risk of PT hepatitis following the transfusion of seronegative blood according to its own results. As zero risk remains a strong objective in blood transfusion, the use of Nucleic Acid Testing (NAT) for screening blood units is of potential value. We recommend NAT screening of anti-HBc positive blood in our region as the presence of HBV DNA has been detected in 1.2% of isolated anti-HBc blood donors. NAT is expected to reduce the pre-antibody seroconversion window period as it was reported for HCV and HIV infected donations [7].

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