RESEARCH ARTICLE

Frequency of true hepatitis B virus positivity in blood donors positive for hepatitis B core antibodies: implications for transfusion practice

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Abstract

Objective: To determine the true positivity of hepatitis b virus in anti-hepatitis B core antigen positive donors, keeping polymerase chain reaction as the gold standard.

Methods: The cross-sectional study was carried out at the Shifa International Hospital, Islamabad, Pakistan, from June 1, 2014, to December 30, 2016, and comprised all blood donors who were positive for hepatitis B core antibody and negative for hepatitis B surface antigenon the basis of by enzyme-linked immunosorbent assay. The smaples were subjected to real-time polymerase chain reaction for hepatitis B deoxyribonucleic acid detection. Data was analysed using SPSS 20.

Results: All the 100 samples came from males subjects who had a mean age of 36.8±10.8 years. Only 2(2%) donors showed hepatitis B virus deoxyribonucleic acid reactivity.

Conclusion: The inclusion of Hepatitis B core antibody screening may make blood transfusions safer for the patients.

Keywords: Occult hepatitis B Infection, Hepatitis B core antibody, Nucleic acid amplification testing.

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Introduction

Hepatitis B virus (HBV) infection is a major global health problem, especially in Asia.[1] Approximately 400 million people worldwide are chronically infected with HBV.[2] Pakistan is a highly endemic area for HBV with increased seroprevalence in the general population. According to a recent World Health Organisation (WHO) survey, approximately 9 million people in Pakistan are infected with this virus.[3]

Parenteral exposure and blood transfusions have been shown to be major sources of HBV infection. Pre-transfusion blood tests have been recommended by the WHO in order to check the frequency of transfusion-transmitted infections (TTI). Initially, only hepatitis B surface antigen (HBsAg) test was introduced for donor screening, but after 1986, the WHO recommended antibody testing to hepatitis B core (HBc) antigen as well to minimise TTIs during the window period when HBsAg is not detectable.[4]

HBV infection is indicated by the presence of HBsAg in serum or plasma, but it may exist in humans without detectable HBsAg, with the presence of HBV deoxyribonucleic acid (DNA) in the serum and/or in the liver, i.e. the occult HBV infection (OBI).[5] Therefore, the absence of HBsAg is not sufficient to ensure the lack of circulating virus. Low-level HBV viremia can be picked if a routine screening of the sera for HBc is done. Blood donors who are HBsAg-negative and anti-HBc-positive can transmit the infection. As HBsAg negativity alone cannot be the criteria to rule out HBV infectivity and transmissibility, testing for anti-HBc can be added to blood donors screening in order to minimise infections during the window period and to pick occult infections in blood donors. Recent data from southeast Asia with regard to true HBV positivity in HBsAg-negative and HBc-positive blood donors is quite varied ranging from 0.2% to 17.2% in good sample-sized studies.[2,6]

In India where the HBV prevalence is 4% in general population, a prospective study was conducted in Chenai, India in 2009, where a total of 9100 donors were screened for the HBsAg and anti-HBc immunoglobulin (Ig) M and IgG) by enzyme-linked immunosorbent assay (ELISA). Anti-HBc-positive samples were subjected to real-time polymerase chain reaction (PCR) for the Hepatitis B DNA detection. Out of 10% donors, who were positive for HBc and negative for the HBsAg, 0.2% showed the presence of viral DNA and true positivity. The study highlighted the importance of correct identification of viral infection by routine screening through HBsAg and HBc antibody.[7]

Another study carried out in Egyptian blood donors to detect OBI showed that 10.45% were anti-HBc-positive and anti-HBsAg-negative. HBV DNA was quantified on 303 blood donors and 52(17.2%) were true positive for HBV by DNA testing.[2]

Most setups in Pakistan rely only on HBsAg for screening of blood, however taking into account the disease burden and morbidity and mortality associated with transmission of this deadly virus, more research in this regard is needed to minimise viral transmission during the window period

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and to consider the inclusion of anti-HBc serological testing and/or DNA testing by PCR. Nucleic acid amplification testing (NAT) is the gold standard, but is extremely expensive for most Pakistani blood banks. The current study was planned to determine the true positivity of HBV in anti-HBc-positive donors, keeping PCR as the gold standard.

Materials and Methods

The prospective cross-sectional study was conducted from June 1, 2014, to December 30, 2016, at the Blood Transfusion Department of Shifa International Hospital, Islamabad, Pakistan. After approval from the institutional ethics committee, the smaple size was calculated using WHO calculator while keeping confidence interval (CI) at 95%, prevalence at 33.3%, and absolute precision at 7.5%.[8]

All blood donors who were anti-HBc-positive and HBsAg-negative were included. After taking informed consent from the subjects, serological marker testing was done prior to inclusion in the study. Confidentiality of the donor was maintained at every step. All donor samples were screened for HBsAg and anti-HBc IgM and IgG using ELISA (Abbott architect anti-HBc II reagent). The samples that were positive for anti-HBc and negative for HBsAg were subjected to real-time PCR for the HBV DNA detection (Abbott real-time HBV amplification reagent kit). Additional serological markers hepatitis B surface (HBs) and hepatitis Be (HBe) antigens on some blood donors to define the true status of positive hepatitis B core antibody.

Data was analysed using SPSS 20. Frequencies and percentages were calculated for qualitative variables, whereas mean and standard deviation were calculated for quantitative variables.

Results

All the 100 samples came from males subjects who had a mean age of 36.8±10.8 years (range: 19-51 years).

Overall, 34(34%) subjects were positive for HBV vaccination, while 66(66%) were negative (Figure 1).

Overall, positivity of HBV DNA was found in 2(2%) donors who tested positive for anti-HBc (Figure 2). The HBV DNA levels in the first and second case were 10IU/mL and <10IU/mL respectively. Both of these donors were not vaccinated. HBs antibody and HBe antibody were negative in both donors in whom HBV DNA was detected, suggesting OBI. Anti-HBs and andti-HBe testing was done in 60(60%) samples.

Anti-HBs was highly reactive in 10(16.6%) donors, while 34(56.6%) were only reactive for anti-HBs.

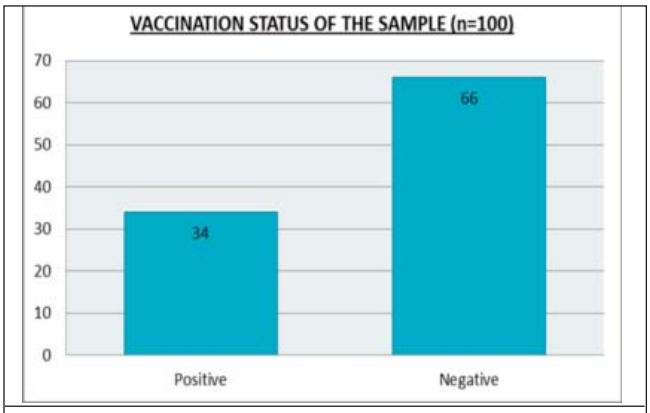

Figure-1: Hepatitis B vaccination status in study population.

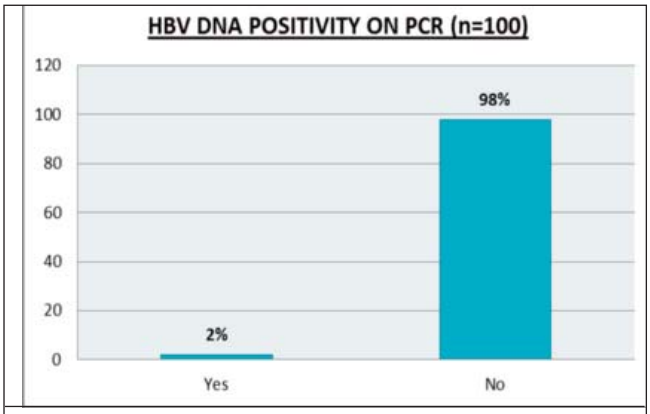

Figure-2: True hepatitis B virus (HBV) positivity by polymerase chain reaction (PCR) in blood donors tested positive for Hepatitis B core antibody..

HBe antibody was reactive in 32(53.3%)donors, while 28(46.6%) were non-reactive.

Both HBs and HBe were positive in 20(33.3%) donors, and 12(20%) were non-reactive for both HBs and HBe antibodies.

Discussion

Safe blood donation depends on proper selection of donors with sensitive screening tests so that exclusion of TTIs can be done. If the donor, while giving blood, is in the window period, then there is a high risk of post-transfusion hepatitis. Following the infection with hepatitis B, the first marker that appears in blood is HBV DNA followed by HBs and HBe antigens. Subsequently, anti-HBc and andti-HBe can be detected.[9]

Candotti et al. carried out a study on transfusion-transmitted HBV infection, and showed that OBIs carrying detectable anti-HBs (approximately 50%) were essentially not infectious by transfusion. It stated that in cases where anti-HBs levels were >100IU/ml, transfusion can be performed. However, in areas where hepatitis B has low prevalence, all HBc-positive blood units are usually

rejected because it is more economical than introducing anti-HBs testing in blood donors.[10]

This strategy cannot be applied in high prevalence areas where deferring anti-HBc reactive units affect negatively the blood supply too severely and at a non-affordable cost. So anti-HBc testing should be followed by anti-HBs testing or ideally more sensitive testing of HBV DNA.

Khalid A et al. described the frequency and significance of isolated HBc antibody, stating that there were some vaccinated patients who were chronically infected with hepatitis B and they usually did not respond well to the vaccines. These carriers are called "cryptic HBV carriers". In such cases, detection of HBV DNA is positive in most cases.[11]

Hu KQ et al. described that OBI in vaccinated persons can mask HBsAg because of HBsAg-anti-HBs immune complexes.[12]

Dwyre et al. described that there was a significant decrease in HBV prevalence during the past few years probably due to the application of universal hepatitis B vaccination as a result of increased awareness and routine screening in young population.[13]

The detection of OBI also depends on the sensitivity and specificity of the assay used. For HBV DNA, the lower limit of detection should be <10 IU/mL.[2]

Launay et al. also emphasised the importance of anti-HBc antibody by establishing very high viral loads in patients with HBsAg escape mutations. It showed that out of 362 core-positive blood donors, 11 were false positive (FP) since their HBV DNA was not detected. Though 10 donors were positive for HBV DNA, when their HBsAg was repeated, it turned out to be positive in only 2 cases. However, 7 cases revealed mutations in the HBsAg immunodominant region. The study concluded that OBI due to escape mutation in HBsAg should also be kept in mind.[14]

Liu et al. reported a frequency of 0.13% (5/2972) of OBI in Chinese blood donors. Their routine screening by HBsAg was negative. When their nested PCR was performed, there was a mutation in "S" determinant region of DNA.[5]

Kuhns et al. compared NAT with immunoassay methods, and reported that although the former is a very sensitive test for screening donors in the window period, anti-HBc antibody is still an important serological test and it should be included in routine screening. Donors who are anti-HBc-positive may have a very low level of HBV DNA and these are often undetectable by NAT. Further, HBsAg screening is also mandatory in centres where combined anti-HBc and NAT are being done. Donors with chronic infection may have positive HBsAg and they can also contain a small amount of HBV DNA in their blood. However, every blood bank should establish its screening protocol depending upon its cost effectiveness, the prevalence of infection and sensitivity of the different assays used.[15]

Rekha et al. described the benefits and limitations of NAT, showing that although NAT is a very sensitive test and it has reduced the window period of HBV to 10 days only, its operation requires high technical skills and every blood bank is not eligible to start this sophisticated technique. Other obstacles in its implementation are high costs, organisation facility and equipment. Hence, to establish NAT, basic standards of blood transfusion service should be in place.[16]

The results of our study validate that the isolated presence of HBc antibody is a well-established phenomenon and is a relatively common occurrence. Isolated presence of anti-HBc antibody in donors, who fail to develop anti-HBs antibody after the standard dose of vaccination for HBV, warrants consideration for diagnosing chronic infection.

OBI is a threat to blood safety. Employing genomic screening through NAT can lead to considerable safe blood transfusions, but despite introducing the individual NAT test, transfusion centres cannot bypass serological tests. No screening test alone can guarantee complete and absolute blood safety. Non-specific nature of Anti-HBc antibody has been one of the main culprits of the increase in blood wastage.

Implementation of NAT screening should be analysed cautiously and a decision of incorporating anti-HBc antibody and HBV NAT in donor screening should be based on large, comprehensive and multicentre studies as the perception of the actual prevalence of OBI in the general population and blood donors is of crucial value for decision-making of screening protocols. The introduction of HBsAg, anti-HBc and HBV DNA by NAT will ensure safer transfusion and it will help to detect viraemia during acute infection, occult infection and infection due to escape mutants due to which there will be negligible chance of introducing infection through transfusion.[17] Not only this can increase blood safety but also decrease the wastage of blood because of anti-HBc reactive status.

Conclusion

The inclusion of HBc antibody screening may make blood transfusions safer for the patients. Depending upon the expenses, the inclusion of HBc antibody screening should be done in all blood banks that cannot afford NAT screening.

Disclaimer: The text is based on a thesis for Fellowship of the College of Physicians and Surgeons, Pakistan.

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