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Significance of measuring Anti-HBc assays in detecting occult Hepatitis B infection in donor samples - An Indian study.

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ABSTRACT

Introduction:
It has been observed that Hepatitis B transmission can occur from HBsAg negative blood donors despite adopting highly sensitive screening measures. Thus, the term occult hepatitis B virus infection was introduced.

Material and Methods:
6000 healthy donor samples were screened for Hepatitis B infection using Hepatitis B markers viz., HBsAg, Total anti-HBc. The percentage of Occult HBV infection with IgM anti-HBc and/or total anti-HBc antibody alone was calculated. Samples which were total anti-HBc reactive and anti-HBs negative (< 12 mIU/mL) were considered Isolated anti-HBc.

Result and Discussion:
A total 500 samples were tested positive for total Anti-HBc (8.3%) while out of these 39 samples were also HBsAg positive. The prevalence of HBsAg in this study is 0.65%. Among 461 samples which were anti-HBc positive were subjected anti-HBs. 80 samples were anti-HBs negative. The prevalance of "Isolated anti- HBc" in healthy donors in this study was 1.33% and prevalence of Occult HBV infection was (19 out of 6000) i.e. 0.316 %.

Conclusion:
Anti-HBc and nucleic acid testing in transfusion settings can be minimized by the use of very sensitive HBsAg screening assays to reduce the false positive OBI.

KEYWORDS :

Introduction:
Hepatitis B virus (HBV) infection is a grave global health predicament and is thought to be a significant cause of cirrhosis, chronic hepatitis and hepatocellular carcinoma (HCC). Serological and viral markers are generally used to diagnose HBV infection. Hepatitis B surface antigen (HBsAg) is the trademark of HBV and is regularly used for demonstrating HBV in blood donors. However, it was observed that HBV transmission can still occur from HBsAg negative blood donors despite above screening measures, accounting for approximately 1 in every 63,000 transfused units [1]. Thereafter, the term occult hepatitis B virus infection (OBI) was introduced [2]. OBI was described for the first time in late 1970s and since then there has been a growing curiosity about this topic. The prevalence of OBI depends upon endemicity of HBV infection, characteristics of the cohort under study and most importantly the sensitivity and specificity of various assays employed for detection. OBI is defined as condition when HBsAg is undetectable in serum, in spite of the presence of HBV DNA in liver or blood [3]. These patients have been additionally sub classified as "seropositive" or "seronegative" HBV depending upon positivity or negativity for other HBV markers, typically hepatitis B core antibody (anti-HBc). Demonstration of HbsAg with or without total anti-HBc specifies constant replication of viral antigens, the existence of anti-HBs and anti-HBc together is thought to be a marker of recent HBV replication. Recent efforts emphasize on additional classification of this pattern, using supplementary test methods viz. anti-HBe and IgM anti-HBc. IgM antibody to hepatitis B core antigen (IgM anti-HBc) is a dependable marker for acute phase of disease in HBV infection. During the progression of infection, when antigenemia (HBsAg) has resolved and anti HBsAg antibody (anti-HBs) is yet to develop (window period), IgM anti-HBc antibody may be the lone marker during this period.

Historically serologic testing for HBsAg and anti-HBc has been the foundation of blood screening, whereas nucleic acid testing (NAT) for HBV has been newly developed for detection of HBsAg-negative blood units donated through earliest phase of an acute infection. While such donors are typically anti-HBc reactive, they would be interdicted by anti-HBc screening, appearing mainly as IgM anti-HBc around 6 to 8 weeks post infection (Fig 1). Anti-HBc classically perseveres for life, but after approximately 6 months the total anti-HBc mostly comprises of IgG type anti-HBc. The demonstration of anti-HBc in the absence of HBsAg as well as anti-HBs is known as isolated anti-HBc [4]. Isolated anti-HBc substantiates three different states of HBV infection, apart from false positivity. It specifies that firstly IgM anti-HBc may be the lone marker present through the "window period", secondly, resolved hepatitis B infection with fading titers of anti-HBs and finally, patient has HBV that is actively replicating (at levels lesser than 10,000 IU/ml), but without the production of demonstrable HBsAg. When HBV serology is requested on a routine basis, majority of the laboratories report out the total levels of anti-HBc, which can comprise of a blend of IgM and IgG antibodies. Accordingly, specific request should be made for measurement of IgM anti-HBc acute HBV infection is suspected.

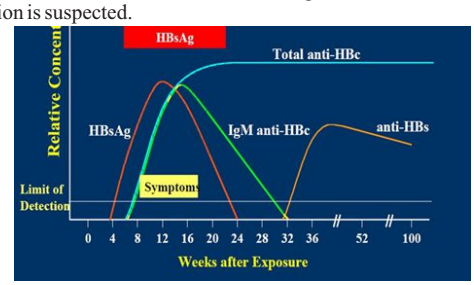

Fig 1: Hepatitis B Infection Serology

Thus, our study intended to establish the prevalence and significance of OBI and isolated anti-HBc detection in the blood donors. This can help to establish whether anti-HBc screening needs to be done in all healthy donors and whether it be followed by HBV DNA analysis, to find the true infective potential of this donated blood.

Material and Methods:
All donors who reported to this blood bank and fulfilled the criteria for blood donation as per Transfusion Medicine Technical Manual, Government of India and the Drugs and Cosmetics Act, 1940 and the amendments made thereafter, through the study period, were included in the study population. The donors who did not satisfy any of these norms were deferred from blood donation and there after left out from

the study. All donors were subjected to thorough history taking and clinical examination by trained staff to screen donors before blood donation. Blood donation was carried out after obtaining consent for blood donation as well as infectious maker testing. Infectious marker testing samples were collected after the blood donation from the diversion pouch of the primary blood collection bag. The serum samples collected from the healthy voluntary donors were screened for the Infectious Disease markers using highly sensitive Enhanced Chemiluminescence Technique (VITROS 3600 Immunodiagnostics system).

The protocol followed for the study is as follows:

1. 6000 healthy donor samples were screened for HBV infection using HBV markers viz., HBsAg, total anti-HBc Ab using Enhanced Chemiluminescence Technique (ECI).
2. HBsAg negative and total anti-HBc reactive sample was subjected to quantification of anti-HBs Antibody using Enhanced Chemiluminescence Technique.
3. anti-HBs positive samples (>12 mIU/mL) were considered as donors with resolved HBV infection while samples with anti-HBs antibody negative (< 12 mIU/mL) were considered as having an ongoing infection donand subjected to HBV DNA Quantitative PCR.
4. Samples positive for HBV DNA were considered as Occult HBV cases. The percentage of Occult HBV infection with anti-HBc IgM antibody and/or anti-HBc total antibody alone was calculated.
5. Samples which were total anti-HBc antibody reactive and anti-HBs antibody negative (< 12 mIU/mL) were considered Isolated anti-HBc detection. Isolated anti-HBc prevalence was then calculated.

The algorithm that was followed in this study is represented in Fig 2.

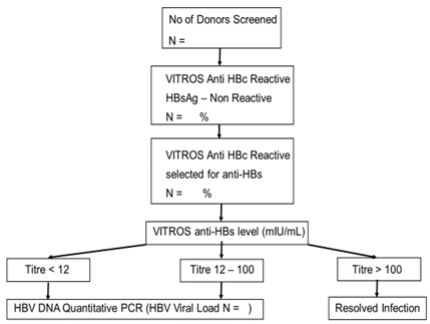

Fig 2: Study Algorithm

Results:

This study was conducted at a premier transfusion center between Jan 2015 and Dec 2015. Total of 6000 samples from healthy donors were screened for infectious markers, including total anti-HBc as an additional marker apart from the routine infectious disease markers. HBV infection using HBV markers viz., HBsAg and total anti HBc using highly sensitive Enhanced Chemiluminescence Technique (ECI). The median age of the donors included in the study was 32 (range 18-65) years with majority the donors being males (89%). 500 samples out of 6000 tested positive for total anti-HBc (8.3%), while out of these 39 samples also tested positive for HBsAg. The prevalence of HBsAg in our study is 0.65%. These HBsAg positive samples were left out from further study. Therefore, there were 461 donors who were total anti-HBc positive and HBsAg negative making the prevalence of total Anti-HBc positivity among all donors to be 7.68%. This group of donors with total anti-HBc positive and HBsAg negative were subsequently tested for quantitative anti-HBS Antibody and anti-HBc IgM. Of these tested samples, only 1 sample tested positive for anti-HBc IgM but HBV DNA was negative.

Amongst 461 samples which were anti-HBc positive 80 samples were anti-HBS antibody negative (Isolated anti-HBc). Thus, the prevalence of "Isolated anti-HBc" in healthy donors in our study was 1.33%. These were further subjected to HBV DNA Quantitative PCR. Out of these, there were 19 samples which were HBV DNA positive and were considered as OBI infection. Hence, the prevalence of Occult HBV infection (19 out of 6000) in our study was 0.316 %.

Discussion:

Our Blood Bank collects approximately 25,000 units of blood from voluntary as well as replacement blood donors each year. To augment the safety of blood for transfusion, donor blood units were screened by ECI for the five mandatory diseases as mandated by the regulatory authorities. Our study was under taken to detect occult hepatitis B infections (OBI) in healthy voluntary blood donors as it has been considered a probable risk to the safety of blood to be transfused to a patient. Though, the exact reason is yet not known, certain theories for the perseverance of HBV DNA in HBsAg negative sample have been proposed viz. the existence of HBV DNA in low copy numbers, S gene variation and the occurrence of immune complexes in which the HBsAg antigen may be hidden [5]. The detection of OBI is dependent on the sensitivity of assays of either or both these markers for HBV [6]. The finding of specific virus nucleic acid not necessarily always translates into infectivity [7]. In OBI, typically anti-HbC is the solitary HBV serological marker when the level of HBV DNA in the serum is generally very low (< 200 IU/ml) [8]. OBI is of significance in numerous clinical conditions. It has been shown to be transmitted via transplantation, hemodialysis or transfusion. Thorough screening of blood donors, prophylaxis for recipients of organ transplantation and infection-control programs specific for dialysis should be considered to minimize the hazard of transmission [9]. Reactivation of OBI is known to cause acute hepatitis in patients whose immunity is compromised. Closely monitoring the HBV DNA and judicious antiviral treatment can help to prevent HBV reactivation and subsequent clinical decline. It is also known to be a risk factor for hepatocellular carcinoma due to the direct proto-oncogenic outcome and by indirect causation of obstinate hepatic fibrosis and inflammation.

In our study, 6000 healthy voluntary donor blood samples were screened for HBV using HBsAg and total anti-HBc on VITROS 3600 Immunodiagnostics system, apart from the other routine infectious disease screening. There were 461 donors who were total anti-HBc positive and HBsAg negative making the prevalence of total anti-HBc positivity among all donors to be 7.68%. This was relatively low in contrast to the study by Makroo et al in which the core positivity rate was 10.22% [10]. Thus, if no further testing of these blood units is done 7.68% of the blood units collected will have to be discarded. Thus, testing for anti-HBC results in donor attrition, thereby leading to a decline in the effective donor pool. As there can be various reasons for the positivity of the anti-HBc Antibody as discussed further, the blood may not always be infective. So further testing with anti-HBS antibody and HBV DNA Quantitative PCR assay, can be carried out to find the prevalence of isolated anti-HBC. Isolated anti-HBC prevalence in our study was found to be 1.33% as compared to the study by Khalid et al which showed its prevalence to be 2% [11]. Demonstration of isolated anti-HBC is possible in three conditions: throughout the window period of acute hepatitis B, it is mainly IgM class; several years post recovery when anti-HBS levels are almost undetectable and lastly, after years of chronic HBV infection when the HBsAg titer has declined below the detectable cutoff level. As noted above, absence of demonstrable HBsAg occurs in roughly 0.5 percent of patients with chronic hepatitis B per year [12]. The clinical implication of isolated anti-HBC is still not clear. Though, HBV DNA has been demonstrated in the serum of individuals with isolated anti-HBC when tested by PCR assays, the frequency of detection varies from 0 to 20 percent [13]. In our study, HBV DNA was detected in 0.036% of the samples. HBN DNA can be demonstrated in the liver of majority of (more than 70 percent) people with isolated anti-HBC [14]. In this study, those samples which were found to be reactive in VITROS anti-HBC antibody assay were further subjected to VITROS anti-HBS assay for quantification of the anti-HBS levels. Samples which were anti-HBS positive (>12 mIU/mL) were considered as donors with resolved HBV infection, whereas samples with anti-HBS antibody negative (<12 mIU/mL) were considered as having ongoing infection ('Isolated anti-HBC') and were further subjected to HBV DNA Quantitative PCR.

The prevalence of OBI in our study was 0.316 %. This figure is relatively higher than 8.55 per 1 million donations according to a 2008 international survey. In a study by Makroo et al on Indian donors, only 0.15% samples were HBV DNA positive. This can be attributed to the fact that India has intermediate to high hepatitis B endemicity [14]. Clinical outcome of occult HBV transmission principally depends on the immune status of the recipient and the copies of HBV DNA present in blood products.

Previous reports have a documented prevalence of occult HBV of 7.5 to 30% in India [15,16]. In contrast, in our study, we found a prevalence of 0.316% occult HBV in anti-HBC seropositive healthy blood donors. Low prevalence of occult HBV infection may be attributed to varying sensitivity of HBsAg assays used for donor screening. Donors may well be HBsAg positive when screened by more sensitive HBsAg assays, hence eliminating them from the category of donors with so-called occult infection.

However, in a context of limited resources, anti-HBC can be an affordable marker for diagnosing OBI infection. However, occult infection in anti-HBC seronegative individuals has been reported. Hence, there is an enduring risk of HBV transmission in transfusion settings that employ anti-HBC alone as a supplementary indicator for occult HBV screening.

CONCLUSION:

To conclude, the presence of HBV DNA in anti-HBC seropositive blood donors was 0.316% in our setting. The different rates of occult HBV infection reported across the country may be due to the varying sensitivity of HBsAg assays used for donor screening. The question that needs to be answered is whether testing for HBV DNA as a screening test in resource limited country like ours, is a viable option. Further the implementation of anti-HBC screening in donor populations is limited, as excluding isolated anti-HBC blood donations will result in higher discard rates of blood units, especially in higher endemicity regions. The requirement of anti-HBC and nucleic acid testing in transfusion settings can be minimized by the use of highly sensitive HBsAg screening assays to reduce the false positive OBI. Larger, multicentre studies are required to understand the actual burden of occult HBV infection in transfusion settings.

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