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RESEARCH ARTICLE

HEPATITIS B SCREENING ACCURACY IN BLOOD BANK

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ABSTRACT

Screening measures help to maximize the safety of blood transfusion. Though there are scientific and technical sophistication in the safety of blood donation, mainly hepatitis B virus (HBV) still remain the most transmitted infectious pathogenic agents. The blood bank testing accuracy cross checked by retesting of donors samples collected during blood donation camp, Raigadh. Total 382 samples were retested by ELISA at National Institute of Immunohaematology, ICMR, Mumbai. There was False Positive 5 (1.30%) and False Negative 5 (1.30%). The anti-HBc ELISA also done for same samples which was 12.82% positive. False negative percentage directly indicates the residual transfusion of HBV 1.30%, whereas the false positive rate (1.83%) indicates the pointless wastage of blood units. External Quality Assurance Program has to be implemented mandatorily to overcome the problem of false reporting, along with intense training of blood bank personals.

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INTRODUCTION

Blood is essential for transporting oxygen, nutrients, and other substances to tissues throughout the body. Donated blood can be lifesaving for individuals who have lost blood because of accidents or surgery, as well as for people who have become severely anaemic or have dangerously low platelet counts because of certain medical conditions and/or treatments. Screening measures help to maximize safety of blood donation for the donor and the recipient. But still Transfusion transmitted infections (TTIs) remain major public health problem encountered by the health delivery systems in many developing countries mainly due to under resourced facilities and lack of requisite staff ¹[Aach RD *et al*, 1983]. Though there are scientific and technical sophistication in the safety of blood donation, viruses and bacteria mainly hepatitis B virus (HBV) still remain the most transmitted infectious pathogenic agents ²[El-Sherif AM *et al*, 2007].

Blood donors like anyone else, could occasionally carry an infectious agent, sometimes for a long period without having any clinical signs or symptoms ³[McClelland DBL 1996.] thus possessing threat to future recipients through the blood products from such donors. Several screening tests/assays have been developed over the years to overcome this threat; Some of these assay techniques used in screening blood/blood products prior to transfusion include immunochromatographic assay,

enzyme-linked immunosorbent assay (ELISA) and nucleic acid test (NAT) or polymerase chain reaction (PCR) assay techniques. The effectiveness of all these tests in interdicting contaminated units of blood/blood products depends in part on the point in time when an infected donor provides the unit relative to the individual's exposure to the virus ⁴[Gallarda JL *et al*], same time it is important to give adequate training to laboratory staff on their ability to follow standardised procedures in applying all these testing technologies as well as adhering to good quality control measures so as to prevent testing/procedural errors ^{5, 6}[Basavaraju SV *et al* 2010; Busch MP *et al*, 2000]

This may leads to critical issue as the outcome of the test if not properly performed can result in serious consequences for either the blood service or the blood donor. False positive result can lead to a larger number of blood donors being deferred, while a false negative testing may jeopardize blood safety ⁷[Rahman M *et al*, 2008]. The main cause behind it is untrained laboratory personnel may also produce a false screening result. Transfusion of an such infected blood to an individual is a crime ^{8,9}[Torane VP *et al*, 2008; Elliot Eli Dogbel *et al* 2015] HBsAg can be detected in the serum from several weeks before onset of symptoms to months after onset. HBsAg is present in serum during acute infections and persists in chronic infections. The presence of HBsAg indicates that the person is potentially infectious. Very early in the incubation period, pre-S1 and pre-S2 antigens are present. They are never detected in

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the absence of HBsAg. Hepatitis B virions, HBV DNA, DNA polymerase, and HBeAg are then also detected. The presence of HBeAg is associated with relatively high infectivity and severity of disease. Anti-HBc is the first antibody to appear. Demonstration of anti-HBc in serum indicates HBV infection, current or past. IgM anti-HBc is present in high titre during acute infection and usually disappears within 6 months, although it can persist in some cases of chronic hepatitis. This test may therefore reliably diagnose acute HBV infection. IgG anti-HBc generally remains detectable for a lifetime.

Anti-HBe appears after anti-HBc and its presence correlates to a decreased infectivity. Anti-HBe replaces HBeAg in the resolution of the disease. Anti-HBs replaces HBsAg as the acute HBV infection is resolving. Anti-HBs generally persists for a lifetime in over 80% of patients and indicates immunity. Acute hepatitis patients who maintain a constant serum HBsAg concentration, or whose serum HBeAg persists 8 to 10 weeks after symptoms have resolved, are likely to become carriers and at risk of developing chronic liver disease. A complication in the diagnosis of hepatitis B is the rare identification of cases in which viral mutations change the antigens so they are not detectable¹⁰. (World Health Organization) This study tries to evaluate the screening practices of the blood bank in Raigadh District of Maharashtra. It shows the significant requirement to train the blood banks personnel.

Methodology

The total 382 blood donor serum samples were collected during blood donation camps conducted by blood bank. The camps were conducted at Raigad district of Maharashtra. As per FDA regulation every unit has to be tested for five major Transfusion Transmitted Infections (TTIs) which are HIV 1&2, Hepatitis B, Hepatitis C, Malaria and Syphilis. The testing of three major viruses HIV1&2, HBsAg and HCV done by Enzyme Linked Immunosorbant Assay i.e ELISA in respective blood bank as per manufacturer instruction and protocol. The same samples were retested using HBsAg ELISA and Anti-HBc ELISA at National Institute of Immunohaematology Institute (NIIH), ICMR, Mumbai to crosscheck the blood bank testing accuracy by ELISA method.

To find out the probability of HBV carrier donor Anti-HBc testing were carried out. All samples were retested by same HBsAg and Anti-HBc ELISA. These results were compared with the results which were tested by blood bank under regular screening testing.

RESULT

Hbsag elisa

Total 382 donor serum sample were tested for HBsAg ELISA. From these 382 samples blood bank reported 9 samples as HBsAg positive but on retesting at NIIH, Mumbai it found that only 4 were found true positive and 5 samples were false positive. Blood bank reported 373 samples as negative samples

but actually it found that 368 samples were true negative and 5 samples were false negative.

Table 1

Total No of Samples(382)	HBsAg ELISA reported by Blood Bank	On Retesting for HBsAg ELISA of same samples by NIIH, Mumbai	Anti-HBc ELISA of same samples
Positive	9	True positive 4(1.05%) False Positive 5 (1.30%)	49 (12.82%)
Negative	374	True Negative 368 (96.34%) False Negative 5 (1.30%)	333(87.18%)

Anti-HBc ELISA

Same 382 samples were tested for anti-HBc ELISA . Out of total 382 samples 49 (12.82%) samples were positive for anti-HBc ELISA. From these 49 positive samples 2 samples were under Grey Zone and 4 samples were also reported positive by Blood Bank by HBsAg ELISA. There were about 4(1.2%) confirm positive samples which were positive for both HBsAg ELISA as well as anti-HBc ELISA, also reported positive by blood bank. Actual 42(10.99 %) samples were only positive for anti-HBc ELISA.

CONCLUSION

The false positive and false negative rate of HBsAg ELISA, reported by blood bank, is very high which was respectively 1.30% each. False negative percentage directly indicates the residual transfusion of HBV 1.30%, whereas the false positive rate (1.83%) indicates the pointless wastage of blood units. If we estimate the sensitivity and specificity of the test performed by the blood bank was 97.7%.¹¹[Ana-Maria Šimundić: Research Gate 2015.] External Quality Assurance Program has to be implemented mandatorily to overcome the problem of false reporting, along with intense training of blood bank personals.

The 49 samples tested positive for anti-HBc ELISA out of which 2 donors were diagnosed under Grey Zone, might be intermediate samples. These donors may be negative for hepatitis B or might be due to nonspecific serological cross reactions. These donors might have history of Hepatitis infection which might or might not resolve. In conclusion, The blood donors who are chronically infected with HBV but without detectable levels of the Hepatitis B surface Antigen (HBsAg) and with antibodies of the Hepatitis B core Antigen (Anti-HBc) contribute to the residual risk of the transfusion transmitted HBV infection.

DISCUSSION

Anti-HBc antibodies are markers of acute, chronic, or resolved HBV infection and remain detectable for life. These can be present in the absence of both HBsAg and anti-HBs antibodies, during the convalescent period following acute hepatitis B before the appearance of anti-HBs antibodies, or in patients who have resolved infections but lost detectable anti-HBs antibodies. Anti-HBc antibodies are, therefore, detected in anyone who has been infected with HBV. A donor with a positive anti HBc-IgG indicates either a past infection or a carrier state. The anti-HBc IgG may remain positive for life in

an affected individual, although the individual has protective levels of anti-HBs and therefore, this does not necessarily mean that the blood of such a donor is infectious. In some countries with a high prevalence of HBV infection, such as Japan, exclusion of all anti-HBcAg positive plasma units would result in a drastic reduction in the number of units available for transfusion. Additional testing, like determination of the level of anti-HBcAg antibody has been included to enable the transfusion of some of the anti-HBcAg-positive plasma units¹²[Lisuka H. *Et al*,1992] But the study in Egypt blood donors shows that the donors diagnosed negative for Hepatitis B surface antigen are positive for HBV DNA and same donors are positive for Anti-HBc¹³ [Silva CMD *et al*, 2005].

The screening of HBV infection in blood bank is largely depend on the sensitivity of the ELISA kit used for screening the blood units. The study was conducted by the International Consortium for Blood Safety (ICBS) to identify high-quality test kits for detection of hepatitis B virus (HBV) surface antigen (HBsAg) for the benefit of developing countries. The 70 HBsAg test kits from around the world were evaluated comparatively for their clinical sensitivity, analytical sensitivity, sensitivity to HBV genotypes and HBsAg subtypes. The range of sensitivity between the most sensitive test kit and the least sensitive HBsAg devices was 0.021 IU/ml to >2.33 IU/ml i.e This represents a >300-fold difference between the most sensitive assay and the least sensitive devices. Diagnostic efficacy of the evaluated HBsAg test kits differed substantially. Therefore, Laboratories should be aware of the analytical sensitivity for HBsAg and check for the relevant HBV variants circulating in the relevant population¹⁴[H. Scheiblauer *et al*, 2010] The significance of anti-HBc in the absence of the surface antigen is somewhat controversial. Such finding could represent 1) a situation where despite anti-HBs and anti-HBc being undetectable, the patient is immunized; 2) a case where HBsAg levels are very low to be detected with routine assays; 3) false-positivity or cross-reactivity of anti-HBc; and 4) an immunological window period, in which HBsAg is already undetectable and the surface antibody is not yet detectable¹⁵[Kleinman S *et al* 1997]. The anti-HBc typically persists for life, but after about 6 months the total anti-HBc mainly consists of IgG anti-HBc. For patients with resolved acute HBV infection, the IgM anti-HBc is not usually detectable after 6 months. With chronic HBV infection, the IgM anti-HBc can remain detectable at very low levels, even years after infection. The anti-HBc typically persists for life, but after about 6 months the total anti-HBc mainly consists of IgG anti-HBc.

Several demographic and behavioural risk factors are associated with HBV status among blood donors like shaving at barber shop, socioeconomic status, body piercing, having a tattoo, consuming alcohol, having sex with multiple partners, and paying for sex. It is very important to introduce individual pre-donation questionnaires to defer risky persons from blood donation. However, it also emphasizes the need for further epidemiologic research and training in the domain of blood safety in India¹⁶ [Latha Jagannathan *et al*, 2010] Anti-HBc has been found to be an excellent indicator of occult HBV infection during the window period^{17,18,19,20} [Margaret Oluwatoyin Japhet *et al* 2011; Hoofnagle JH *et al*, 1978; Douglas DD *et al*, 1993;

Muhlbacher A *et al* 2001] Potential Transmission of HBV by donors with anti-HBc positive is not clear and no large-scale investigations using extensive methods to detect HBV have been performed to provide a precise estimate of the infectious risk in patients with isolated anti-HBc. Detectable serum HBV DNA has been reported in some patients, even those with high titers of anti-HBs. Although nucleic acid amplification assays detect HBV DNA in up to 14% of patients with isolated anti-HBc, the detectable HBV DNA generally occurs at relatively low levels. In addition, several reports have documented HBV transmission from blood and organ donors who had isolated anti-HBc, but other relatively large studies have shown no risk of HBV transmission from donors with isolated anti-HBc to kidney allograft recipients. Nevertheless, based on available data, it appears that most persons with isolated anti-HBc have very low risk of transmitting HBV, except in settings involving potential transfer to susceptible individuals of substantial quantities of virus, such as with blood transfusion.²¹ (Discussion : Hepatitis Web Study)

Other markers for detecting occult HBV infection in an HBsAg negative blood donor such as detection of HBV DNA by polymerase chain reaction (PCR) may not be cost effective²² [Stevens CE *et al* 1984] Detection of anti-HBc has contributed significantly in reducing the incidence of post transfusion hepatitis B amongst patients^{23,24,25} (Mosley JW *et al*, 1995; Grob P *et al*, 2000; Allain JP, 2004]

The IgM class of the anti-HBc is a marker that indicates recent infection. The IgG variety of anti-HBc appears later during the infection and points to a past HBV infection. Individuals with IgG variety of anti-HBc may not be infectious as they may have sufficiently high titres of antibodies to HBsAg (anti-HBs), which are protective in nature and the affected individuals may actually be disease free. The sero-prevalence rate of anti-HBcIgM in the general population in Nigeria is not available. To the best of our knowledge, the only published data on estimation of anti-HBc in Nigeria was on the prevalence of anti-HBc total (i.e. both IgG and IgM) amongst patients with chronic liver disease and it was estimated as 90%²⁶ [Ojo OS *et al*, 1995] Anti-HBc IgG may remain positive for life in an infected individual although the individual has protective levels of anti-HBs and therefore does not necessarily mean that blood of such a donor is infectious. Consequently, anti-HBcIgM is considered to be a more specific marker for HBV infection during the window period^{27,28,29} [Neirmeijer P *et al*, 1978; Van Ditzhuijsen TJ *et al*, 1985; Sawke Nilima G *et al*; 2010]

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