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PCR-based efficacy assessment of hepatitis B core antibody and hepatitis B surface antigen screening tests in the blood donor population

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Author`s Contribution

[2,3] designed the study,
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ABSTRACT

Objectives: To assess the screening efficacy of HBsAg and HBcAb serological assays in comparison with Polymerase Chain Reaction (PCR), as the gold standard.
Methodology: This was a single centre, cross-sectional prospective study conducted from November 2018 to February 2019, at the Department of Pathology and Transfusion Medicine, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan. A total of 7,550 donations were screened for HBsAg through Chemiluminescence Microparticle Immunoassay. Out of 7,550, 186 HBsAg reactive, and 208 HBsAg non-reactive samples were selected randomly for the study. The screening tests for HBsAg and HBcAb were run in parallel with PCR as the gold standard. In the statistical analyses, the specificity, sensitivity, and positive and negative predictive values were calculated using the PCR results as the gold standard. Kappa agreement was also calculated. Ethical approval was granted by the Ethics Committee of the Shaheed Zulfiqar Ali Bhutto Medical University.
Results: A total of 394 blood samples were tested with reactivity rate of HBsAg (n= 186) 47.2% (186/394), HBcAb (n= 210) 53.2% (210/394) and PCR (n= 188) 47.7% (188/394). Kappa agreement for HBsAg and HBcAb were calculated as 0.961 and 0.886 respectively. The results showed 5.5% false positive results by HBcAb test.
Conclusion: HBcAb screening showed false-positive results. HBsAg screening found to be the best possible choice that can give credible results, considering the high cost of the molecular assays.
Keywords: HBV, Donor, HBsAg, HBcAb, Pakistan

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Introduction

Every year, more than 118.4 million blood units are collected globally with 58% in low and middle-income countries.[1] Even though blood transfusions can enhance the quality of patients' lives, it remained one of the foremost sources of the transmission of infections. As an integrated strategy for the provision of safe blood, the WHO recommends the quality-assured screening of all donated blood for five transfusion transmissible infections; namely hepatitis C virus, hepatitis B virus, human immunodeficiency virus, syphilis, and malaria.[2] Despite opting for improved eligibility criteria for blood donors and the development of more advanced screening methods, transfusion transmitted infectious agents present a serious threat to blood safety.

Hepatitis B is a leading life-threatening infection found in many developing countries including Pakistan where its prevalence is estimated to be 1.98%.[3] WHO has reported that approximately 12 million population is suffering from hepatitis B or C and each year 150,000 new cases are adding in the pool.[4]

Across the blood transfusion system in Pakistan, there is a lack of proper initial screening of donors for high-risk behaviours through blood donor interviews and physical health examination. As a result, even blood from high-risk donors is collected increasing manifold the risk of transmission of infections through transfusions. The National Blood Policy and Strategic Framework (2014-20) is the national narrative to achieve safe blood transfusion. The section "Cluster: 3, Core Business" underlines the importance of TTI screening and ensure 100% screening of TTIs on donated blood as well as ensure proper resource allocation for adequate and sustainable supply of validated screening assay and required accessories. The legislative framework enacted in the country also highlights the mandatory screening for five TTI markers including HBV in addition to HCV, HIV, syphilis, and malaria.[5]

The unscreened blood transfusion, reuse of unsterilized syringes and medical equipment, ear piercing, tattooing, are the major causes of the rapid Hepatitis B transmission.[6] Serological markers found in individuals chronically infected by HBV include Hepatitis B surface antigen (HBsAg), antibody to Hepatitis B core antigen (anti-HBc/HBcAb), Hepatitis B antigen (HBeAg), antibody to Hepatitis B antigen (anti-HBe/HBeAb), and antibody to Hepatitis B surface antigen (anti-HBs/HBsAb).[7] HBsAg is the differentiating marker of HBV infection, is considered an initial scrutiny of HBV acute infection, and is used globally to screen donated blood in the blood banks.[8] When it persists for more than six months, it is indicative of chronicity.[9]

HBsAg may not be found at the later stage of the infection in some patients due to gradual clearance in an immunocompetent individual; leading to negative results in the screening of HBV in such patients.[10] Anti-HBs are produced against HBsAg followed by its expression within 1 to 4 weeks after the beginning of symptoms. It can also indicate clinical recovery and subsequent immunity as it can neutralize HBV.[11] After successful vaccination against HBV, the anti-HBs are also produced.[12] Hepatitis B core antigen is found in HBV infection essentially as infectious virions and contains an inner “core particle” in which the genome of the virus is enclosed. The corresponding antibody to HBcAg is the anti-HBc which belongs to the class of IgM and IgG but cannot neutralize the virus. However, the presence of IgM can be used to identify HBV infection at early stages.[13]

Various assay systems with variable sensitivities and specificities are available for HBV screening in blood banks.[14] The common initial analysis for Hepatitis B screening is based on the detection of HBsAg. However, some blood banks also screen for HBcAb in addition to HBsAg. Though testing for anti-HBc may result in a significant loss of large number of donors, consequently shrinkage of the effective donor pool.[15]

The current study was performed to assess the screening efficacy of HBsAg and HBcAb serological assays compared to the gold standard - the Polymerase Chain Reaction.

Methodology

This was a single centre, cross-sectional, prospective study, performed at the Department of Pathology and Transfusion Medicine, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan from November 2018 to February 2019. During the study period, 7,550 donors donated blood in the blood bank. All donated blood was screened for transfusion-transmitted infections including the HBsAg through Chemiluminescence Microparticle Immunoassay (CMIA) (Abbott Architect i-2000 SR system). The Architect HBsAg assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum or plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. All tests were performed according to the manufacturer's instructions.

Out of 7,550, 186 HBsAg reactive, and 208 HBsAg non-reactive samples (total=394) were selected randomly for the study. HBcAb assay was performed on these 394 samples by CMIA technology using commercially available anti-HBc kits and later confirmed by Polymerase Chain Reaction (PCR). The DNA extraction protocol by Sambrook and Russell (2001) was followed.[16] PCR was performed by using forward primer (HBVF1) 5′-TCA CCA TAT TCT TGG GAA CAA GA-3′ (nt 2823–2845, universal, sense) and Reverse Primer

(HBVR1) 5′-CGA ACC ACT GAA CAA ATG GC-3′ (nt 685–704, universal, antisense). The DNA product (amplified) was run on 1.5% percent agarose gel with 150 V and analyzed with a UV transilluminator.

In the statistical analyses, the specificity, sensitivity, and positive and negative predictive values were calculated using the PCR results as the gold standard. Kappa agreement was also calculated.

Results

Out of 7,550 donations screened, 394 samples (186 HBsAg reactive and 208 HBsAg non-reactive) were randomly selected for the study. The same samples were tested for HBcAb. The results showed 210 HBcAb reactive samples while the remaining samples (n=184) were non-reactive. The majority of donors enrolled in the study were males, i.e. 354 (90%) while females were only 40 (10%). The positivity and negativity of the samples were observed according to standard serum cutoff value (s/co).

When these samples were tested by PCR, it showed the presence of HBV DNA in 188 samples (47.7%) out of 394 samples. The sensitivity of HBsAg was 98.9% while that of HBcAb was 89.5%. On the other hand, the specificity of the HBsAg was 99.03% while the specificity of the HBcAb was 90.3%. The sensitivity and specificity with 95% confidence interval of the assays are shown in Table I.

The positive predictive values (PPV) were 90.38% for the HBsAg and 89.5% for HBcAb. The negative predictive value (NPV) for the HBsAg was 99.0 % while that of 90.35% for HBcAb. The positive and negative predictive values and positive and negative likelihood ratios for both assays are shown in Tables II and III.

Kappa agreement was used to check that one assay and the other assay had a probability of agreeing. Kappa agreement for HBsAg and HBcAb was calculated by using a 2x2 table shown in Figure 1.

The calculated results of the kappa agreement for HBsAg and HBcAb were 0.961 and 0.886 respectively.

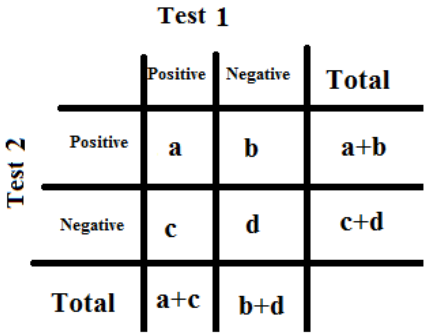

Figure 1: 2x2 table for Kappa calculation

Discussion

Pakistan has a demand-driven fragmented blood transfusion system relying predominantly on the 'family replacement donors'[17] instead of the internationally accepted 'voluntary non-remunerated regular blood

Table I: Summary of sensitivity and specificity of the HBV assays evaluation (n= 394)

Assays	Reactive	Non-Reactive	Sensitivity (%)	CI (%)	Specificity (%)	CI (%)
HBsAg	186	208	98.9%	94.2 - 99.9	99.03	96.2 - 99.9
HBcAb	210	184	89.5 %	82.0 - 94.6	90.35	83.3 - 95.0

Table II: Summary of positive and negative predictive values of HBV assays evaluation (n= 394)

Assays	Reactive	Non-Reactive	PPV (%)	CI (%)	NPV (%)	CI (%)
HBsAg	186	208	90.38	94.2 - 99.9	99.0	94.7 - 99.9
HBcAb	210	184	89.5	82.0 - 94.6	90.35	83.3 - 95.0

Table III: Positive and negative likelihood ratio (n= 394)

Parameters	Kits	95% CI		
		Estimated value	Lower limit	Upper limit
Positive Likelihood Ratio	**HBsAg**	10.29	14.63	723.77
	HBcAb	9.28	5.27	16.33
Negative Likelihood Ratio	**HBsAg**	0.01	0.00	0.07
	HBcAb	0.12	0.07	0.20

donors' which are the safest. The availability of blood is not equitable across the country. According to the data collected by the national Safe Blood Transfusion Programme, 2.7 million blood donations were collected in Pakistan in 2018 from almost 650 blood centres of varying workloads.[18] Safety of blood has particular relevance in Pakistan where hepatitis B and C infections are widespread, the dengue epidemic is a regular phenomenon and HIV/AIDS is present in the form of a 'concentrated epidemic' with the potential to spill over into the general population.

The current study assessed the screening efficacy of HBsAg and HBcAb serological assays in comparison with Polymerase Chain Reaction (PCR), as the gold standard. The findings indicated that 5.5% of donors were reactive for HBcAb while negative for HBsAg and HBV DNA. This results in a loss of potential blood donors in a country where the blood donor recruitment and retention programme is still in infancy. These are the donors who are potentially non-infectious and may be returned to the donor pool.

In many countries, the HBcAb screening strategy has been assessed through molecular biological markers (DNA testing). All such studies report wide-ranging findings dependent on the assays used and the regions' endemicity of HBV. Earlier regional studies have reported the prevalence of HBcAb as 8.4%, 18.3%, 19.8%, and 15.9%.[19-22] A study from Cameroon has reported the prevalence of HBcAb in HBsAg negative blood donors as a high as 43.24%.[23] An Iranian study on healthy blood donors found 6.55% HBsAg-negative blood samples to be positive for HBcAb antibody[24] while a study from Saudi Arabia reported 6.2% reactivity for HBcAb.[25] There is only one study from Pakistan reporting HBcAb in HBsAg and HBV DNA negative blood donors. The study reported a high percentage of 17.28% for HBcAb reactivity.[26] It is pertinent to mention that the seroprevalence of HBcAb in the general population in Pakistan is not available.

In the present study, only two samples that were positive for HBcAb and negative for HBsAg were found PCR positive for HBV DNA highlighting the need for a strict and better screening system to avert occult HBV infection.

Our study also suggested that as HBV infection is endemic in the Pakistani population, screening for HBcAb will result in the discarding of a large unit of blood collected by blood bank because of false-positive results. Nucleic acid testing (NAT) technology is believed to be more useful for the detection of HBV DNA in the window period. However, this places additional expenditure on the blood banks and ultimately on the patients and not feasible especially in a country like Pakistan where the blood transfusion system is fragmented and dominated by the private/NGO sector blood establishments.[27]

Conclusion

Our results indicated that implementation of the hepatitis B core antibody test in routine along with the Hepatitis B surface antigen test may improve safety but it loses a significant amount (5.5%) of donated blood. The best alternative is to include PCR/NAT, which of course has cost implications. A multi-centre study with larger sample size is proposed to authenticate our findings.

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