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RESEARCH ARTICLE

CORRELATION BETWEEN HEPATITIS B CORE ANTIBODIES TEST WITH HEPATITIS B SURFACE ANTIGEN

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ABSTRACT

The Hepatitis B core antibody screened in various blood banks in India to attractive the safety of blood transfusion. However, In India, HBcAb is not a mandatory test as per Drug and Cosmetic Act, 1940. Now a day, HBcAbIgM and IgG testing have been adopted for routine screening but it leads to increased cost of transfused blood. Therefore, this study was carried out to look into the possibility of obviating the need of screening of HBcAb testing is necessary or not, so as to optimize the resource utilization and reduce the cost without compromising the blood safety.

Aim: To find out the agreement of Hepatitis B core antibody test with HBsAg.

Methods: Screening results were evaluated for HBV by immunoassay, HBsAg & Anti-HBcAbIgM and IgG. Initial reactive samples were confirmed by ID-NAT (TMA) discriminatory HBV assay.

Results: Value of kappa indicates that there is no significant agreement between HBcAb and HBsAg. The diagnostic accuracy of HBcAb is very bad (8.26%) when compared with HBsAg. However, the sensitivity of the test is good (85.71%).

Conclusion: The value of Kappa indicates that there is no significant agreement between HBcAb and ID-NAT with HBsAg. In fact the HB core antibody IgM can enhance the blood safety. The facility for HBV core total screening is same as that of HBsAg, HIV & HCV, whereas, HBcAb is capital extensive but give help to extra safety of blood.

INTRODUCTION

Post transfusion Hepatitis is a serious global health problem which is affecting two billion people worldwide, and 350 million people suffer from chronic HBV infection. This is about five percent of the world population (Zarski *et al.,* 2002). Transmission of hepatitis through blood transfusion was first reported in 1943 (Beeson, 1934 and Morgan, 1943). There are over 4 million new cases of acute clinical hepatitis yearly. About one million of those infected die annually from chronic hepatitis B infection or one of its complications- cirrhosis and hepatocellular carcinoma (Previsani *et al.* (2004). Post transfusion Hepatitis by B virus (HBV) infection via blood transfusion is a serious health problem in India due to the unstandardized testing procedure. Different methods give different result due to presence of concentration of antibody. The incubation period that can range between 50 to 180 days, this infection has an insidious onset. In some cases Hepatitis B viruses are detectable in the blood for several months or even years and about 5-10% of individuals become chronic carriers.Chronic infection by Hepatitis B Virus could lead to the development of Liver cirrhosis and hepatic malignancy (Narayan, 2001). Screening for hepatitis B infection by surface antigen (HBsAg) started in 1971 (Stramer, 2005). Usually testing for HBsAg is performed using Immunochromatography, EIA, radioimmunoassay or other immunological based assays. The latest, most sensitive HBsAg detection method uses chemiluminescence and this method is widely used in USA, Canada, Europe, and some parts of Asia (Biswas, *et al.,* 2003).

In India, Method for detection of HBsAg is not yet standardized by FDA. The only requirement now for HBsAg testing is licensed or accreditation of the test in this country became a selection of the test different in different countries. In some tropical countries where blood donor centres have limited resources, rapid and less expensive immuno filtration, latex based, or immuno chromatographic methods are used, without any confirmatory testing techniques (WHO, 2001). Screening of HBsAg by ELISA is a mandatory test for over 20 years, but still transfusion-associated HBV (TAHBV) continues to be a major problem in India, and more so in patients receiving repeated blood transfusions (Chaudhuri *et al.,* (2003). However, it has been observed that some HBsAg-negative individuals continue to replicate Hepatitis B Virus (Nanu*et al.,* 1997 and Yotsuyanagi *et al.,* 2001). Hence, the HBsAg Negative result in the blood cannot reflect that the person is completely free from Hepatitis B Virus. Blood containing anti-HBc with or without the detectable presence of HBsAg might be infectious. In 1992, antibody to the core protein of hepatitis B (anti HBc total) was introduced in the screening process as a surrogate marker for post-transfusion none A none B hepatitis (NANB) (NIH, Consens statement,

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1995). Therefore, routine blood donor screening for anti-HBc was implemented to reduce the risk of post-transfusion HBV infection (Kleinman *et al.,* 2003). Screening of Hepatitis B Virus practices of depends on pathogenicity, morbidity, mortality, endemicity of pathogen. Ethics vs. financial viability has led to varied practices across the globe. Hepatitis B core antibody screening is controversial in blood banks worldwide, because Hepatitis B core antibody gives false positive results. Recently introduced ID- NAT testing has reduced the window period but does not completely eliminate HBV. Hepatitis B virus infection has a worldwide prevalence due to its infectivity, chronicity and ability to cause substantial morbidity and mortality, in the form of cirrhosis and Hepatocellular Carcinoma (Beasley, 1988).

However, recent development of ID-NAT technology can be proved more sensitive testing for safe blood supply by HBV infection, but it's expensive. It can also be an effective test to rule out occult hepatitis, HBsAg negative and anti-HBc positive infections with low levels of viraemia. The complete elimination of traditional testing methods in the near future is difficult (Busch, 2004). Screening of HBsAg and HBV DNA by ID-NAT would appear preeminent in the countries with a normal to high prevalence of HBsAg. On the other hand, HBV DNA by NAT and anti-HBc testing may be superior to HBV DNA by NAT and HBsAg testing in countries with a low prevalence rate (Klein, 2005).In India, screening for HIV 1 & 2, HCV, HBsAg, RPR and MP is mandatory by law. However, the screening for Anti HBV core antibodies IgM & IgG is employed by some advance blood banks to ensure the decrease of transmission of HBV in case of HBsAg negative blood. In Recent time, the use of nucleic acid testing (ID-NAT) of blood donors has been adopted to detect the presence of occult hepatitis B virus in blood donors which is considered a Potential risk for transfusion of hepatitis B virus (Chaudhuri 2003). Though the use of ID- NAT to prevent transfusion of hepatitis B virus may offer a new opportunity to blood screening programmes, the feasibility of implementing it should be fully considered, since the requirements for infrastructure, financing, staffing levels, training and quality systems and the overall costs of implementation may far outweigh any potential benefit in terms of increased blood safety. The present study was carried out to find out the correlation between the Hepatitis core antibody IgM and IgG (HBcAbIgM&IgG) with Hepatitis surface antigen (HBsAg) of the need of screening HB core antibody along with HBsAg.

MATERIALS AND METHODS

Donated blood was evaluated and collected between October 2009 and April 2011 covering 581 HBV reactive samples. The hospital centers to patient population from different parts of country and world such as USA, Canada, Iraq, Nigeria, Oman, Kazakhstan etc. All samples were screened for serology and ID- NAT. Serological screening was performed by random access ChemiluminescenceImmuno Assay (CLIA) for TTI including HBsAg & HBcAb – Total (IgM & IgG), (Vitros ECI; OCD; JNJ from USA). Nucleic acid screening (Supplementary test) Individual donor ID-NAT was performed by Transcription Mediated Amplification (TMA) technology (Novartis Diagnostic from USA).

RESULTS

Screened 581 samples were reactive by HBV markers, screened reactive samples by serological markers- HBsAg & Anti-HBcAb, were compared with ID-NAT Discriminatory. Of all the samples reactive for HBV, about 07 (1.2%) samples reactive by only HBsAg assay. About 17 (2.93%) reactive by HBcAb Total and HBsAg and 513 (88.3%) of solitary HBcAb reactive samples. However, HBsAg, HBcAb along with ID-NAT were reactive 31 (5.34%). No non- reactive sample was observed to be reactive by ID-NAT, during the study period. Hence, the find out the agreement between HBcAb with HBsAg wit using the statistical tool, Cohen's kappa measures the agreement between the evaluations of two raters when both are rating the same object. A value of 1 indicates perfect agreement. A value of 0 indicates that agreement is no better than chance. So that compared HBcAb with HBsAg by using this statistical test. The value of Kappa indicates that there is no significant agreement between HBcAb and HBsAg. The diagnostic accuracy of HBcAb is very bad (8.26%) when compared with HBsAg. However, the sensitivity of the test is good (85.71%) Table & Figure 1.

Table 1. Agreement and sensitivity analysis of HBcAb with respect to HBsAg

		HBsAg		Total
		Non-Reactive	Reactive	
HBcAb	Non- Reactive	0	8	8
		0.0%	14.3%	1.4%
	Reactive	525	48	573
		100%	85.7%	98.6%
	Total	525	56	581
		100%	100%	100%

Symmetric measures

		Value	Asymp. Std. Error [a]	Approx. T [b]	Approx. Sig.
Measure of Areement	Kappa	-.028	.010	-8.721	.000

a. Not assuming the null hypothesis.
b. Using the asymptotic standard error assuming the null hypothesis.

Sensitivity	85.71428571
Specificity	0
Predictive Value of Positive test	8.376963351
Predictive value of Negative test	0
% of False Negative	14.28571429
% of False Positive	100
Diagnostic Accuracy	8.2616179
Relative Risk =	0.083769634
95% confidence interval for Relative Risk =	0.146262,0.047978
Chi-square =76. 04712042	p-value = 0.000000

DISCUSSION

In the present study screened 581 samples were reactive by HBV markers, screened reactive samples by serological markers- HBsAg & Anti-HBcAb, were compared with ID-NAT Discriminatory. Of all the samples reactive for HBV, about 07 (1.2%) samples reactive by only HBsAg assay. About 17 (2.93%) reactive by HBcAb Total and HBsAg and 513 (88.3%) of solitary HBcAb reactive samples. However, HBsAg, HBcAb along with ID-NAT were reactive 31

(5.34%). No non- reactive sample was observed to be reactive by ID-NAT, during the study period.The above mentioned fact suggests that though ID-NAT is not supporting to eliminate anti HB core but still is required validity based upon time to time, because mutation of the viral pathogen. Safety of blood is a challenge in India because of the high prevalence of HBV, HCV and HIV, the relatively low percentage of volunteer donors and the lack of standardization of screening procedures among the multitude of blood collection centres (Kapoor *et al.,* 2000). The potential of NAT yield in India is staggering when compared to other countries that have already implemented the technology. My previous study showed that only 0.2% of the positive samples were HBsAg reactive/ ant-HB core non reactive/ ID-NAT reactive and none of the sample (0%) was found to be HBsAgnon reactive/ anti-HB core non reactive/ ID-NAT reactive (Choudhary *et al.,* 2013). These findings clearly indicate that NAT is capital extensive in the present scenario. Cost effectiveness of introducing universal anti-HBc screening and discarding a large number of blood units versus considering ID NAT needs to be assessed.

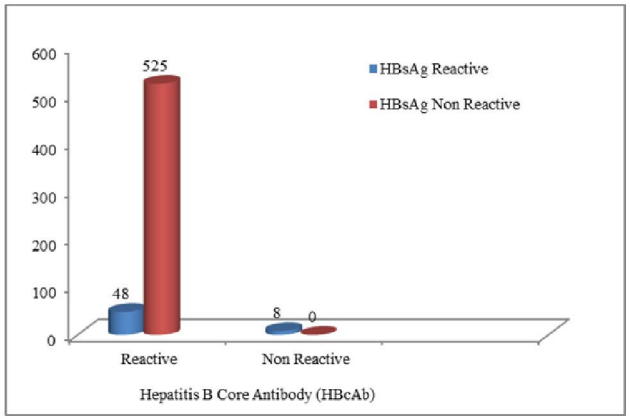

Fig. 1.Comparison of HBcAb with HBsAg

The value of Kappa indicates that there is no significant agreement between HBcAb and HBsAg. The diagnostic accuracy of HBcAb is very bad (8.26%) when compared with HBsAg. However, the sensitivity of the test is good (85.71%). Another Indian study on large sections of blood donors demonstrated 4.2 per cent anti-HBc only positivity (Chaudhuri *et al.,* 2003). Hence, Previously we have observed 6.24% prevalence of anti-HBc positivity, which supports with our finding. The other study also corroborates the presence of occult HBV infections in blood donors positive for antibodies against hepatitis B core antigen and emphasizes on the need for establishing sensitive screening modalities for blood transfusion (Asim *et al.,* 2010). In our study, we also find that the presence of occult HBV infections in the blood donor's positive hepatitis B core antibody and focuses on the need for establishing sensitive screening modalities for blood transfusion.It is commonly followed that the diagnosis of infection with HBV is based on the presence of the HBsAg in the bloodstream (Badur and Akgun 2001). However, screening of blood bank donors for HBsAg does not totally minimize the chances of the risk of HBV infection through blood transfusion (Conjeevaram and Lok 2001), since the absence of HBV marker in the serum does not exclude the presence of HBV DNA (Comanor and Holland 2006). It is in this context it can be said that, donors with occult HBV infection, who lacked detectable HBsAg but whose exposure to HBV infection was indicated by a positive anti-HBc and HBV DNA, may be a potential source of HBV infection. Various studies have reported that the rate of detection is high of HBV- DNA in liver or peripheral mononuclear cells compared with serum or plasma in occult hepatitis (Conjeevaram and Lok 2001). The other study shown that only serum or plasma sample detected only 0.2% of the positive samples were HBsAg reactive/ HB core non reactive/ ID-NAT reactive and none of the sample (0%) were found to be HBsAgnon reactive/ HB core non reactive/ ID-NAT reactive, these finding also indicate the HBV-DNA detection is very low. Occult HBV infection exists and that most cases are related to very low levels of HBV rather than to HBV mutants that do not express or produce abnormal surface proteins and consequently are undetected by standard method (Bre`chot *et al.,* 2001). Because HBV-DNA detection is the gold standard method for diagnosis of occult HBV infection, the type of assay used and its sensitivity must be specified. The sensitivity of PCR assays for HBV DNA in studies on occult HBV infection varies from 101 to 103 copies/mL (Pawlotsky *et al.,* 2000).

However, most of the PCR assays including commercially available assays are not standardized Choudhary *et al.,* 2010).In India, anti-HBc screening is not mandatory. However, higher prevalence of only anti-HBc (58.8%) cases with 22.8% HBV DNA reactivity from Kolkata (Banerjee *et al.,* 2007). Similarly, other studies also substantiate with Pakistani and Egyptian studies showing 17.2 and 21.47 per cent anti-HBc prevalence in HBsAg-negative, HBV DNA-negative blood donors (Bhatti *et al.,* 2007). In India where the anti-HBc reactivity ranges from 4.2% to 18.3 % in healthy donors (Bhattachrya *et al.,* 2007). In this study also found that between the above findings. Our earlier study shows out of 2740 samples (n= 186) 6.7% sample reactive bydetection of liver or peripheral mononuclear cells compared with serum or plasma (Choudhary *et al.,* 2010). The result of study on only a serum or plasma sample showed that only 0.2% of the positive samples were HBsAg reactive/ HB core non reactive/ ID-NAT reactive and none of the sample (0%) was found to be HBsAgnon reactive/ HB core non reactive/ ID-NAT reactive, these finding also indicate the HBV-DNA detection is very low.Worldwide literatures show presence of anti-HBc in HBsAg-negative blood donors. The incidence of anti-HBc in blood donors varies from 0.07% to 18%, and 0.3%-38% of these donors show the presence of HBV DNA in their blood, depending on the type of blood donors and the endemicity of disease in the study population (MadhuriAgrawal *et al.,* 2013). The present study showed compared with HBsAg the sensitivity is 53.57%, specificity 97.33% so that diagnostic accuracy is very good, however, the sensitivity of the test is mediocre. Specificity was found good 97.33%. A study reported from New Delhi (Northern India) by (Chaudhuri *et al.,* 2003) revealed that the prevalence of anti-HBc was 10.82% with distribution of 6.92% in voluntary donors and 12.53% in replacement donors. In contrast, a study of West Bengal (Eastern India) by (Bhattacharya *et al.,* 2007) showed an anti-HBc positivity as high as 18.3% in voluntary blood donors. Prevalence of anti-HBc reported by (Behzad-Behbahani *et al.,* 2006) in Iran was 6.55%, in this study replacement and voluntary donors were included. High prevalence of anti-HBc (17.28%) was reported by (Bhatti

et al., 2007) from Pakistan. The prevalence of anti-HBc in Europe and North America is quite low, an anti-HBc prevalence of 0.07% in the UK and 1.5% in Germany has been reported (Bhatti *et al.,* 2007). However, after observation of various above facts that there is no any clear suggestion lear to value to screening of HBcore is Our study indicates that there is no significant agreement between HBcAb and HBsAg. The diagnostic accuracy of HBcAb is very bad (8.26%) when compared with HBsAg. However, the sensitivity of the test is good (85.71%) so that there is no clear

Conclusion

The value of Kappa indicates that there is no significant agreement between HBcAb and ID-NAT with HBsAg. In fact the HB core antibody IgM can enhance the blood safety. The facility for HBV core total screening is same as that of HBsAg, HIV & HCV, whereas, HBcAb is capital extensive but give help to extra safety of blood.

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