Original Article

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Microbiology Section

The Prevalence of the Hepatitis B Core Antibody and the Occult Hepatitis B Infection Among Voluntary Blood Donors in Chennai, India

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ABSTRACT

Introduction: The infection with the Hepatitis B Virus (HBV) is a global health problem which affects 2 billion people worldwide. In India, the prevalence of the Hepatitis B infection is 4% in the general population. The prevalence of the HBV infection in voluntary blood donors is 1-3%. It has been reported that the viraemia continues even after the clinical recovery from the acute HBV infection. Some blood donors who were negative for the surface antigen but positive for the core antibody have been reported to transmit HBV, leading to acute hepatitis. This study was done to determine the seroprevalence of the hepatitis B core antibody in voluntary blood donors in Chennai, India.

Materials and Method: This prospective study was conducted in our department during 2008-2009. A total of 9100 donor samples were screened for the Hepatitis B surface antigen and the Hepatitis B core antibody (IgM and IgG) by ELISA. The samples which were positive for the core antibody were subjected to Real-time PCR for the Hepatitis B DNA detection.

Results: Among the 9100 donors, 911 (10.01%) donors were positive for the core antibody. The Hepatitis B Surface antigen was positive in 199 (2.18%) donors. Among the 911 donors who were positive for the core antibody, 820 (90.01%) donors were negative for the HBsAg and 2 donors were positive for Hepatitis B DNA.

Conclusion: If a routine screening of the sera for the core antibody is not done, the low-level HBV viraemia may not be identified. The absence of the surface antigen in the blood of apparently healthy individuals may not be sufficient to ensure the lack of the circulating virus.

Key Words: Blood donors, Core antibody, Hepatitis B, Seroprevalence

INTRODUCTION

The Hepatitis B Virus (HBV) infection is a global health problem which affects 2 billion people worldwide and 350 million people suffer from the chronic HBV infection [1]. In India, the prevalence of the Hepatitis B infection is 4% in the general population, which means that 40 million people are infected with HBV in our country. The prevalence of the HBV infection in the voluntary blood donors is 1-3% and it is 10-12% in the commercial donors [2].

The safety of the blood components depends on a proper donor selection which is complemented by sensitive screening tests to exclude the transmission of infective agents. Despite the screening of HBsAg by ELISA for over 20 years, transfusion associated HBV (TAHBV) continues to be a major problem in India, more so in patients who receive repeated transfusions. The prevalence of the post transfusion Hepatitis B in India is 1-5% [2]. The Occult Hepatitis B Infection (OBI) is defined as the presence of the HBV DNA in blood or liver tissues without detectable Hepatitis B surface antigens (HBsAg), with or without antibodies to the Hepatitis B core antigen (Anti-HBc) and the Hepatitis B surface antigen (Anti-HBs) [3]. As the free HBcAg does not circulate in significant quantities in the blood, it is not detected on the serum tests. So, the antibodies to the core antigen are usually tested and detected.

It has been reported that viraemia continues even after the clinical recovery from the acute HBV infection in some blood donors who were negative for HBsAg but positive for anti-HBc and can transmit HBV, leading to acute hepatitis [3]. Vaishali et al., reported the prevalence of anti-HBc in northern India as 10.82% [4]. Since there was no published data for finding out the prevalence of the Hepatitis B core antibody among the voluntary, non remunerated blood donors in Chennai,India, this study was undertaken to detect the Hepatitis B core antibody among healthy voluntary blood donors and to detect the HBV-DNA in the samples which were positive for the Hepatitis B core antibody.

MATERIALS AND METHODS

This prospective study was conducted over a period of one year from 2008 to 2009 in the Department of Transfusion Medicine, The Tamilnadu Dr. MGR Medical University, Guindy, Chennai, India. A total of 9100 voluntary blood donors were selected. This study was approved by the ethical committee of the institution and a written informed consent was obtained from the donors. 5ml of blood from each donor was collected from the collection bag into a sterile capped tube. It was then centrifuged and the plasma was separated and stored as two aliquots at -70°C till further use.

The samples that were frozen earlier were thawed and used. The screening for the Hepatitis B core antibody (anti-HBc IgM and IgG) was done by a 3rd generation ELISA by using Biorad's Monolisa Anti-HBc Plus kit. It is an indirect type of ELISA which is based on the use of a solid phase which is prepared with the recombinant HBc antigen. All the steps were followed as per the manufacturer's

instructions.

The screening for the Hepatitis B surface Antigen (HBsAg) was done by a 3rd generation ELISA by using J.Mitra's Hepelisa kit.

The detection of the HBV DNA in the Hepatitis B core antibody positive samples was done by real time PCR by using a Roche light cycler. The DNA was extracted from the plasma by using a QIAamp DNA kit from Qiagen (Germany). All the steps were followed according to the manufacturer's instructions.

The statistical analysis was done by using the SPSS software. For relating the variables with each other, a multivariate analysis was done. The Chi square test was employed to detect any significant correlation between the different variables.

RESULTS

Among the total 9100 donors in the study, 8399 (92.29%) were males and 701(7.7%) were females. Of the total 9100 donor blood samples which were tested for anti-HBc (IgM and IgG), 911 samples (10.01%) were found to be positive and 199 samples (2.18%) were found to be positive for HBsAg. Out of 199 HBsAg positive samples, 91 were positive for the core antibody [Table/Fig-1]. So, 820 samples were positive for the core antibody alone and they were negative for HBsAg. The gender distribution and the age group distribution of the anti-HBc antibody has been shown in the [Table/Fig-2 & 3] respectively.

HBsAg	Anti-HBc	No.of donors	Percentage
Positive	Negative	108	1.18%
Positive	Positive	91	1%
Negative	Positive	820	9.01%

[Table/Fig-1]: HBsAg and Core antibody result

	Anti-HBc antibody		Percentage	p-value
	Positive	Negative		
Male	846	8399	10.07%	0.32
Female	65	701	9.27%	1

[Table/Fig-2]: Demographic factors of Anti-HBc antibody

Anti-HBc Positive					
Age group	Male	Female	Total Anti-HBc positive/ Total donors	Percentage	p-value
18-20 years	360	22	382/4045	9.44%	
20-30 years	273	31	304/3012	10.09%	0.002
30-40 years	150	12	162/1527	10.60%	
40-50 years	63	0	63/516	12.20%	

[Table/Fig-3]: Anti-HBc seropositivity among different age groups

HBsAg	Anti-HBc	No.of donors
Dhawan et al.,[5]	Chandigarh	8.4%
Bhattacharya et al., [6]	West Bengal	18.3%
Seo et al., [8]	Korea	13.5%
Makroo et al., [9]	New Delhi	10.22%
Margaret et al., [14]	Nigeria	13%
Present study	Chennai	10.01%

[Table/Fig-4]: Prevelance of Hepatits B Core antibody in various studies

The 820 samples which were positive for the core antibody and negative for HBsAg were subjected to Real time PCR (RT-PCR) for the HBV-DNA detection. Of the 820 samples, 2 samples (0.24%) were found to be positive for the HBV-DNA.

DISCUSSION

The blood donors who are chronically infected with HBV but without detectable levels of the Hepatitis B surface Antigen (HBsAg) and with or without antibodies to the Hepatitis B core Antigen (Anti-HBc) contribute to the residual risk of the transfusion transmitted HBV infection.

In our study, of the 9100 blood donors who were tested for the Hepatitis B core antibody, 911 (10.01%) were positive. Of the 911 core antibody positive donors, 820 donors were HBsAg negative, whereas the remaining 91 were HBsAg positive. The varied prevalence of the core positivity has been reported in Indian studies. Dhawan et al., [5] from Chandigarh reported a core positive rate of 8.4% which was comparable to our data, whereas a high positivity rate of 18.3% was observed by Bhattacharya et al., [6] in West Bengal [Table/Fig-4].

The demographic analysis of the 911 donors who were positive for the anti-HBc antibody showed that it had a higher preponderance in males (10.07% in males vs 9.27% in females; p=0.32; [Table/Fig-2]. A study which was done by Asim et al showed a difference in the seroprevalence of the core antibody between the male and the female donors (19.3% vs 18%), but as in our study, the difference was statistically not significant [7]. We observed a significantly increasing prevalence of the core antibody with the increasing age of the donor population [Table/Fig-3], which was similar to the findings of the study which was done by Seo et al., [8]. In our study, 2.18% of the donors were positive for HBsAg. This was in concordance with the study which was done by Bharat et al., in New Delhi, who had reported a 2.2% seropositivity for HBsAg in the blood donors [2].

In our study, of the 820 samples that were positive for the anti-HBc antibody and negative for HBsAg, 2 (0.24%) samples were found to have HBV-DNA positivity by the RT-PCR method. Makroo et al reported 0.15% of the core antibody positive samples to have the HBV DNA [9]. Gutierrez et al., from Venezulla reported a 6% prevalence of the HBV-DNA in samples which were positive for anti-HBc, only when the titres of the antibody was equal to or higher than 1/10 [10]. Kleinman et al reported a 0.24% detection rate of the HBV-DNA among the anti-HBc reactive donors [11]. Amini et al., in a study which was done on 4930 healthy blood donors, found that 5.1% were positive for anti-HBc without having any detectable HBsAg; however, they did not determine the presence of the HBV-DNA [12]. The reasons for the non detection of the HBV DNA in the samples may be that the donor may have been in the recovery phase of an acute infection or that the donor may have been immune due to an earlier infection and with undetectable levels of HBV-DNA now.

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. However, unless we do a sensitive Nucleic Acid Amplification Test (NAT), we can't confirm the presence or absence of the virus in such a donor.

But in a majority of the blood banks in the developing countries like India, NAT may not be feasible. Unlike IgG anti-HBc, the IgM core antibody is a marker of a recent Hepatitis B infection. Sawke et al felt that the screening of the donor blood for the total anti-HBc was not practical, as it could result in the discarding of huge amounts of blood, whereas the screening for the IgM- anti- HBc of the blood units which were negative for HBsAg could identify the potentially infectious units [13].

These observations suggested that the HBV DNA screening would be more effective in preventing the transfusion transmitted Hepatitis B infections. But the anti-HBc screening would be a good screening marker where the NAT screening is not feasible. But due to the varying specificities of the current antibody screening assays, the absence of an acceptable confirmatory test strategy and the high anti-HBc seroprevalence rates in the HBV endemic regions make the screening problematic due to the high donor loss rates.

CONCLUSION

This study helps in determining the current status of the Hepatitis B infection and the prevalence of the occult HBV infection in our blood donor population. The screening of the blood units for the core antibody adds to the cost, but it is definitely useful in reducing the residual risk of post transfusion hepatitis. Though the HBV DNA was detected only in 0.24% of the core antibody positive donors, they were still considered as infectious unless they were proved as negative by PCR for the HBV DNA. Considering the loss of the donor pool due to the high prevalence rate of anti-HBc among the voluntary blood donors in our study, the subject of including the anti-HBc testing as a routine screening test is debatable.

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