

RAPID COMMUNICATION

Significant increase in HBV, HCV, HIV and syphilis infections among blood donors in West Bengal, Eastern India 2004-2005: Exploratory screening reveals high frequency of occult HBV infection

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Abstract

AIM: To evaluate the prevalence of markers of hepatitis B virus (HBV) and hepatitis C virus (HCV) and human immunodeficiency virus (HIV) among blood donors in Kolkata, Eastern India for two consecutive years and to conduct a pilot study to explore the presence of HBV DNA among hepatitis B surface antigen (HBsAg) negative but anti-HBc positive blood donors.

METHODS: Seroprevalence of HBsAg, anti-HCV and anti-HIV was studied among 113 051 and 106 695 voluntary blood donors screened in 2004 and 2005, respectively. Moreover, a pilot study on 1027 HBsAg negative donors was carried out for evaluating the presence of HBV DNA by PCR on HBsAg negative/anti-HBc positive donors.

RESULTS: A statistically significant increase in the prevalence of HBV (1448 vs 1768, $P < 0.001$), HIV (262 vs 374, $P < 0.001$), HCV (314 vs 372, $P = 0.003$) and syphilis (772 vs 853, $P = 0.001$) infections was noted among blood donors of Kolkata West Bengal in 2005 as compared to 2004. Moreover, the exploratory study on 1027 HBsAg negative donors revealed that 188 (18.3%)

of them were anti-HBc positive out of which 21% were positive for HBV DNA.

CONCLUSION: The findings of this study underscore the significantly increasing endemicity of hepatitis viruses, syphilis and HIV among the voluntary blood donors of our community. The pilot study indicates a high rate of prevalence of HBV DNA among HBsAg negative/anti-HBc positive donors and thus emphasizes the need for a more sensitive and stringent screening algorithm for blood donations.

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Key words: Hepatitis B virus; Human immunodeficiency virus; Hepatitis C virus; Blood donation; Occult HBV infection

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INTRODUCTION

Hepatitis B is one of most common infectious diseases of the world and has infected 2 billion people worldwide; including an estimated 400 million chronically infected cases^[1]. Individuals with chronic infection have a high risk of developing liver cirrhosis and hepatocellular carcinoma. Hepatitis C virus (HCV) infection is another common chronic blood borne infection with an estimated 3.9 million persons infected by the virus and a high rate of development of liver cirrhosis. Infection by hepatitis B virus (HBV) and HCV causes serious mortality, morbidity and financial burden and are thus a major global health problem^[2]. In addition, prevalence of human immunodeficiency virus (HIV), is increasing in the world. India, with an estimated 5.7 million cases of HIV

infection, is the second highest pool of these patients in the world.

Evaluation of data on the prevalence of these transfusion transmitted infections (TTIs), namely HBV, HCV, HIV and syphilis, among blood and plasma donors permits an assessment of the occurrence of infections in the blood donor population and consequently the safety of the collected donations. It also gives an idea of the epidemiology of these diseases in the community.

Among these infections HBV is more infective than the other viruses^[1]. Detection of hepatitis B surface antigen (HBsAg) in blood is diagnostic for infection with HBV and in the blood banks screening for HBsAg is carried out routinely to detect HBV infection. Occult HBV infection is defined as the presence of HBV DNA in blood or liver tissues in patients negative for HBsAg but who may or may not be positive for HBV antibodies^[1].

Thus some HBsAg negative individuals, positive for antibodies against HBV core antigen (anti-HBc) and/or HBsAg (anti-HBs) continue to be positive for HBV DNA. Due to limitations in current blood screening practices in developing countries, donation by such individuals is a potential source of HBV transmission to the recipients^[3-6]. Such occult hepatitis B infection may be detected in (1) Individuals with resolving HBV infection positive for both antiHBc and anti-HBs (2) "anti-HBc-only" carriers in a window period of infection who are seronegative for HBsAg (3) carriers in whom HBsAg is not detectable due to presence of escape mutants^[5]. High frequencies of HBV DNA positivity (10% to 40%) have been described among anti-HBc only sera^[3]. Routine anti-HBc screening of individual blood donations and nucleic acid amplification testing (NAT) by pooling of sera is done in some countries to exclude these donations^[7-9]. In India, detection of HBV infection among blood donors is carried out by HBsAg screening while detection of anti-HBc is rarely done^[10].

In this study we aimed to assess the prevalence and trends of the transfusion-transmitted infections (TTIs) in two consecutive years, 2004 and 2005, among blood donors of West Bengal. Moreover, in a pilot study, we also explored the prevalence of occult HBV infection among 1027 randomly selected HBsAg negative donor samples, without sera pooling.

MATERIALS AND METHODS

The Institute of Blood Transfusion Medicine and Immunohematology (IBTMI), is located in Kolkata (Calcutta). It is under the Department of Health and Family Welfare, Ministry of Health, West Bengal. It is the leading organization that coordinates blood transfusion services throughout the state of West Bengal. The state has a population of 80 176 197 according to the 2001 census. The majority of contributors to this blood bank are voluntary donors. The voluntary donations primarily were obtained from blood donation camps, mostly organized by clubs, colleges, political parties, religious organizations *etc.*

The screening of blood donations for HBsAg, anti-HIV, syphilis and anti-HCV is mandatory in IBTMI. Blood donations from individuals who are found to be positive

Table 1 Prevalence of TTIs among the blood donors in the years 2004 and 2005

	Total No of samples studied		No of samples reactive (%)		Statistical significance	
	2004	2005	2004	2005	RR (95% CI)	P values
HBsAg	113 051	106 695	1448 (1.28)	1768 (1.66)	1.29 (1.21-1.39)	< 0.001
Anti-HCV	113 051	106 695	314 (0.28)	372 (0.35)	1.26 (1.08-1.46)	0.003
Anti-HIV	113 051	106 695	262 (0.23)	374 (0.35)	1.51 (1.29-1.77)	< 0.001
Rapid plasma reagin (Syphilis)	113 051	106 695	772 (0.68)	853 (0.80)	1.17 (1.06-1.29)	0.001

for any of the above infections previously were deferred at IBTMI. Moreover, the donors were pre-counseled regarding their health status and also required to fill out a donor screening registration form as part of a routine blood donation screening procedure. During the 2-year period, blood samples from 1027 HBsAg negative blood donors (564 in the year 2004 and 463 in the year 2005) were chosen at random, at various periods of time for the pilot study. Informed consent was obtained from all the donors. The study abided by the rules of the Ethical Committee of IBTMI.

All the donor samples were examined in IBTMI, using commercial ELISA, HBsAg and anti-HCV (Span diagnostics, India), anti-HIV (General Biologicals, Taiwan) and RPR (Rapid Plasma Reagin) for Syphilis (Span diagnostics, India). Anti-HBc and anti-HBs (Organon Teknika, Boxtel, The Netherlands) was detected in a subset of 1027 donors for exploratory screening at ICMR Virus Unit. All anti-HBc positive samples were retested in duplicate for HBsAg as well as for anti-HBc. Only repeat HBsAg negative/anti-HBc positive samples were considered to be positive for anti-HBc. HBV DNA was detected by in-house nested PCR, amplifying two different regions of the HBV genome as described earlier^[11].

RESULTS

A total of 219 746 blood units were collected at IBTMI, from January 2004 to December 2005, 94.6% of which were collected from voluntary donors. Analysis of the prevalence of TTIs among them revealed a statistically significant increase in the occurrence of all the blood borne infections in the year 2005 as compared to 2004 (Table 1); frequency of co-infection of these viruses was negligible. Furthermore, relative risk (RR) of HIV infection was 1.51 (95% confidence interval, $P < 0.001$). In 2005 the prevalence rates (per 100 000 donations) were 350 for HIV, 350 for HCV, 1660 for HBV and 800 for syphilis.

Out of the 1027 HBsAg negative blood samples screened, 18.3% were found to be anti-HBc positive (Table 2). Notably, 21.3% of the anti-HBc positive samples were HBV DNA positive by PCR. Among the 'anti-HBc only' subgroup, a marginal increase in the proportion of HBV DNA positive samples was noted in 2005 as

Table 2 Detection of HBV DNA in anti-HBc positive blood donors

Samples collected in the year	Total number of HBsAg negative samples collected	No of Anti HBc positive samples (irrespective of anti HBs status) (%)		No of 'Anti HBc only' samples (anti HBs negative) (%)	
		Total	Presence of HBV DNA	Total	Presence of HBV DNA
2004	564	95 (16.8)	23 (24.2)	36 (6.4)	11 (30.5)
2005	463	93 (20.1)	17 (18.3)	25 (5.4)	8 (32.0)
Total	1027	188 (18.3)	40 (21.3)	61 (5.9)	19 (31.1)

compared to 2004 (Table 2). None of the HBV DNA positive samples were co-infected with HCV or HIV.

DISCUSSION

Our study was aimed at analyzing two blood transfusion related issues—one was to assess the trends in TTIs in two consecutive years and the other was to evaluate the prevalence of occult HBV infection among HBsAg negative donors in a pilot study.

We examined the occurrence of HBV, HCV, HIV and syphilis infections among blood donors in Kolkata, West Bengal by serological methods and compared the results to assess the trends in two consecutive years, 2004 and 2005. 94.6% of the blood donors have donated voluntarily in the blood donation camps organized by different clubs, religious organizations, offices, political parties, etc. Therefore, the prevalence of viral carrier rates in blood donors is similar to that of the general population. Thus, the data highlighting the increase of TTIs among the blood donors is of concern. It indicates that the occurrence of these infections among the voluntary donors should be monitored carefully and the possible causes evaluated. The Report of National AIDS Control Organization^[12], shows a considerable increase in HIV prevalence in the year 2005 among antenatal clinics (0.5% to 0.84%) and sexually transmitted diseases clinics (0.88% to 2.16%) from the state of West Bengal, whereas adult HIV prevalence in the rest of the country was comparable to the previous years. Simultaneous increase of HBV, syphilis and HIV infections indicate that sexual transmission might be a possible route.

It is generally accepted that the diagnosis of infection by HBV is based on the presence of the HBsAg in the bloodstream^[13]. However, screening of blood bank donors for HBsAg does not totally eliminate the risk of HBV infection through blood transfusion^[14,15], since the absence of this marker in the serum does not exclude the presence of HBV DNA^[16-19]. It is possible 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, are a potential source of HBV infection^[20-22]. Our pilot study revealed that 188 of 1027 (18.3%) HBsAg negative blood donors were anti-HBc positive and thus were exposed to HBV infection. Occult HBV infection was observed in approximately 21% of those anti-HBc positive donors subjected to exploratory screening (Table 2). Thus these donors have the potential to transmit HBV contaminated blood through the

public blood supply. Recent reports actually documented transmission of occult HBV by transfusion^[22]. Moreover, in the 'anti-HBc only' group 31% were positive for HBV DNA (Table 2). The infectivity of anti-HBc positive/HBV DNA positive blood components is reported to be low^[24]. In contrast, 'anti-HBc only' blood products, which are HBV DNA positive, are more prone to transmit HBV infection^[6]. Presence of occult HBV infection has also been reported in blood donors from other Asian countries; HBV DNA was detected among 16 of 131 (12.2%) anti-HBc positive donors in Iran, 7 of 250 (2.8%) in Lebanon and 5 of 167 (2.9%) in Pakistan^[25-27]. A previous study from Delhi, Northern India showed prevalence of HBV DNA to be 25% among the 'anti-HBc only' donors; another study from Chandigarh in Northwestern India showed 0% prevalence while the present study has revealed 31% prevalence from West Bengal, Eastern India^[28,29].

Our study raises serious concerns regarding the safety of the blood supply in our community, even after donor screening for HBsAg. In India transfusion associated HBV is estimated to be approximately 50% or more in multiply transfused patients and approximately 1.5% in post surgical recipients^[30]. Thus the absence of HBsAg in the blood of apparently healthy individuals may not be sufficient to ensure lack of circulating HBV. Blood containing anti-HBc with or without detectable presence of HBsAg might be infectious, therefore routine blood donor screening for anti-HBc has been implemented in some countries resulting in a decrease in the risk of post-transfusion HBV infection^[2]. The trends in the two years in IBTMI, suggest that routine anti-HBc screening of blood donations could possibly prevent some transfusion-transmitted HBV infections in this population. However, the usefulness of screening for anti-HBc as an additional screening test to improve the safety of the blood supply in India deserves further analysis. A national study, including a statistically significant number of blood donors from different blood donation centers across the country, should determine whether screening for anti-HBc in addition to HBsAg detection and introduction of PCR based screenings like NAT should also be considered for the Indian blood donors.

Our study underscores the increasing endemicity of TTIs in our community and the need for a sensitive screening algorithm of blood donations to improve blood safety.

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COMMENTS

Background

Transfusion of blood and blood product is a life saving measurement and benefits numerous patients worldwide. At the same time blood transfusion is an important mode of transmission of infection to the recipients.

Research frontiers

For evaluation of safety of blood transfusion in West Bengal, Eastern India, both

the markers of transfusion transmitted infections (TTIs) in human immunodeficiency virus, hepatitis B virus, hepatitis C virus and syphilis virus infection among the donors and the prevalence of occult hepatitis B virus infection by the presence of HBV DNA in absence of HBsAg were studied.

Innovations and breakthrough

There is an increasing prevalence of TTIs in Kolkata, West Bengal, India in the two years. An exploratory study highlights a high rate of occult HBV infection among the blood donors.

Applications

High prevalence of occult infection indicates a need to reconsider the current policy of blood donor screening.

Peer review

This is an important and timely paper documenting the prevalence of viral hepatitis and HIV in the West Bengali blood donor population, written by an authoritative Indian group from the major city, Kolkata. It deserves publication.

REFERENCES

- Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. *N Engl J Med* 1996; **334**: 1685-1690
- Kleinman SH, Kuhns MC, Todd DS, Glynn SA, McNamara A, DiMarco A, Busch MP. Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-HBc: implications for transfusion transmission and donor screening. *Transfusion* 2003; **43**: 696-704
- Weber B, Melchior W, Gehrke R, Doerr HW, Berger A, Rabenau H. Hepatitis B virus markers in anti-HBc only positive individuals. *J Med Virol* 2001; **64**: 312-319
- Wang JT, Wang TH, Sheu JC, Shih LN, Lin JT, Chen DS. Detection of hepatitis B virus DNA by polymerase chain reaction in plasma of volunteer blood donors negative for hepatitis B surface antigen. *J Infect Dis* 1991; **163**: 397-399
- Weinberger KM, Bauer T, Böhm S, Jilg W. High genetic variability of the group-specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. *J Gen Virol* 2000; **81**: 1165-1174
- Allain JP. Occult hepatitis B virus infection: implications in transfusion. *Vox Sang* 2004; **86**: 83-91
- Goodnough LT, Brecher ME, Kanter MH, AuBuchon JP. Transfusion medicine. Second of two parts--blood conservation. *N Engl J Med* 1999; **340**: 525-533
- Stramer SL. Nucleic acid testing for transfusion-transmissible agents. *Curr Opin Hematol* 2000; **7**: 387-391
- Nationwide nucleic acid amplification testing of hepatitis B virus, hepatitis C virus and human immunodeficiency virus type 1 for blood transfusion and follow-up study of nucleic acid amplification positive donors. *Jpn J Infect Dis* 2000; **53**: 116-123
- Chattopadhyay S, Rao S, Das BC, Singh NP, Kar P. Prevalence of transfusion-transmitted virus infection in patients on maintenance hemodialysis from New Delhi, India. *Hemodial Int* 2005; **9**: 362-366
- Datta S, Banerjee A, Chandra PK, Chowdhury A, Chakravarty R. Genotype, phylogenetic analysis, and transmission pattern of occult hepatitis B virus (HBV) infection in families of asymptomatic HBsAg carriers. *J Med Virol* 2006; **78**: 53-59
- National AIDS Control Organization. Available from: URL: <http://www.nacoonline.org>
- Badur S, Akgün A. Diagnosis of hepatitis B infections and monitoring of treatment. *J Clin Virol* 2001; **21**: 229-237
- Allain JP. Occult hepatitis B virus infection. *Transfus Clin Biol* 2004; **11**: 18-25
- Conjeevaram HS, Lok AS. Occult hepatitis B virus infection: a hidden menace? *Hepatology* 2001; **34**: 204-206
- Comanor L, Holland P. Hepatitis B virus blood screening: unfinished agendas. *Vox Sang* 2006; **91**: 1-12
- Bréchet C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchet P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; **34**: 194-203
- Lai ME, Farci P, Figus A, Balestrieri A, Arnone M, Vyas GN. Hepatitis B virus DNA in the serum of Sardinian blood donors negative for the hepatitis B surface antigen. *Blood* 1989; **73**: 17-19
- Wang JT, Lee CZ, Chen PJ, Wang TH, Chen DS. Transfusion-transmitted HBV infection in an endemic area: the necessity of more sensitive screening for HBV carriers. *Transfusion* 2002; **42**: 1592-1597
- Yotsuyanagi H, Yasuda K, Moriya K, Shintani Y, Fujie H, Tsutsumi T, Nojiri N, Juji T, Hoshino H, Shimoda K, Hino K, Kimura S, Iino S, Koike K. Frequent presence of HBV in the sera of HBsAg-negative, anti-HBc-positive blood donors. *Transfusion* 2001; **41**: 1093-1099
- Allain JP, Hewitt PE, Tedder RS, Williamson LM. Evidence that anti-HBc but not HBV DNA testing may prevent some HBV transmission by transfusion. *Br J Haematol* 1999; **107**: 186-195
- Dreier J, Kröger M, Diekmann J, Götting C, Kleesiek K. Low-level viraemia of hepatitis B virus in an anti-HBc- and anti-HBs-positive blood donor. *Transfus Med* 2004; **14**: 97-103
- Hennig H, Puchta I, Luhm J, Schlenke P, Goerg S, Kirchner H. Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core antigen. *Blood* 2002; **100**: 2637-2641
- Mosley JW, Stevens CE, Aach RD, Hollinger FB, Mimms LT, Solomon LR, Barbosa LH, Nemo GJ. Donor screening for antibody to hepatitis B core antigen and hepatitis B virus infection in transfusion recipients. *Transfusion* 1995; **35**: 5-12
- Behzad-Behbahani A, Mafi-Nejad A, Tabei SZ, Lankarani KB, Torab A, Moaddeb A. Anti-HBc & HBV-DNA detection in blood donors negative for hepatitis B virus surface antigen in reducing risk of transfusion associated HBV infection. *Indian J Med Res* 2006; **123**: 37-42
- Bhatti FA, Ullah Z, Salamat N, Ayub M, Ghani E. Anti-hepatitis B core antigen testing, viral markers, and occult hepatitis B virus infection in Pakistani blood donors: implications for transfusion practice. *Transfusion* 2007; **47**: 74-79
- Ramia S, Ramlawi F, Kanaan M, Klayme S, Naman R. Frequency and significance of antibodies against hepatitis B core (anti-HBc) antigen as the only serological marker for hepatitis B infection in Lebanese blood donors. *Epidemiol Infect* 2005; **133**: 695-699
- Chaudhuri V, Nanu A, Panda SK, Chand P. Evaluation of serologic screening of blood donors in India reveals a lack of correlation between anti-HBc titer and PCR-amplified HBV DNA. *Transfusion* 2003; **43**: 1442-1448
- Duseja A, Sharma S, Subramanian PG, Agnihotri SK, Chakraborti A, Chawla Y. Occult hepatitis B virus (HBV) infection in healthy blood donors. *Indian J Pathol Microbiol* 2003; **46**: 690-692
- Saraswat S, Banerjee K, Chaudhury N, Mahant T, Khandekar P, Gupta RK, Naik S. Post-transfusion hepatitis type B following multiple transfusions of HBsAg-negative blood. *J Hepatol* 1996; **25**: 639-643

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