

Seropositivity of Hepatitis B Surface Antigen (HBsAg) Among Blood Donors at The Blood Bank of A Tertiary Care Hospital

Asha Purohit, Puja Jarwani* and S.M.Patel

Dept. of Pathology, GCS Medical College, Hospital and Research centre, Ahmedabad, Gujarat, India

ABSTRACT

Background: Blood is a scarce, but life saving resource. Hepatitis B virus (HBV) is transmitted by blood and blood products. Hence screening of hepatitis B surface antigen (HBsAg) among blood donors is important for safe blood transmission. The reports about the prevalence of HBV in the blood donors of Gujarat are limited and this study was conducted with an aim to find the seropositivity of HBV in the blood donors in a tertiary care hospital at Ahmedabad.

Methods: This is a retrospective analytical study conducted over a period of five years from 1st January 2012 to 31st December 2016. A total of 9353 donor blood samples were screened for HBsAg status using enzyme linked immunosorbent assay (ELISA). Reactive samples were retested in duplicate. The samples reactive in all three tests were considered positive. The samples which were reactive only in first test and non reactive on repeat testing were labeled as false positive. The samples reactive in any one of repeat testing were considered as positive.

Results: Out of 9353 blood donors screened 137(1.46 %) donors were initially reactive and 70(0.75 %) donors were reactive after triple testing. The observed seroprevalence of HBsAg was higher in replacement donors than in voluntary donors (0.60% vs. 0.15%, respectively). 100% seroreactivity was in male donors and no seroreactivity was observed in female donors

Conclusions: Our study showed similar HBsAg seroprevalence as reported by World Health Organization (WHO) statistics in low prevalence zone (less than 2%).

Keywords: Hepatitis B Surface Antigen, Seroprevalence, Blood Donors.

Introduction

HBsAg (Australia antigen) was found in serum of patients with multiple transfusions in hemophiliacs in 1965 in Philadelphia, in 1977 Blumberg was awarded noble prize for his discovery.^[1] Hepatitis B virus (HBV) is a highly infectious DNA virus from the Hepadnaviridae family that comprises eight genotypes (A to H). Hepatitis B has a prolonged incubation period (2 to 26 weeks) and remains in the blood until and during active episodes of acute and chronic hepatitis. HBV induced chronic liver disease is also an important precursor for the development of hepatocellular carcinoma even in the absence of cirrhosis. Countries with high prevalence rates of HBV usually also have high incidences of Hepatocellular Carcinoma (HCC), about 80% of HCC cases worldwide occur in developing countries, and nearly half of these are associated with chronic HBV infection. The disease is therefore important candidate for public health measures aimed at prevention, early diagnosis and treatment. Despite education and availability of drugs and vaccines it is estimated that 2 billion people have evidence of past or present infection with HBV worldwide and 248 million are chronic carriers of HBV surface antigen (HBsAg), particularly in low and middle-income countries (LMICs).^[2] 40 million chronic

carriers of HBV arise in India itself.^[3] Transfusion of infected blood is a key transmission route of HBV; other practices that confer risks for HBV infections include tattooing, piercing, acupuncture, multiple sex partners, surgeries and occupational and vertical transmission. Blood safety therefore remains an issue of major concern in transfusion medicine. Screening of blood donors for the presence of the Hepatitis B surface antigen (HBsAg) in their serum is an important method for preventing HBV transmission by blood transfusion.^[4] In all settings, screening of blood donors for HBsAg should be mandatory with linkage to care, counselling and treatment for those who are test positive.^[5] The incidence of transfusion-related spread has reduced greatly in recent decades due to screening of donated blood for HBsAg and exclusion of paid blood donors. All HBsAg positive donations should be considered to be at high risk of transmitting HBV and should not be released for transfusion.^[6] Countries are classified on the basis of endemicity of hepatitis B virus (HBV) infection into high (8% or more), intermediate (2-7%) or low (less than 2%) prevalence countries. India has intermediate endemicity of hepatitis B with HBsAg prevalence of 2-10% among the study population.^[7] The reports about the prevalence of HBV in the blood donors of Gujarat are limited and this study was conducted with an

aim to find the seropositivity of HBV in the blood donors in a tertiary care hospital at Ahmedabad. The study is aimed to determine the trend in hepatitis B infection, to compare the prevalence with that of other areas in India and with other countries. The results of these prevalence studies should help in the creation of long-term strategies to improve public health and to prevent spreading of the disease in the local population.

Materials and Method

This is a retrospective analytical study conducted over a period of five years from 1st January 2012 to 31st December 2016 in the blood bank of tertiary care hospital in Ahmedabad. A total of 9353 donors eligible to donate blood as per the criteria based on technical manual for transfusion medicine were included in the study.^[8] The family members, friends or relatives of the patients were categorized as replacement donors. People who donate blood without expecting any favor in return or in voluntary blood donation camps were classified as voluntary blood donors. At the end of the blood collection, donor samples were obtained for serological testing. Donor blood samples were screened for HBsAg status using Monolisa HBsAg ULTRA assay from Bio-Rad based on “sandwich enzyme linked immunosorbent (ELISA) principle” using monoclonal and polyclonal antibodies selected for their ability to bind themselves to the various subtypes of HBsAg recognized by World health organization (WHO) and most part of variant HBV strains. Test ELISA procedure was performed by strictly following manufacturer’s instructions. Sensitivity of the serological assay used was 100% and the analytical sensitivity was less than 0.06 ng/ml as per the kit insert. Initially reactive samples were retested in duplicate. The samples reactive all three times were considered positive. The samples which were reactive only in first test and nonreactive in any duplicate testing were labeled as false positive, the samples which were reactive in first test and reactive in one of the repeat testing were also considered as positive. Samples which were reactive at any stage and all the blood components

derived from it were discarded as per National Biomedical Waste Management policies.^[9] Confirmatory testing of reactive donations was undertaken for donor notification, counseling, referral for treatment, deferral or recall for future donation, and look-back on previous donations as per WHO guidelines.^[7] The year-wise statistics were calculated and prevalence rates are shown as percentages.

Results

Out of the total 9353 blood donors, 9277 (99.19%) were males and 76 (0.81%) were females. Table 1 shows that 69.42% of donors were categorized as replacement donors and the rest were voluntary blood donors. The initial screening test revealed that 137 (1.46%) were found to be initially seroreactive for HBsAg in the first assay. When the reactive samples were further tested by the ELISA in duplicate, 70 (0.75%) were found repeat reactive (seropositive as per WHO criteria) and 67 (0.72%) samples were negative. On repeat testing we were able to eliminate the false positives which accounted for 48.91% of all HBsAg reactive in first ELISA testing (Figure- 1).

Varying rates of HBsAg seroreactivity from 0.56% to 1.28% were observed during this period (2012-2016) (Table 2). When the trend was observed in the study period a decline was found in the seroprevalence rate of HBsAg in later years as compared to earlier years (Figure 2). The observed seroprevalence of HBsAg was higher in replacement donors than in voluntary donors (0.60% vs. 0.15%, respectively, Table 3). HBsAg seroreactivity was observed only among male donors and no female blood donors were positive for HBsAg (0.75% versus 0% respectively).

Discussion

Nowhere in the world is transfused blood considered 100% safe. HBV infection is common serious complication of blood transfusion. The current study presents the prevalence of HBV infection in blood donors of a tertiary care referral teaching hospital in Ahmedabad. Donor blood samples were screened for HBsAg status using Monolisa HBsAg ULTRA assay from Bio-Rad. Out of the 9353

Table 1: Donor category and gender distribution.

YEAR	Total donors	Voluntary		Replacement	
		Male	Female	Male	Female
2012	857	32	1	817	7
2013	1040	41	1	994	4
2014	1357	91	2	1252	12
2015	2724	699	17	1999	9
2016	3375	1955	21	1397	2
Total	9353	2818	42	6459	34

Table 2: Comparison of HBsAg reactive cases in five years (2012 – 2016).

Year	Total donors Screened	Total no. of HbsAg Reactive cases(no.)	Total no. of HbsAg Reactive cases (%)
2012	857	11	1.28
2013	1040	11	1.06
2014	1357	9	0.66
2015	2724	20	0.73
2016	3375	19	0.56
Total	9353	70	0.75

Table 3: Comparison of seroprevalence in various donor categories

Donors	HBsAg Positive	Prevalence
Replacement	56	0.60%
Voluntary	14	0.15%
Males	70	0.75%
Females	00	0%

Table 4: Comparison of HBsAg prevalence rate in different parts of India

Place	Prevalence
New Delhi ^[10]	2.23%
Kerala ^[11]	3.1%
Rural India ^[12]	7.62%
Maharashtra ^[13]	2.15%
Tamilnadu ^[14]	4.33%
Dehradun ^[15]	0.99%
Kolkatta ^[16]	1.66 %
Kanpur ^[17]	2.25%
Bangalore ^[18]	1.86%
Ahmedabad (Present study)	0.75%

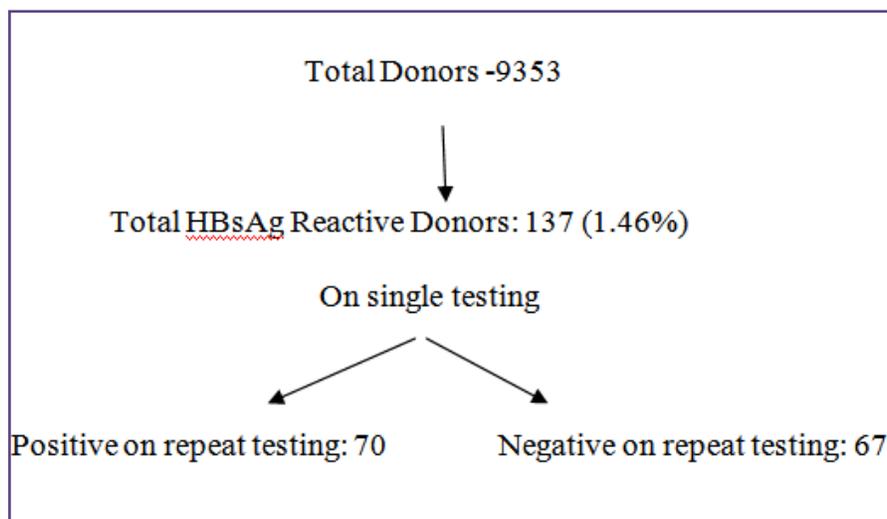


Fig. 1: Comparison between HBsAg reactivity by single/triple testing of donor samples.

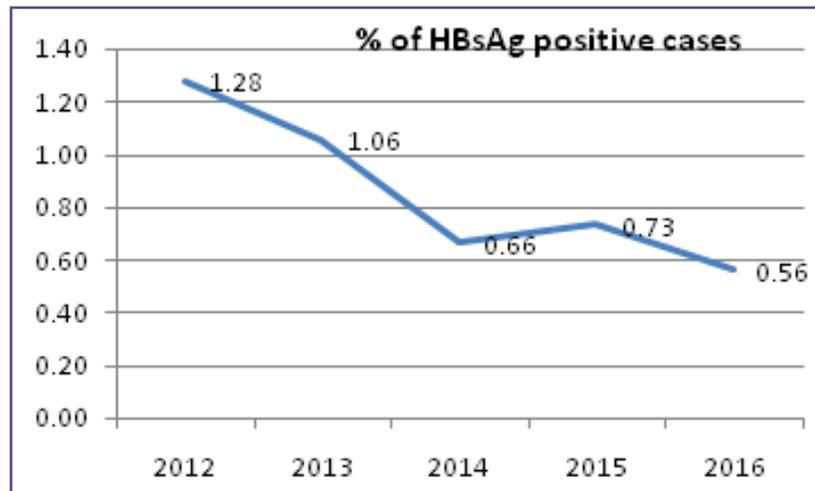


Fig. 2: Trend in seroprevalence of HBsAg over five years (Prevalence: 0.75%).

blood donations screened, 137(1.46%) were found to be initially seroreactive for HBsAg in the first assay. When the reactive samples were further tested by the same ELISA kit in duplicate, 70 (0.75%) were found repeat reactive and 67 (0.72%) samples were negative. Hence the seroreactivity of HBsAg was 0.75%. We observed the trends of HBsAg seroreactivity and it was found that it was variable but always in low prevalence zone (less than 2%) according to WHO statistics during the entire period.^[5] Decline in HBsAg seroreactivity was found in the later years. Among population-based studies HBsAg prevalence among general population groups ranged from 0.1% to 11.7%, being between 2% to 8% in most studies, HBsAg prevalence rate among blood donors ranged from 1% to 4.7%,^[7] our study showed seroreactivity in low prevalence zone (0.75%). In comparison with the other parts of India also the present study shows low seroprevalence of hepatitis B infection (Table no.4).^[10-18] The prevalence of HBsAg observed either higher or lower may depend on actual prevalence of HBV infection in general population, repetition of the initial seroreactive samples and technical errors causing high or low absorbance value. The present study revealed that HBV infection was more prevalent among replacement blood donors than voluntary donors (0.60% vs. 0.15%, respectively) as also noted in the study of Sonwane et al.^[12] In our scenario low prevalence of HBV might be due to strict screening of all the blood donors according to the technical manual for transfusion medicine and encouragement of voluntary donation. The awareness about the disease and modes of prevention may also be one reason for the low prevalence and declining trend in HBV infection. Seroprevalence was significantly high in male donors as compared to female donors. It is to be noted that the majority of our study population were males. A significantly higher HBsAg seroprevalence in

males than in females is also reported in other studies.^[19] It has been reported that the seroprevalence of HBsAg among blood donors is also lower in our neighboring countries, in Pakistan seroprevalence rate of HBV is 1.71% (Karachi).^[20] The seroprevalence rate of HBV was 0.35%-1.2% among blood donors in Nepal.^[21] Seroprevalence of HBsAg among blood donors was found to be 0.11% in Mexico (Veracruz).^[22] Nigeria has shown higher prevalence of hepatitis B antigen in blood donors i.e. 15% as reported by Ado A et al, 2010,^[23] it could be due to the higher prevalence of HIV, such countries come under high endemic countries. HIV and HBV are known to be transmitted sexually. The prevalence of HBV infection blood donors in Ghana was estimated to be 10.79%-11.59%.^[24] Studies in Egypt show 1.4% seroreactivity for HBsAg in blood donors.^[25] Considering the vast population of the country, even low prevalence amounts to large number of infected people. Prevention of transfusion-transmitted HBV infection in developed countries has been achieved by reducing unnecessary transfusions, using only regular voluntary donors, excluding donors with specific risk factors and systematic screening of all units of donated blood for HBV infection. By contrast, in many developing countries none of these interventions is applied uniformly and the risk of transfusion-transmitted infections remains high. If high sensitivity serological assays are not used, the safety of the blood for transfusion may become a big concern. Sensitivity of the serological assay used in our study was 100% and the analytical sensitivity was less than 0.06 ng/ml.

Strict measures in donor screening including better donor recruitment, promoting voluntary blood donation, screening of blood and blood products using high sensitivity serological assays, other infectious diseases

markers and more recently nucleic acid amplification technology (NAT) would considerably improve the current screening procedure for blood donation and enhance the safety of the blood intended for transfusion. Technological advancements have led to the development of more sensitive methods to detect various infectious disease markers, e.g., viral specific antigens, antibodies and nucleic acids in order to enhance the safety of blood transfusion. The implementation of a nucleic acid test (NAT) in the blood banks of developing countries requires known prevalence rates, pilot testing, and cost-benefit studies. It has been reported that NAT can detect hidden cases of hepatitis B (HBsAg-negative, NAT-positive), but it does not greatly reduce the window period (time from infection to first reactive test) compared to serological testing.^[26] Thus, the blood banks should consider the prevalence in each region. As the prevalence of HBV in our region is relatively low and has decreased over time, the potential impact of NAT may not justify the cost. Rational use of blood also needs to be promoted. Recent modifications to the official Indian regulations relating to transfusion medicine have ensured the adequate monitoring of blood banks and the safety of blood units. However, early detection of infection remains elusive goal to the existing problem of “Window period,” false negative results due to the limitation in the screening assays, genetic modifications in viral strains, and laboratory errors. Also these technologies are costly and not easily accessible in developing countries. Indeed, there is still a great difference in the safety of blood transfusions in developed versus developing countries, as the latter typically rank lower in donations, facilities, equipment, training, and supplies.^[27] Further studies are recommended among other blood donors and among different population groups in order to know the real prevalence and to study the epidemiology of disease. As hepatic diseases are already among the principal mortality causes in the Indian population, future research should also examine additional risk factors and seek to develop methods for early detection of HBV infections.

Abbreviations

ELISA: Enzyme linked immunosorbent assay

HBV: Hepatitis B virus

HBsAg: Hepatitis B surface antigen

HCC: Hepatocellular carcinoma

HCV: Hepatitis C virus

LMICs: low- and middle-income countries

WHO: World Health Organization

Acknowledgements

We thank the authorities of the G C S Hospital especially Dr S. M. Patel, Professor and head Department of Pathology. We also thank all the Blood Bank staff of GCS Hospital, for their support for present study.

References

1. The Liver, Biliary tract and Exocrine Pancreas. In: Harsh Mohan, editor. Harsh Mohan Text book of pathology. 7th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2015: 592.
2. WHO guidelines on hepatitis B and C testing. Geneva: World Health Organization; 2017.
3. Datta S. An overview of molecular epidemiology of hepatitis B virus (HBV) in India. *Viral J.* 2008; 5:156–67.
4. Pathak S, Chandrashekhar M. Nucleic acid testing: redefining security in blood screening. *Max-medical-journal.* 2010 September.
5. WHO guidelines on Screening donated blood for transfusion transmissible infections. Geneva: World Health Organization; 2010.
6. Neil D. Theis. Liver and Gall bladder. In: Kumar, Abbas, Aster, editors. Robbins and Cotran Pathologic Basis of Disease. 9th ed. Philadelphia: Elsevier Saunders; 2015:831-2.
7. Prevention of Hepatitis B in India-An Overview by World Health Organization. New Delhi : World Health Organization; 2002 August.
8. Saran RK. Transfusion Medicine: Technical Manual. 2nd ed. New Delhi: Directorate General of Health Services, Ministry of Health and Family Welfare, Govt. of India; 2003.
9. National Biomedical Waste Management policies. New Delhi: Ministry of Environment, Forest and Climate Change, Govt. of India; 2011.
10. Pahuja S, Sharma M, Baitha B, Jain M. Prevalence and trends of markers of Hepatitis C Virus, Hepatitis B Virus and Human Immunodeficiency Virus in Delhi blood donors: A Hospital Based Study. *Jpn J Infect Dis.* 2007; 60: 389- 91.
11. Mathai J, Sulochana PV, Satyabhama S, Nair PK, Sivakumar S. Profile of transfusion transmissible infections and associated risk factors among blood donors of Kerala. *Indian J Pathol Microbiol.* 2002; 45: 319-22.
12. Sonwane BR, Birare SD, Kulkarni PV. Prevalence of seroreactivity among blood donors in rural population. *Indian J of Med Sci.* 2003; 57: 405-7.
13. Prevention of hepatitis B in India, An overview. New Delhi: World Health Organization; 2002.
14. Singhvi A, Pulimood RB, John TJ, et al. The prevalence of markers for hepatitis B and human immunodeficiency viruses, malarial parasites and microfilaria in blood donors in a large hospital in south India. *J Trop Med Hyg.* 1990; 93: 178-82.

15. Chattoraj A, Behl R, Kataria VK. Infectious Disease Markers in Blood Donors. *Med J Armed Forces India*. 2008; 64: 33-5.
16. Bhattacharya P, Chandra PK, Datta S, et al. 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. *World J Gastroenterol* .2007; 21: 3730-3.
17. Behal R, Jain R, Behal KK, Bhagoliwal A, Aggarwal N, Dhole TN .Seroprevalence and risk factors for hepatitis B virus infection among general population in Northern India. *Arq Gastroenterol*.2008; 45: 137-40.
18. Srikrishna A, Sitalakshmi S, Damodar P .How safe are our safe donors? *Indian J Pathol Microbiol*.1999; 42: 411-6.
19. Karki S, Ghimire P, Tiwari BR, Rajkarnikar M. HBsAg serosurveillance among Nepalese blood donors. *Ann Trop Med PH*. 2008; 1: 15-8.
20. Nazar H, Nadia N, Shazia N, Zulfiqar A, Farhat A. Prevalence of hepatitis B and hepatitis C in blood donors of Karachi. *Biomedica*. 2008; 24: 116-7.
21. Tiwari et al. Seroprevalence of HBV and HCV in blood donors: A study from regional blood transfusion services of Nepal. *Asian J Transfus Sci*.2010; Jul 4 Suppl 2: 91-3.
22. López-Balderas et al. Hepatitis viruses in blood donors. *J Infect Dev Ctries*. 2015; 9 Suppl 3:274-82.
23. Ado A, Alhassan S, Chonoko UG, Samaila AU. Seroprevalence of hepatitis B surface antigen (HBsAg) among blood donors attending Ahmadu Bello University Teaching Hospital (Abuth), Zaria, Nigeria. *BAJOPAS*. 2010 ; 3: 20-2.
24. Dongdem et al. Prevalence of hepatitis B virus infection among blood donors at the Tamale Teaching Hospital, Ghana (2009). *BMC Research Notes*. 2012; 5:115.
25. Wasfi et al. Prevalence of hepatitis B surface antigen and hepatitis C virus antibodies among blood donors in Alexandria, Egypt .*EMHJ* .2011; 17 Suppl 3 :238-42.
26. Dwyre DM, Fernando LP, Holland PV. Hepatitis B, hepatitis C and HIV transfusion-transmitted infections in the 21st century. *Vox Sang*. 2011; 100: 92-8.
27. Global status report on blood safety and availability. Geneva: World Health Organization ; 2013

***Corresponding author:**

Dr. Puja Jarwani, 40, Niharika Bunglows, Nr.Azad Society,Nr.Himmmatlal Park, Ambawadi,Ahmedabad-380015,Gujarat,India.

Phone: +91 9825320312

Email: pujajarwani@gmail.com

Financial or other Competing Interests: None.