Original Article ISSN (O):2395-2822; ISSN (P):2395-2814

Seroprevalence of Hepatitis B and Association Among Various Factors Affecting Management of Hepatitis B in Blood Donors of a Tertiary Care Hospital in Western India.

Sushil Narang[1], Sonny Bherwani[2], Purvi R Dave[3], A S Saumya[4], Kaushal Y Vyas[5]

[1]Assistant Professor, Department of Gaastroenterology, V. S. Hospital, Gujarat.
[2]Resident, Department of Gaastroenterology, V. S. Hospital, Gujarat.
[3]Consultant Pathologist, Green Cross Laboratory.
[4]Pathologist, Apollo Hospital.
[5]Professor & HOD, Department of Gaastroenterology, V. S. Hospital, Gujarat

Received: March 2017
Accepted: March 2017

ABSTRACT

Background: Hepatitis B surface antigen (HBsAg) is a known indicator of chronic hepatitis B virus (HBV) infection. Also, levels of HBV DNA, alanine aminotransferase (ALT), hepatitis B e antigen (HBeAg), and liver biopsy support clinical decision making. This study was conducted to estimate the prevalence of HBsAg among blood donors in Western India, status of HBeAg in them, and association of HBeAg, DNA, ALT, and biopsy results. **Methods:** This was a prospective observational study. HBsAg positive blood donors were identified using blood bank records. Levels of DNA, ALT, status of HBeAg and liver histopathology were determined using standard methods. Association between different parameters was estimated using Fisher's exact test and multiple logistic regression model. **Results:** Out of 32518 blood donors screened, 234 (0.72%) were found to be HBsAg positive and 123 agreed to participate in the study. Eight patients (6.5%) were found to be HBeAg positive. High HBV DNA (>2000 IU/ml) was observed in 98 (79.7%) patients. 9 patients gave consent for biopsy and 8 of them had abnormal histopathological findings. We found lower odds (0.3901) of HBeAg positive status with lower DNA levels while, odds were higher with abnormal ALT levels (1.0144) and abnormal histopathology results (1.7519). ALT and DNA levels showed a trend of positive correlation. **Conclusion:** HBsAg prevalence was lower in blood donors and very few of them were HBeAg positive. Association study of DNA, ALT, HBeAg and biopsy status indicates that all of these parameters should be considered while treating chronic HBV.

Keywords: Blood donors, Hepatitis B, Seroprevalence, Transfusion.

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem with around 300 million people afflicted with chronic infection and at the risk of developing end-stage liver diseases.[1] Chronic infection accounts for over 686000 deaths annually due to complications of hepatitis B, including cirrhosis and liver cancer.[2] It is estimated that 10-15% of world's HBV carriers reside in India, which sums to around 40 million people.[3] About 15-20% of hepatitis B surface antigen (HBsAg) carriers are estimated to die prematurely due to cirrhosis and hepatocellular carcinoma (HCC).[3]

Name & Address of Corresponding Author
Dr. Sonny Bherwani
Resident, Department of Gaastroenterology,
V. S. Hospital, Gujarat.

HBV infection follows a complicated natural history that is influenced by many factors: those associated with host (such as age at infection, sex, immune status), those associated with virus (such as genotype of the virus, mutation, and serum levels of viral DNA), and miscellaneous factors (such as other hepatic infections or alcoholism).[4] A poor understanding of the natural history, heterogeneity of the disease, and asymptomatic presentation at the early stage have rendered the disease a clinical challenge for management.

According to World Health Organization (WHO), the presence of 'e' antigen of HBV (HBeAg) generally marks the high viral DNA levels in the host.[5] The inactive HBsAg carrier is diagnosed by levels of HBeAg, HBV DNA, alanine amino transferase (ALT); necroinflammation, fibrosis and histology.[4] HBsAg and anti-HBe positive carriers who have normal ALT, HBV DNA <2000 IU/ml and minimal or no hepatic histopathology are defined as inactive carriers.[1] All of the three factors, HBV DNA levels, ALT levels, and HBeAg status form the basis of decision to treat chronic hepatitis B.[6]

HBV is transmitted by exchange of body fluids. Blood transfusion is a common mode of HBV transmission therefore, transfusion medicine

focuses on the safety of blood and blood products.[7] Increasing demand of blood transfusion secondary to various medical conditions increases the risk of HBV infection through contaminated blood, therefore, WHO recommends testing blood donors for HBsAg.[8] To best of our knowledge, there are only a few Indian studies focusing specifically on blood donors to investigate the various epidemiological parameters and especially the association of indicators of chronic HBV infection. Therefore, this study was conducted to estimate the seroprevalence of HBsAg in blood donors of the Western India, HBeAg status in HBsAg positive blood donors and its association with HBV DNA level, ALT level, age of patients, and hepatic histopathology.

MATERIALS AND METHODS

Study design

This prospective observational study was conducted at Department of Gastroenterology, VS Hospital, Smt. NHL Municipal Medical College, Ahmedabad, India, during September 2015 to September 2016. The study was constructed of four visits: a screening visit and three follow up visits. Patients were interviewed and all the baseline data were collected at first visit. Based on ALT, HBV DNA, HBeAg, and histopathology, we categorized the patients who needed treatment. Such patients were started with treatment; change in ALT levels from baseline was determined at subsequent follow up visits every three months till a period of one year.

Patients

Blood donors who were found HBsAg positive during pretransfusion screening and had consented to be informed about their HBsAg status in their requisition forms, filled prior to the donation were included, irrespective of their age and sex. Few donors who came to the out-patient department and were detected HBsAg positive during blood donation at some other place were also included.

Study protocol was approved by the Institutional Review Board and was the study conducted in agreement with the ethical principles that have originated from Declaration of Helsinki and are consistent with Good Clinical Practices and applicable regulatory requirements. All the patients provided informed consent before enrolment.

Assessments

HBsAg prevalence

HBs Ag prevalence was estimated retrospectively. Data over the previous 5 years (form the study commencement date) were collected from blood banks of VS Hospital, NHL Medical College, Ahmedabad, India. Donors who were detected HBs Ag positive during pretransfusion screening were contacted via post and/or telephone and were requested to contact the department of Gastroenterology, those who responded positively by coming to the department were evaluated.

Patient Interview

Patients were interviewed on the basis of a predesigned questionnaire to investigate various factors associated with HBV infection including, possible modes of transmission, personal, social and vocational high risk behaviour (such as alcohol use or smoking, sexual behaviour, tattooing) and medical history (such as jaundice, blood transfusion, surgery). Physical and systemic examinations were also carried out to detect the signs and symptoms of liver disease.

Laboratory tests and biopsy

Various tests were conducted on all the patients at first visit including liver function tests (Beckman kit and auto-analyser; Beckman coulter Inc., USA), HBeAg seropositivity estimation (Bioline test kit; Standard diagInc, Korea), test for HBV DNA (real-time polymerase chain reaction; COBAS TaqMan, Roche Diagnostics, Indianapolis, USA), and ultrasonographic screening of the liver. Normal ALT was defined up to the upper limit of testing for the available Kit which was 42 IU/L. The lower limit of detection of the real-time polymerase chain reaction test was 3.8IU/ml.

Liver biopsy was advised to patients older than 40 years with normal ALT who had high HBV DNA levels (>20000 IU/ml for HBeAg positive and >2000 IU/ml for HBeAg negative patients) and patients older than 40 years with abnormal ALT who had low HBV DNA levels (<20000 IU/ml for HBeAg positive and <2000 IU/ml for HBeAg negative patients).[9] Patients were explained about the need for liver biopsy, the procedure involved and the possible risk of the procedure. Those who consented, underwent liver biopsy using an 18 gauge Trucut needle as an inpatient procedure. Adequate liver biopsy was considered if more than 3 portal triads were present for assessment. Histological activity index (HAI) was assessed using the modified Knodell score;[10] a score more than 3 was considered abnormal.

Statistical Analysis

Data were presented using descriptive statistics generally. Continuous HBV DNA values were transformed into dichotomous data as high (≥2000IU/ml, a strong predictor of HCC development) or low,[11] baseline ALT values were dichotomized as abnormal (≥42 IU/ml) or normal, histopathology results as abnormal (HAI>3) or normal, and age of the patients as greater or equal to 40 years or lesser. Association between two categorical variables was assessed using Fisher's exact test at two-tailed P values. A multivariable logistic regression model was applied to study the association among HBeAg status, HBV DNA load, Age, and histopathological results. Odds ratios (OR) were calculated at 95% confidence interval (CI). A p value of <0.05 was considered statistically

significant. SPSS version 16 (SPSS, Inc., Chicago, IL, USA) was used to analyse data.

RESULTS

During the study period, 32518 blood donors were screened and 234 (0.72%) were found to be HBsAg positive. Out of these, 123HBsAg positive donors agreed to participate in the study. The mean age of the patients was 35.11 years (8.074) and most of them (n=94, 76.4%) were men [Table 1].

Table 1: Demographics and baseline characteristics of study patients, (n = 123).

Demographics	
Age, mean (SD)	35.11 (8.074)
Sex, male, n (%)	94 (76.4)
Personal and social behaviour n (%)	
Alcohol consumption	13 (10.5)
Smoking	2 (1.6)
High risk sexual behaviour	11 (8.9)
Tattooing	21 (17.0)
Medical history n (%)	
Jaundice	9 (7.3)
Blood transfusion	3 (2.4)
Surgery	6 (4.9)
IV therapy	6 (4.9)
Dental procedure	12 (9.8)
Hepatitis B in family	8 (6.5)
Family history of HBeAg positivity	6 (4.99)
Signs and symptoms of liver disease n (%)	
Pallor	7 (5.7)
Icterus	0
Pedal edema	0
Ascites	0
Hepatomegaly	0
Abnormal ultrasonography	13 (10.6)

Baseline characteristics

Among all the high risk behaviours presumed to be associated with HBV infection, most of the patients (n=21, 16.85%) were associated with tattooing and had a history of dental procedures (n=12, 10.11%). Other than pallor, no sign or symptom of liver disease was observed. Twenty six patients (21.1%) had abnormal ALT at baseline, eight patients (6.5%) were HBeAg positive, ninety eight (79.7%) had high HBV DNA [Table 1].

Ultrasonography findings were normal in 110 (89.9%) of the patients. The other thirteen patients had fatty liver as the only abnormal finding on ultrasonography. Based on the pre-decided criteria, liver biopsy was suggested for 29 (23.6%) donors. Of these 9 gave consent for biopsy and 8 of them had abnormal histopathological findings (HAI >3). Seventy nine (64.2 %) were truly inactive carriers (based on the serological, biochemical and DNA assays) that had persistently normal ALT with low HBV DNA levels.

Only one of the 9 patients who underwent biopsy had low HAI and no fibrosis scores [Table 2]. Of the 9 patients who underwent biopsy, 5 had abnormal ALT and all of them had abnormal histopathology, while 4 patients had normal ALT but 3 of them had abnormal histopathology. Six patients had high HBV DNA levels and all had abnormal histopathology, while 3 had low HBV DNA levels and of these 2 had abnormal histopathology. Of the 8 patients who had abnormal histopathological findings, 7 were HBeAg negative and one was HBeAg positive. The patient with normal histopathological findings was also HBeAg negative.

Table 2: Histopathological finding of patients, (n=9).

Patient no	HAI	Fibrosis score
1	4	F1
2	6	F2
3	2	F0
4	4	F2
5	7	F2
6	4	F1
7	4	F1
8	7	F3
9	4	F4

Association of HBeAg, HBV DNA, ALT,and age

No statistically significant association was found between the HBeAg status and HBV DNA (Fisher's exact P value = 0.2595), HBeAg status and ALT values (Fisher's exact P value = 0.7353), HBeAg status and age (Fisher's exact P value = 0.7203), and HBeAg status and histopathological results (Fisher's exact P value = 0.4656).

The ALT level and mean HBV DNA levels seemed to have a positive correlation. In patients with normal ALT the mean HBV DNA levels were 1.83x105IU/ml while, 2.56x107IU/ml in those with abnormal baseline ALT. Similarly, in patients with a high HBV DNA, the mean baseline ALT value was 50.71 IU/ml and 29.97 IU/ml in those with low HBV DNA levels. Lower odds (OR, 0.3901) of HBeAg positive status was obtained with lower DNA levels. Conversely, odds were higher with abnormal ALT (OR, 1.0144) and abnormal histopathology (OR, 1.7519), however the odds ratios were not statistically significant [Table3].

Table 3: Multiple logistic regression analysis of HBeAg positive status with HBV DNA, Age, ALT, and histopathological results, (n = 123).

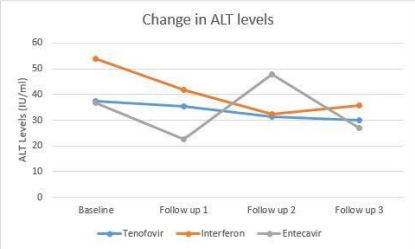
Variable	Coefficient	SE	OR	95% CI	P value
HBV DNA (Low vs high)	0.9414	0.8815	0.3901	(0.0693 - 2.1954)	0.2856
Age (>40 vs <40)	-0.0249	0.0412	0.9754	(0.8997 - 1.0575)	0.9754
ALT (Abnormal vs normal)	0.0143	0.0090	1.0144	(0.9968 - 1.0324)	0.1100
Histopathology results (Abnormal vs normal)	0.5607	1.1439	1.7519	(0.1862 - 16.4877)	0.6240

Treatment and follow up

Based on the status of ALT, HBV DNA, HBeAg, and histopathology, 11 patients were started with

antiviral therapy. Four patients were treated with tenofovir, three patients were treated with interferon, and four patients were treated with entecavir. ALT was reduced clearly from baseline to third follow up visit in patients treated with tenofovir. Patients treated with interferon also showed decrease in ALT until second follow up visit, which increased slightly at last visit. Patients treated with entecavir also had a decrease in ALT level from baseline to third follow up visit [Figure 1].

Figure 1. Change in ALT levels in patients treated with antiviral therapy, (n=11)



DISCUSSION

This study was conducted among blood donors in Western India to determine various epidemiological parameters of HBV infection. We estimated the seroprevalence of HBsAg, HBeAg status amongst HBsAg positive donors and the association of HBeAg status with age of the patients, host HBV DNA load, ALT levels, and the hepatic histopathology.

In our study the prevalence of HBsAg was found to be (0.72%). This is lesser than few of the previously reported prevalence in Indian studies on blood donors that ranges from 3.36% to 2.25%.[12,13] However, results from another Indian study on same population group reported an HBsAg seroprevalence of around 0.62%,which is comparable to our estimate.[14] The lower prevalence could be explained by the increasing awareness about the disease and the modes of prevention among general population. Also, the implementation of strict pre-donation counselling and donor selection criteria helped in excluding possibly infected donors.

Male blood donors have been reported to predominate and also have a greater seroprevalence compared with females in previously reported studies.[13,15] In our study also, males were more common than females (76.4%). Further, in HBeAg positive chronic hepatitis patients, male have been reported to be more common than female.[16.17] We also found comparable results (male: female = 2) in HBeAg positive patients. An Indian study involving 20000 blood donors found that the maximum seroprevalence of HBsAg was in the age group of 35-45 years, which was comparable to the mean age of our study population.[13] This again corroborates with the statement of Sharma et al that HBeAg positive chronic hepatitis is usually presented during third to fourth decade of life.[4]

6.5% patients were found to be HBeAg positive in our study. Two of the studies conducted among Indian patients report a higher HBeAg prevalence. Kurien et al. found an HBeAg positivity of 23.5% among those who were HBsAg positive while,[18] Kumar et al. found a HBeAg positivity rate of 43.5%.[19] Lower prevalence rate observed in our study may be due to smaller sample size compared with that of the previous studies.

Abnormal baseline ALT was found in 21.1% of patients, comparable results were observed by Chan et al. who reported abnormal ALT in 22% of HBeAg negative hepatitis B carriers.[20]

Every patients who had abnormal histopathology did not have abnormal ALT of higher DNA levels. This indicates that ALT or HBV DNA may not be alone indicators of disease activity and histopathology is also an important predictor, however, due to the small number of biopsies being performed, we cannot draw a strong conclusion.

Various researchers have found a strong positive correlation between abnormal liver biopsy finding, high HBV DNA load and abnormal ALT levels.[19,21,22] No correlation was found between abnormal histopathological findings and HBeAg status in our study. Kumar M et al. also did not find correlation between abnormal histopathology and HBeAg status.[19] Also, DNA levels did not show a statistically significant association with HBeAg status. Koyuncuer and colleagues also did not find a significant association between these two markers. However, they found an association between ALT and HBeAg status.[23] We also found a trend of greater odds of HBeAg positive status with abnormal values. The number of HBeAg positive patients in our study was very small for any actual comparison.

Patients who were started with treatment based on status of ALT, DNA, HBeAg and histopathology, showed a tendency of decrease in ALT during follow up visits. However, conclusive results could not be drawn due to shorter duration of follow up.

There were a few limitations of our study. The biopsy was not done is most of our donors so the actual disease activity could not be assessed. The follow up period was short to actually evaluate the progression of the inactive carriers. The number of HBeAg positive donors was very few making it difficult to compare the association of ‘e’ antigen presence or absence to disease activity.

CONCLUSION

Results of this study show that prevalence of hepatitis B among the blood donors is overall low but higher in males. Also, a smaller proportion of HBsAg positive patients is HBeAg positive. Abnormal ALT correlated positively with the high

HBV DNA levels thus, both are indicators of HBV infections however, histopathology should also be taken into consideration. Other studies with larger sample size and longer duration of follow up could further validate these results.

Acknowledgement

We are thankful to all the patients who consented to participate in this study.

REFERENCES

1. Invernizzi F, Viganò M, Grossi G, Lampertico P. The prognosis and management of inactive HBV carriers. Liver IntOff J IntAssoc Study Liver. 2016 Jan;36Suppl 1:100–4.
2. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet. 2015 Jan 10;385(9963):117–71.
3. Puri P. Tackling the Hepatitis B Disease Burden in India. J ClinExpHepatol. 2014 Dec;4(4):312–9.
4. Sharma SK, Saini N, Chwla Y. Hepatitis B Virus: Inactive carriers. Virol J. 2005 Sep 28;2:82.
5. WHO | Hepatitis B [Internet]. WHO. [cited 2016 Nov 27]. Available from: http://www.who.int/mediacentre/factsheets/fs204/en/
6. Nguyen MH, Keeffe EB. Are Hepatitis B e Antigen (HBeAg)–Positive Chronic Hepatitis B and HBeAg-Negative Chronic Hepatitis B Distinct Diseases? Clin Infect Dis. 2008 Nov 15;47(10):1312–4.
7. Dongdem JT, Kampo S, Soyiri IN, Asebga PN, Ziem JB, Sagoe K. Prevalence of hepatitis B virus infection among blood donors at the Tamale Teaching Hospital, Ghana (2009). BMC Res Notes. 2012 Feb 22;5:115.
8. ScreeningDonatedBloodforTransfusion.pdf [Internet]. [cited 2016 Nov 27]. Available from: http://www.who.int/bloodsafety/ScreeningDonatedBloodforTransfusion.pdf
9. Hadziyannis SJ, Papatheodoridis GV. Hepatitis B e antigen-negative chronic hepatitis B: natural history and treatment. Semin Liver Dis. 2006 May;26(2):130–41.
10. Manesis EK, Papatheodoridis GV, Sevastianos V, Cholongitas E, Papaioannou C, Hadziyannis SJ. Significance of hepatitis B viremia levels determined by a quantitative polymerase chain reaction assay in patients with hepatitis B e antigen-negative chronic hepatitis B virus infection. Am J Gastroenterol. 2003 Oct;98(10):2261–7.
11. Chu CM, Karayiannis P, Fowler MJ, Monjardino J, Liaw YF, Thomas HC. Natural history of chronic hepatitis B virus infection in Taiwan: studies of hepatitis B virus DNA in serum. HepatolBaltim Md. 1985 Jun;5(3):431–4.
12. Thakur TS, Goyal A, Sharma V, Gupta ML, Singh S. Incidence of australia antigen (HBs Ag) in Himachal Pradesh. J Commun Dis. 1990 Sep;22(3):173–7.
13. 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. ArqGastroenterol. 2008 Jun;45(2):137–40.
14. Singh K, Bhat S, Shastry S. Trend in seroprevalence of Hepatitis B virus infection among blood donors of coastal Karnataka, India. J Infect Dev Ctries. 2009 Jun 1;3(5):376–9.
15. Chandrasekaran S, Palaniappan N, Krishnan V, Mohan G, Chandrasekaran N. Relative prevalence of hepatitis B viral markers and hepatitis C virus antibodies (anti HCV) in Madurai, south India. Indian J Med Sci. 2000 Jul;54(7):270–3.
16. Burk RD, Hwang LY, Ho GY, Shafritz DA, Beasley RP. Outcome of perinatal hepatitis B virus exposure is dependent on maternal virus load. J Infect Dis. 1994 Dec;170(6):1418–23.
17. Cacciola I, Cerenzia G, Pollicino T, Squadrito G, Castellaneta S, Zanetti AR, et al. Genomic heterogeneity of hepatitis B virus (HBV) and outcome of perinatal HBV infection. J Hepatol. 2002 Mar;36(3):426–32.
18. Kurien T, Thyagarajan SP, Jeyaseelan L, Peedicayil A, Rajendran P, Sivaram S, et al. Community prevalence of hepatitis B infection and modes of transmission in Tamil Nadu, India. Indian J Med Res. 2005 May;121(5):670–5.
19. Kumar M, Sarin SK, Hissar S, Pande C, Sakhuja P, Sharma BC, et al. Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. Gastroenterology. 2008 May;134(5):1376–84.
20. Chan HL, Leung NW, Hussain M, Wong ML, Lok AS. Hepatitis B e antigen-negative chronic hepatitis B in Hong Kong. HepatolBaltim Md. 2000 Mar;31(3):763–8.
21. Dixit VK, Panda K, Babu AV, Kate MP, Mohapatra A, Vashistha P, et al. Asymptomatic chronic hepatitis B virus infection in northern India. Indian J GastroenterolOff J Indian SocGastroenterol. 2007 Aug;26(4):159–61.
22. Madan K, Batra Y, Jha JK, Kumar S, Kalra N, Paul SB, et al. Clinical relevance of HBV DNA load in patients with chronic hepatitis B infection. Trop Gastroenterol Off J Dig Dis Found. 2008 Jun;29(2):84–90.
23. Koyuncuer A. Associations between HBeAg status, HBV DNA, ALT level and liver histopathology in patients with chronic hepatitis B. Sci J Clin Med. 2016;3(6):117–23.

How to cite this article: Narang S, Bherwani S, Dave PR, Saumya AS, Vyas KY. Seroprevalence of Hepatitis B and Association Among Various Factors Affecting Management of Hepatitis B in Blood Donors of a Tertiary Care Hospital in Western India. Ann. Int. Med. Den. Res. 2017; 3(3):ME13-ME17.

Source of Support: Nil, **Conflict of Interest:** None declared