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Original Research Article

# Prevalence of Antibody to Hepatitis B Core Antigen among Healthy Blood Donors in Western Rajasthan

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**Abstract**

**Background:** Hepatitis B is a serious global infectious disease and a major cause of acute and chronic hepatitis, cirrhosis and primary hepatocellular carcinoma worldwide. Transfusion-transmitted HBV infection is increasingly becoming a major mode of transmission developing countries like India. Since the presence of HBsAg cannot be detected in the window period, detection of Anti-HBc can be useful in detecting infection.

**Methods:** The study was conducted on 900 healthy blood donors using 5 mL of venous blood collected in a clean sterile glass tube. Anti-HBc was detected using an invitro detecting kit and confirmation of infection was done using NAT analysis.

**Results:** There were 69 samples that were reactive for anti-HBc but negative for HBsAg. That is 7.6% of the total sample size (900).The incidence of HBV DNA positivity in anti HBc positive sample (77) was 29.87%.

**Conclusion:** The blood donors should therefore be checked for anti-HBc in addition to HBsAg, and those found positive for anti-HBc should submitted to HBV DNA testing before giving their blood to the recipients.

**Keywords:** HBsAg, Anti-HBc, HBV-DNA, window period, blood donors.



## Introduction

Hepatitis B is a serious global infectious disease causing significant morbidity and mortality. It is the tenth leading cause of death in the world, with two billion people infected, and an estimated 400 million suffering from chronic hepatitis B virus (HBV) infection [1,2]. Hepatitis B virus causes acute and chronic hepatitis, cirrhosis and primary hepatocellular carcinoma worldwide [3].

HBV is highly contagious. The routes of HBV transmission are percutaneous, permucosal routes, infected blood or body fluids, sexual contact or by the use of contaminated needles. Transfusion-transmitted HBV infection is increasingly becoming a major mode of transmission in the high-prevalence areas in developing countries like India. There is high incidence of medical and surgical conditions in India that demand blood transfusion, and so the possibility of the transmission of HBV (and other blood-

pathogens) through contaminated blood increases several fold.

The discovery of the Hepatitis B surface antigen (HBsAg) was a major breakthrough in decreasing the incidence of post transfusion hepatitis. Following infection by the Hepatitis B virus (HBV), the first serological marker to appear in the blood is the HBV DNA, followed by HBsAg, the DNA polymerase and the hepatitis B 'e' antigen (HBeAg). Thereafter, the antibodies to the hepatitis B core antigen (anti-HBc), hepatitis B 'e'-antigen and the HBsAg can be detected. Screening of donated blood by Enzyme-linked immune sorbent assay (ELISA) for HBsAg is the common method for detecting hepatitis B infection. Screening of blood for the detection of this viral marker, however, does not rule out the risk of transmission of hepatitis B, because during the host serological response to infection, there is a phase during which the HBsAg cannot be detected in the blood. This phase is called the 'window period', which represents the carrier state of the disease [4].

During the window period, detection of the antibody to the hepatitis B core antigen (anti-HBc) serves as a useful serological marker for hepatitis B infection. The IgM class of the anti-HBc is the first to appear, and indicates a recent infection. The IgG variety of anti-HBc appears later during the infection and points to a past HBV infection. Individuals with IgG variety of anti-HBc may not be infectious as they may have sufficiently high titers of antibodies to HBsAg (anti- HBs), which are protective in nature and the affected individuals may actually be disease free.

It is strongly felt that Anti-HBc should be utilized as a serological marker to detect active infection during the window period in high endemic countries like India to decrease the transmission of Hepatitis B further.

The aim of the present study is to determine the prevalence of anti-HBc antibody among blood donors, and its correlation with the presence or absence of HBsAg antigen and HBV DNA, to establish the relevance of Anti-HBc testing in HBsAg negative blood.

## Material & Methods

After obtaining the institutional ethical clearance and informed consent of the participants, a total of 900 healthy blood donors were included in the study, conducted at the blood bank, Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan).

Out of 900, 851 participants were males and 49 were females.

All these blood donors were screened for Anti-HBc along with other mandatory screening tests (Antibody to HIV 1 and 2, HBsAg, Antibody to HCV, Malaria and Syphilis)

At the time of blood donation, 5 mL of venous blood was collected in a clean sterile glass tube for screening of the above mentioned markers using ELISA. An in vitro diagnostic kit was used for the qualitative detection of Anti-HBc concentrations. The test is an enzyme immunoassay based on competitive inhibition principle.

The test was considered valid if the Mean absorbance of Negative Controls was greater than 0.8 and Mean absorbance of Positive Controls was less than 0.1. Cut-off values were calculated as 0.3 times mean absorbance of Negative Controls. Specimens with an absorbance greater than or equal to the cut-off value were considered Non-Reactive and those with an absorbance less than the cut-off value were considered Reactive.

The samples were then sent to the Advance Basic Sciences and Clinical Research Laboratory, Department of Microbiology and Immunology, S.M.S. Medical College, Jaipur, for further

confirmation of Hepatitis B Virus infection by NAT Testing.
Data was collected and statistical analysis was performed.

## Results

The age and sex wise distribution of the participants is shown in Table no. 1. Majority of the donors were males (851 out of 900) and only 49 were females. 60% of the donors were in the age group of 18 to 30 years.

Figure no. 1 demonstrates the proportion of samples that were reactive for various markers. Only 1.22% samples were positive for HBsAg whereas 8.55% samples (77 out of 900) were positive for Anti-HBc. Out of 77, 35 patients were in the age group of 18-30 years, and 27 were in the group of 31-40 years. Remaining 15 were 41 or older. 75 out of 77 were males and 2 were females.

Table no. 2 shows the correlation of Anti-HBc positivity with the presence or absence of HBsAg antigen. There were 69 samples that were reactive for anti-HBc but negative for HBsAg. That is 7.6% of the total sample size (900). Only 26% (18/69) showed the presence of HBV DNA by NAT Testing, while the remaining 73% (51/69) were HBV DNA negative. These constitute 5.6% (51/900) of total donor population.

**Table 1: Age and Sex wise distribution of Blood Donors**

| S. No. | Age (year) | Sex | | Total | Percentage (%) |
|---|---|---|---|---|---|
| | | Male | Female | | |
| 1. | 18-30 | 525 | 19 | 544 | 60.00 |
| 2. | 31-40 | 235 | 19 | 254 | 28.22 |
| 3. | 41-50 | 79 | 09 | 88 | 9.78 |
| 4. | 51-60 | 12 | 02 | 14 | 1.56 |
| Total | | 851 | 49 | 900 | 100 |

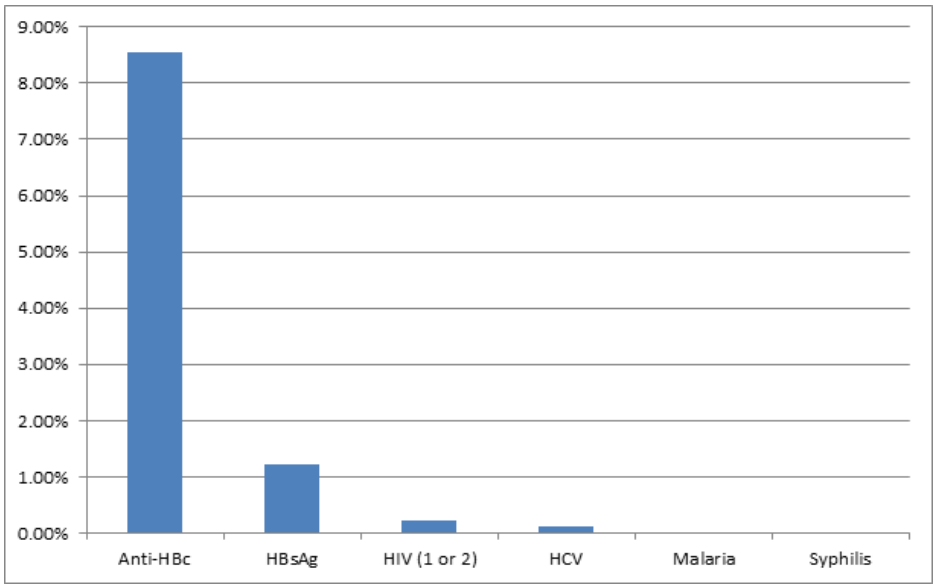


**Figure 1: Serum reactivity of various Markers**

**Table 2: Correlation of HBs Ag and Anti HBc (n=900)**

| HBs Ag | Anti HBc | No. | Percentage |
|---|---|---|---|
| Positive | Non Reactive | 3 | 0.33% |
| Positive | Reactive | 8 | 0.88% |
| Negative | Reactive | 69 | 7.6% |

## Discussion

The diagnosis of HBV infection is based on the presence of HBsAg in the bloodstream [5]. The introduction of reliable serologic screening of blood before donation has made post transfusion hepatitis rare, however, the occult HBV infection (donors who are negative for HBsAg but have detectable circulating HBV DNA) is still a concern regarding the safety of blood supply [6]. Donors with occult HBV infection who lack detectable HBsAg, but exposed to HBV infection (as indicated by a positive anti HBc and HBV DNA) are a potential source of HBV infection [7].

Anti-HBc screening is not mandatory in India. The prevalence of anti-HBc in the prospective blood donors is proportional to the incidence of HBsAg in the general population. It varies greatly in different ethnic groups and geographical locations. It is higher among groups known to be at increased risk of HBV infection e.g. intravenous drug abuse, sexual promiscuity, low economic status, health care workers etc. Studies conducted across India show significance prevalence of antiHBc in the population. Chaudhuri et al [8] revealed the prevalence of anti-HBc to be 10.82% in 2003, and Bhattacharya et al [9] reported a high positive rate of 18.3% in 2007 in West Bengal. Dhawan et al [10] from Chandigarh reported a core positive rate of 8.4% (2008) and Makroo et al [11] from New Delhi reported a core positive rate of 10.2% (2012). These rates are comparable to our study where we found the prevalence rate of antiHBc to be 8.5% (77 out of 900) in 2013.

Since HBsAg detection is being routinely done, it is essential to establish the proportion of antiHBc positive patients who are HBsAg negative. In our study, the proportion of such patients is 7.6%. Amini et al [12] in 1993 found in that 5.1% of their subjects were positive for anti-HBc without having any detectable HBsAg, however, they did not determine the presence of HBV-DNA. R.N. Makroo et al [13] reported the proportion to be 8.13%. Banerjee et al [14] reported a very high prevalence of 'antiHBc only' cases (58.8%) in Kolkata with 22.8% positive for HBV DNA also. In our study 29.87% (23/77) of anti-HBc positive cases were also positive for HBV DNA. Similarly, a study done by Mohammad Asim et al (2010) [15] showed 7.5% (31/413) samples that were positive for both AntiHBc and HBV DNA.

In our study, 18 patients (out of 69 anti-HBc positive and HBsAg negative) were positive for HBV DNA by NAT testing. That is 2% of the total study sample. This result is higher than but comparable to the study done by Makroo et al [11] in 2012 at New Delhi, where the results were 0.15% (13/8660). The subjects that are negative for HBV DNA can be considered safe and their blood fit for donation. A number of studies report cases of HBV transmission after the transfusion of anti-HBc-positive blood [16].

Bhattacharya et al [9] from eastern India reported 18.3% HBV DNA positivity in 'anti-HBc only' group. Duseja A et al [17] from Chandigarh (India) showed 0% prevalence of HBV DNA.

A study by Satake et a1 [18] from Japan found that only 11 of 33 recipients of anti-HBc positive, HBV DNA positive blood became HBV infected, while 11 of 22 persons who were anti-HBc negative but HBV DNA positive became infected. Therefore, HBV DNA positivity is associated with an increased risk of transmission.

Sawke and Sawke [19] observed that as India has a high prevalence of anti-HBc, screening of donor blood for total anti-HBc is not practical and should not be used as a criterion to discard blood. Screening for IgM anti- HBc of blood units negative for HBsAg, on the other hand, could identify potentially infectious units. El-Sherifet al [20] have proposed a policy of testing blood donors for anti-

HBc in addition to HBsAg and those found positive for anti-HBc to be submitted to HBV DNA testing. This approach would be less expensive, would reduce the risk of transfusion-transmitted HBV infection, and decrease the rejection rate of the precious units of collected. [21]

The exclusion of anti-HBc positive units from the donor pool is not practical in areas with intermediate HBsAg prevalence rates such as India as this would result in unacceptably high rates of donor rejection. As the western countries have a low incidence of HBsAg, the prevalence of anti-HBc in their blood donors is also low.

Our present study highlighted the serious concerns regarding the safety of the blood supply seven after donor screening for HBsAg. The incidence of HBV DNA positivity in anti HBc positive sample was 29.87%. Residual risk due to donations of blood during the window period can therefore be managed by using NAT assays.

## Conclusion

There were 69 samples that were reactive for anti-HBc but negative for HBsAg. That is 7.6% of the total sample size (900).The incidence of HBV DNA positivity in anti HBc positive sample(77) was 29.87%. The blood donors should therefore be checked for anti-HBc in addition to HBsAg, and those found positive for anti-HBc should submitted to HBV DNA testing before giving their blood to the recipients.

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