

## Seroprevalence Study : Antibodies to Hepatitis B Core Antigen among Blood Donors in Chattogram

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### Abstract

**Background:** Hepatitis B Virus (HBV) remains a major global health concern, particularly in regions with limited screening. Antibodies to the Hepatitis B core antigen (anti-HBc) indicate past or ongoing infection and help identify potential carriers. This study aims to assess the seroprevalence of anti-HBc among HBsAg-negative blood donors in Chattogram.

**Materials and methods:** This cross-sectional observational study included 100 voluntary blood donors who were screened for anti-HBc (IgM and IgG) and anti-HBs using Electrochemiluminescence Immunoassay (ECLIA) and Chemiluminescent Microparticle Immunoassay (CMIA) respectively.

**Results:** The results revealed that 21% of the HBsAg-negative donors were positive for anti-HBc, indicating previous or ongoing hepatitis B virus (HBV) infection. Notably, 76.2% of the anti-HBc-positive individuals were negative for anti-HBs, suggesting a potential risk of HBV reactivation or occult infection. Homemakers had the highest anti-HBc positivity rate (25%) while students had the lowest (15.8%). Blood transfusion recipients (33.3%) and those with a history of surgery (30.8%) showed higher anti-HBc positivity rates compared to other risk factors. Unvaccinated individuals exhibited a higher anti-HBc positivity rate (26.3%) than vaccinated individuals (14.0%). Prior blood donors accounted for 76.2% of all anti-HBc-positive cases.

**Conclusion:** These findings highlight the hidden burden of past HBV exposure among asymptomatic blood donors and emphasize the need for enhanced vaccination coverage, safe transfusion practices and the potential inclusion of anti-HBc testing in blood donor screening

protocols to reduce the risk of occult HBV infections in endemic areas.

**Key words:** Anti-HBc screening; Anti HBc total seroprevalence; Blood donors; Blood safety; HBV transmission; Hepatitis B virus; Occult hepatitis B infection.

### Introduction

WHO estimates that 296 million people were living with chronic hepatitis B infection in 2019, with 1.5 million new infections each year.<sup>1</sup> HBV infection is now less common than it once was in Bangladesh. HBV was found to be prevalent in 4.90% of persons who went to a hospital in Dhaka for regular checkups, according to a 2013 study.<sup>2</sup>

HBV persists as a disease that can be transmitted by coming into contact with infected organic fluids or blood. Both the transfusion of blood drawn from infected people during the window phase and the presence of occult HBV increase the risk of HBV transmission.<sup>3</sup> The significant risk of infections transmitted during transfusions prompts the implementation of steps to improve blood safety.<sup>4</sup> In order to detect occult Hepatitis B infection, routine HBV screening favors testing HBsAg exclusively and refuses to look into alternative assays.

Despite the fact that the use of screening tests for hepatitis B surface antigen (HBsAg) has greatly decreased the transmission of HBV infection among blood donors, these tests fail to identify cases of Occult HBV Infection (OBI).<sup>5</sup> A patient with acute hepatitis who tested positive for anti-HBc but negative for HBsAg in the 1970s was found to have a novel kind of clinical HBV infection.<sup>6</sup> Anti-HBc testing is an effective method for detecting OBI, however it only detects roughly 80% of OBI patients.<sup>7</sup> The use of anti-HBc screening causes a significant deferral rate of donors who had not been previously rejected in a location with a high frequency, negatively affecting blood collection.<sup>8,9</sup>

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Anti-HBc is produced in infected persons both in the chronic carrier condition and at the conclusion of acute resolving infection. Additionally, it is a crucial component of the screening of blood donors in many nations as it is regarded as a reliable marker for occult HBV infection. Anti-HBc positivity manifests six to twelve weeks after infection, is detectable for the rest of one's life, and can identify infections in HBsAg-negative blood donors.<sup>10,10,11</sup> Brazil started using anti-HBc testing in 1993 and Hungary follows in 2006.<sup>12,13</sup> Since 2006, anti-HBc screening in Germany has increased the country's blood safety.<sup>14</sup> According to the research by Mosley et al. anti-HBc screening would decrease the amount of HBV that needed to be processed by virus inactivation and enhance the level of anti-HBs in plasma pools in the United States.<sup>15</sup>

Early research on HBV seroprevalence in Bangladesh reports extremely high levels of HBsAg among professional or commercial blood donors (18–29%.<sup>16,17</sup> Because of their representativeness in the general population and the possible danger of HBV transmission by transfusion, blood donors are an essential sample for research on the prevalence of antibodies to Hepatitis B core antigen (Anti-HBc). According to "Safe" blood donations having a transfusion-mediated HBV infection prevalence rate of 1.8%, is possible.<sup>17</sup> To determine the scope of this risk in Bangladesh, very little information is currently available.

Positive for both anti-HBcore and anti-HBs less than 100 mIU/mL, according to WHO recommendations, is usually accepted as the lowest protective level in the context of blood screening.<sup>18</sup> The study's objectives include identifying the seroprevalence of anti-HBcore in a subgroup of blood donors and assessing the main risk variables connected to positive anti-HBc markers. By measuring the seroprevalence, we can get a clearer idea of how widespread any current or previous HBV infections are in this community. Additionally, this research will shed important light on the efficiency of current preventative and control measures, such as blood screening procedures and immunization programs, and it will serve as a roadmap for future initiatives to lessen the prevalence of HBV infection in Chattogram.

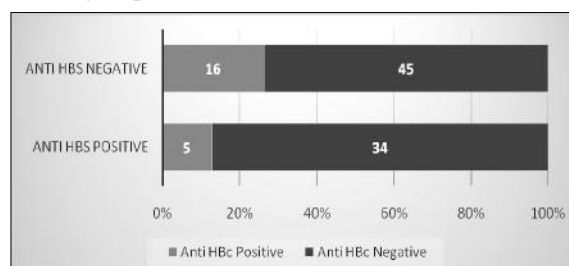
## Materials and methods

This cross-sectional observational study was conducted at the Department of Transfusion Medicine and the Sandhani Unit of Chittagong Medical College over a one-year period from July 2023 to June 2024, following approval from the institutional ethical review committee. The study population comprised voluntary blood donors in Chattogram. A convenient sampling technique was employed and all eligible participants presenting during the study period were included. Inclusion criteria were HBsAg-negative voluntary blood donors, while exclusion criteria included individuals who were HBsAg-positive, had a history of hepatitis B vaccination within the previous six months, had ongoing infections or fever, known chronic liver disease or liver dysfunction, prior anti-HBc positivity, or were unable to consent. Data were collected using a predesigned, structured case record form. After obtaining informed consent, approximately 5 ml of blood was collected from each participant, processed, and stored at –70°C until testing. Anti-HBc (IgM and IgG) was measured using Electrochemiluminescence Immunoassay (ECLIA) on the Cobas E411 platform, and anti-HBs titers were determined using Chemiluminescent Microparticle Immunoassay (CMIA) with the Architect i1000SR system, following manufacturer protocols. Demographic variables such as age, sex, and residential location were recorded. Statistical analysis was performed using SPSS version 26.

## Results

We conducted an analysis of data from 100 blood donors who tested negative for HBsAg over a year-long study. The participants had an average age of about 34.69 years, with ages spanning from 18 to 55, indicating a relatively young group. Among them, 61 were male and 39 were female. A slightly higher percentage of females (23.1%) were positive for anti-HBc compared to males (19.7%). Homemakers showed the highest rate of anti-HBc positivity at 25.0%, followed by business professionals at 23.1%, service holders at 19.4%, and students at 15.8%. Students exhibited the lowest rate of past Hepatitis B exposure, potentially due to their younger age, reduced occupational exposure, or better vaccination

coverage. Out of the 100 HBsAg-negative donors, a minority (21%) tested positive for anti-HBc, indicating previous or ongoing hepatitis B virus infection, while the majority (79%) were negative. Furthermore, 39% of participants were positive for anti-HBs, suggesting immunity from past infection or vaccination. A significant portion (23.8%) of those with positive anti-HBc results also tested positive for anti-HBs, implying resolved infections or vaccination. Conversely, the majority (76.2%) of anti-HBc-positive individuals were negative for anti-HBs, which might suggest an active infection or lack of immunity. Various risk factors linked to hepatitis B virus infection did not show a strong correlation with anti-HBc positivity and these associations were not statistically significant. A slightly lower percentage of anti-HBc positivity was found among IV drug users (16.7%) and those with a history of sexual exposure (11.8%). However, blood transfusion recipients had a notably higher percentage of anti-HBc positivity (33.3%) compared to those who had undergone surgery (30.8%). The percentage of anti-HBc positivity was nearly identical for individuals with a family history of chronic Hepatitis B (20.0%) and those without it (21.3%). A higher percentage of unvaccinated individuals (26.3%) tested positive for anti-HBc compared to vaccinated individuals (14.0%) suggesting that vaccination may reduce the prevalence of anti-HBc positivity by lowering exposure to Hepatitis B infection. Blood donors exhibited a significantly higher percentage of anti-HBc positivity (30.2%) than non-donors (10.6%). Notably, 76.2% of all anti-HBc-positive cases were from individuals who had previously donated blood. The elevated rate of anti-HBc positivity among blood donors suggests that past Hepatitis B exposure might be more prevalent, possibly due to undetected past infections before donation or increased exposure risk within certain donor groups.



**Figure 1** Seroprevalence of Anti HBc and Anti HBs

**Table I** Demographic profile

| Age             | Anti HBc Positive | Anti HBc Negative |
|-----------------|-------------------|-------------------|
| 25th Percentile | 29.00             | 28.00             |
| 50th percentile | 36.00             | 34.00             |
| 75th Percentile | 44.00             | 40.00             |
| Gender          |                   |                   |
| Male            | 12 (19.7%)        | 49 (80.3%)        |
| Female          | 9 (23.1%)         | 30 (76.9%)        |
| Occupation      |                   |                   |
| Student         | 3 (15.8%)         | 16 (84.2%)        |
| Service Holder  | 6 (19.4%)         | 25 (80.6%)        |
| Business        | 6 (23.1%)         | 20 (76.9%)        |
| Home Maker      | 6 (25%)           | 18 (75%)          |

**Table II** Risk factor for anti HBc positivity

| Risk Factor                          | Total | Anti HBc Positive | Anti HBc Negative |
|--------------------------------------|-------|-------------------|-------------------|
| <b>Previous Blood Transfusion</b>    |       |                   |                   |
| Yes                                  | 18    | 06 (28.6%)        | 12 (15.2%)        |
| No                                   | 82    | 15 (71.4%)        | 67 (84.8%)        |
| <b>Intravenous Drug User</b>         |       |                   |                   |
| Yes                                  | 12    | 02 (9.5%)         | 10 (12.7%)        |
| No                                   | 88    | 19 (90.5%)        | 69 (87.3%)        |
| <b>Sexual Exposure</b>               |       |                   |                   |
| Yes                                  | 17    | 02 (9.5%)         | 15 (19%)          |
| No                                   | 83    | 19 (90.5%)        | 64 (81%)          |
| <b>Previous Surgery</b>              |       |                   |                   |
| Yes                                  | 26    | 08 (38.1%)        | 18 (22.8%)        |
| No                                   | 74    | 13 (61.9%)        | 61 (77.2%)        |
| <b>Family History of Hepatitis B</b> |       |                   |                   |
| Yes                                  | 20    | 04 (19%)          | 16 (20.3%)        |
| No                                   | 80    | 17 (81%)          | 63 (79.7%)        |

## Discussion

This research examined the presence of antibodies to the hepatitis B core antigen (Anti-HBc) in blood donors who tested negative for HBsAg in Chattogram and discovered that 21% of the donors were positive for anti-HBc. This indicates that a notable segment of the donor population had either previous or ongoing exposure to the Hepatitis B Virus (HBV). These findings align with other studies in the region that have reported similar rates of anti-HBc positivity among asymptomatic individuals, particularly in areas with moderate endemicity.<sup>19,20,21</sup> The slightly higher rate of Anti-HBc positivity in females (23.1%) compared to males (19.7%) which is not supported by most of previous study.<sup>22,23</sup> But female predominant in our study might be due to variations in immune response or previous

exposure pathways, although the limited sample size affects the robustness of this conclusion. Among various occupational categories, homemakers had the highest anti-HBc positivity rate (25%), possibly due to reduced access to health education and preventive measures.<sup>24</sup> Conversely, students had the lowest positivity rate (15.8%), likely because of their younger age, recent vaccinations and limited exposure to occupational or surgical risks.<sup>25</sup> Notably, a significant portion (76.2%) of anti-HBc-positive individuals were anti-HBs-negative, indicating a potential risk of reactivation or ongoing infection without immunity. This underscores the need for further clinical assessment of Anti-HBc-positive donors, as they might carry Occult Hepatitis B Infection (OBI) despite being HBsAg-negative. The examination of risk factors revealed a significant association between previous blood transfusions (33.3%) and surgical history (30.8%) with increased Anti-HBc positivity, suggesting the potential for hospital-acquired transmission in medical settings and underscoring the necessity for rigorous screening and infection control protocols.<sup>26</sup> Conversely, intravenous drug use (16.7%) and sexual exposure (11.8%) did not show a significant correlation with Anti-HBc positivity in this research, possibly due to underreporting or limited sample sizes in these groups.<sup>27</sup> Vaccination status emerged as another key factor affecting anti-HBc prevalence. Those who were not vaccinated exhibited a significantly higher rate of anti-HBc positivity (26.3%) compared to vaccinated individuals (14.0%), highlighting the protective benefits of hepatitis B vaccination and supporting initiatives to boost vaccine coverage, especially among high-risk adult populations. Chang et.al. notes that the prevalence of hepatitis B core antibody in the vaccinated birth cohort was significantly lower than in those born before the vaccination initiative (2.2% vs 50.8%).<sup>28</sup> Notably, blood donors exhibited a higher anti-HBc positivity rate (30.2%) compared to non-donors (10.6%), with the majority of anti-HBc-positive individuals (76.2%) having previously donated blood. This raises concerns about the potential transmission from asymptomatic carriers with past infections, emphasizing the need to include Anti-HBc

screening in standard blood donor screening protocols in endemic areas. Overall, this study highlights the hidden burden of past hepatitis B exposure among seemingly healthy blood donors. The high rate of anti-HBc positivity, especially among those without anti-HBs, indicates a population at risk of HBV reactivation or transmission. Public health strategies should focus on enhancing vaccination coverage, ensuring safe transfusion practices and considering the inclusion of anti-HBc testing in blood donor screening to reduce the risk of occult infections.

### Limitation

The study was carried out in a designed area within a short time frame including relatively small sample. So the current study may not accurately represent the Bangladeshi population.

### Conclusion

This study reveals a considerable seroprevalence of Anti-HBc among HBsAg-negative blood donors in Chattogram, indicating past exposure to Hepatitis B Virus (HBV) in a notable portion of the donor population. The presence of Anti-HBc in the absence of Anti-HBs in most cases raises concern about the potential for occult HBV infection and underscores the importance of additional serological screening. The results further highlight the protective role of vaccination and the association of certain risk factors, such as previous surgery and blood transfusions, with increased HBV exposure. These findings suggest that current donor screening protocols may need to be revisited to enhance transfusion safety in this setting.

### Recommendation

Multicenter larger sample sized study all over Bangladesh can be performed which make the study complete, among Bangladeshi population.

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**Contribution of authors**

MAF-Design, acquisition of data, data analysis, interpretation of data, drafting & final approval.

EUA-Conception, design, data analysis, interpretation of data, critical revision & final approval.

MR-Conception, acquisition of data, data analysis, interpretation of data, drafting, critical revision & final approval.

MNM-Acquisition of data, data analysis, drafting & final approval.

SHH-Data analysis, drafting & final approval.

**Disclosure**

All the authors declared no conflicts of interest.

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