Research Article

Seroprevalence of Hepatitis B Core IgM Antibodies Among Apparently Healthy Blood Donors: An Enzyme Immunoassay-Based Cross-Sectional Study

Devendra Singh[1], Prof. Dr. Navneet Kumar[2]

[1]Ph.D. Scholar, College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India. E-mail: devsdhillon78@gmail.com
[2*]Professor, College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India. E-mail: principal.paramedical@tmu.ac.in

Abstract

Background & objectives: Hepatitis B virus (HBV) remains a major transfusion-transmitted infection despite routine screening for hepatitis B surface antigen (HBsAg). The present study aimed to determine the seroprevalence of anti-hepatitis B core immunoglobulin M (anti-HBc IgM) antibodies among HBsAg-non-reactive blood donors using ELISA. Methods: In this prospective cross-sectional study, 115 apparently healthy HBsAg-non-reactive blood donors were recruited from a tertiary care blood centre in Moradabad, Uttar Pradesh, India. Donor demographic and clinical characteristics were recorded. Serum samples were screened for anti-HBc IgM antibodies using a commercially available ELISA kit according to the manufacturer's instructions. Descriptive statistics were used to summarize the study findings. Results: The mean age of the donors was 33.2 ± 7.0 years, and 97.4% were male. Blood group A (39.1%) was the most frequent ABO type, while 94.8% of donors were Rh positive. Anti-HBc IgM antibodies were detected in 9 of 115 HBsAg-non-reactive donors, yielding a seroprevalence of 7.8%, whereas 106 (92.2%) donors were non-reactive. conclusions: Detection of anti-HBc IgM antibodies among HBsAg-non-reactive blood donors suggests that exclusive HBsAg screening may not identify all potentially infectious donations. Supplementary serological screening may improve blood transfusion safety, particularly in settings where nucleic acid testing is not routinely available.

Keywords: Hepatitis B virus, Anti-HBc IgM, Blood donors, ELISA, Seroprevalence

1. Introduction

Ensuring the microbiological safety of blood transfusion remains a major priority for transfusion services worldwide[1]. Although mandatory screening for hepatitis B surface antigen (HBsAg) has substantially reduced the transmission of hepatitis B virus (HBV), residual transfusion risk persists because HBsAg alone cannot identify all infected donors[2]. Individuals in the serological window period, those with occult hepatitis B infection (OBI), or donors with low circulating HBsAg levels may escape routine screening and remain potentially infectious[2]. Antibody to hepatitis B core antigen (anti-HBc) is a sensitive marker of natural HBV infection. In particular, anti-HBc immunoglobulin M (IgM) is associated with recent or acute HBV infection and may be the only detectable serological marker during the window period before the appearance of protective antibodies[3]. Detection of anti-HBc IgM by enzyme immunoassay (EIA) can therefore improve the identification of potentially infectious blood donors who would otherwise be classified as eligible based solely on HBsAg testing. While nucleic acid testing (NAT) offers the highest sensitivity for detecting occult infection, its implementation is constrained in many resource-limited settings because of cost and infrastructure requirements, making additional serological markers clinically relevant[4]. The prevalence of anti-HBc among blood donors varies considerably across regions owing to differences in HBV endemicity, donor selection policies, vaccination coverage, and screening algorithms[5]. Several countries have incorporated anti-HBc testing into donor screening strategies, whereas its routine use remains controversial in many developing countries because of concerns regarding donor loss and cost-effectiveness[6]. Consequently, region-specific epidemiological data are essential for evaluating the potential utility of anti-HBc screening in enhancing blood safety[7]. Data on the prevalence of anti-HBc IgM among apparently healthy blood donors from northern India remain limited[8]. Estimating its seroprevalence in the donor population may help identify residual HBV risk not detected by conventional HBsAg screening and provide evidence for strengthening transfusion screening

policies[9]. Therefore, the present study aimed to determine the seroprevalence of anti-HBc IgM antibodies among apparently healthy blood donors using enzyme immunoassay and to describe the demographic characteristics of seroreactivity donors.

2. Materials & Methods

Study design and setting: This hospital-based cross-sectional study was conducted at the Blood Centre, Bright Star Hospital, Moradabad, Uttar Pradesh, India, from December 2023 to March 2024. The study was undertaken as part of the doctoral research programme of the College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad.

Study population: Apparently healthy voluntary and replacement blood donors attending the blood centre during the study period were consecutively recruited. Donor eligibility was assessed according to the National Blood Transfusion Council (NBTC), Government of India, and World Health Organization (WHO) guidelines. Donors fulfilling the eligibility criteria and providing written informed consent were included in the study.

Data collection: Demographic and clinical characteristics, including age, sex, haemoglobin concentration, body weight, blood pressure, pulse rate, ABO blood group, Rh type, history of blood transfusion, hepatitis B vaccination status, and relevant medical history, were recorded using a standardized donor questionnaire.

Sample collection and processing: Approximately 5 mL of venous blood was collected aseptically from each donor during routine blood donation. Serum was separated by centrifugation at 3000 rpm for 10 min and analysed on the same day. Samples requiring delayed analysis were stored at 2–8°C until testing.

Serological analysis: Routine screening for HBsAg was performed according to standard blood bank protocols. Detection of immunoglobulin M anti-HBc IgM was carried out using a commercially available ELISA kit (Erba Diagnostics, Germany) in accordance with the manufacturer's instructions. Positive and negative controls supplied with the kit were included in each assay run, and results were interpreted using the recommended cut-off criteria.

Outcome measures: The primary outcome was the seroprevalence of anti-HBc IgM among apparently healthy blood donors. Secondary outcomes included the distribution of anti-HBc IgM seroreactivity according to demographic characteristics and blood group profile.

Statistical analysis: Data were analysed using IBM SPSS Statistics version XX (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were summarized as frequencies and percentages. A two-tailed P value <0.05 was considered statistically significant.

3. Result

Donor characteristics: A total of 120 apparently healthy blood donors were recruited during the study period. Five samples were excluded because of haemolysis, inadequate sample volume, or failure to meet the study eligibility criteria. Consequently, 115 blood donors were included in the final analysis. The mean age of the participants was 33.20 ± 7.03 years. The mean height and body weight were 161.95 ± 6.98 cm and 69.35 ± 6.35 kg, respectively. The mean systolic and diastolic blood pressures were 131.94 ± 10.98 mmHg and 84.74 ± 5.54 mmHg, respectively. The mean pulse rate was 80.00 ± 8.79 beats/min, while the mean haemoglobin concentration was 14.19 ± 0.80 g/dL. Of the 115 donors included in the analysis, 112 (97.4%) were male and 3 (2.6%) were female. The baseline demographic and clinical characteristics of the study participants are summarized in Table 1

Table 1. Baseline demographic and clinical characteristics of the study participants (n = 115)

Characteristic	Value
Age (years),	33.20 ± 7.03
Height (cm),	161.95 ± 6.98
Weight (kg),	69.35 ± 6.35
Systolic blood pressure (mmHg),	131.94 ± 10.98
Diastolic blood pressure (mmHg),	84.74 ± 5.54
Pulse rate (beats/min),	80.00 ± 8.79
Haemoglobin (g/dL),	14.19 ± 0.80
Sex, n (%)	
Male	112 (97.4)
Female	3 (2.6)

Data are presented as mean ± standard deviation (SD) or number (%).

ABO and Rh blood group distribution

Among the 115 study participants, blood group A was the most prevalent [(39.1%)], followed by blood groups B [38 (33.0%)], O [17 (14.8%)], and AB [15 (13.0%)]. Rh-positive blood type was observed in 109 (94.8%) donors, whereas 6 (5.2%) donors were Rh negative (Table 2).

Table 2. ABO and Rh blood group distribution of the study participants (n = 115)

Characteristic	n (%)
ABO blood group	
A	45 (39.1)
B	38 (33.0)
O	17 (14.8)
AB	15 (13.0)
Rh type	
Rh positive	109 (94.8)
Rh negative	6 (5.2)

Serological screening

A total of 115 apparently healthy blood donors were screened for hepatitis B surface antigen (HBsAg). All 115 donors were found to be non-reactive for HBsAg and were subsequently tested for anti-HBc IgM antibodies by enzyme-linked immunosorbent assay (ELISA). Among the HBsAg-non-reactive donors, 9 (7.8%) were reactive for anti-HBc IgM antibodies, whereas 106 (92.2%) were non-reactive (Table 3)

Table 3. Seroprevalence of anti-HBc IgM among HBsAg-non-reactive blood donors (n = 115)

Parameter	Number of donors (n)	Percentage (%)
HBsAg status		
Non-reactive	115	100.0
Anti-HBc IgM antibody status		
Reactive	9	7.8
Non-reactive	106	92.2
Total	**115**	**100.0**

Values are presented as number and percentage.

4. Discussion

The present study demonstrated an anti-HBc IgM seroprevalence of 7.8% among HBsAg-non-reactive blood donors. Detection of anti-HBc IgM in HBsAg-negative donors is clinically important because it may indicate recent HBV infection during the serological window period, when HBsAg is undetectable but the donor may remain infectious. Similar concerns regarding residual transfusion-transmitted HBV infection despite routine HBsAg screening have been highlighted by **Kumar et al. (2007)** who emphasized the value of anti-HBc testing as an additional screening marker in blood donors[10]. The donor population in the present study was predominantly male (97.4%), with a mean age of 33.2 years. This demographic profile is consistent with **Dhawan et al. (2008)** studies, where males constitute more than 90% of the donor population because of higher voluntary donation rates and frequent deferral of female donors due to anaemia and other eligibility criteria[11]. In the present investigation, anti-HBc IgM antibodies were detected in 9 of 115 HBsAg-non-reactive donors (7.8%). **Kumar et al. (2007)** reported anti-HBc IgM positivity in 0.39% of HBsAg-negative blood donors. The higher seroprevalence observed in the **Shastry and Bhat (2011)** study, which may be attributed to regional variation in HBV epidemiology, differences in donor characteristics, assay performance, sample size, and study design. Importantly, comparisons should be interpreted cautiously because several published studies evaluated total anti-HBc rather than anti-HBc IgM specifically[12]. Anti-HBc IgM is considered a useful marker of acute or recent HBV infection and may remain detectable during the serological window period **de Almeida Pondé et al. (2025)[13]**. Consequently, donors who are HBsAg negative but anti-HBc IgM positive may still pose a risk for transfusion-transmitted HBV infection **Sawke and Sawke (2010)**[14]. **Jindal et al. (2025)** study have therefore suggested incorporating additional serological markers or NAT to improve blood safety, particularly in regions with intermediate or high HBV endemicity[15]. The present study has certain limitations. It was conducted at a single centre with a relatively small sample size, and HBV DNA testing was not performed to confirm occult HBV infection. Nevertheless, the findings provide regional evidence regarding anti-HBc IgM seroreactivity among HBsAg-negative blood donors and support further multicentre studies incorporating molecular confirmation to determine the diagnostic utility of anti-HBc IgM in routine donor screening.

5. Conclusion

The present study demonstrated that anti-HBc IgM antibodies were detected in 7.8% of HBsAg-non-reactive blood donors, indicating that a subset of apparently healthy donors may exhibit serological evidence of recent HBV infection despite being negative for HBsAg. These findings highlight the limitations of relying solely on HBsAg-based screening for blood donor selection and suggest that additional serological markers may improve the detection of potentially infectious donations. Incorporation of anti-HBc IgM testing, particularly in settings where nucleic acid testing is not routinely available, may strengthen transfusion safety. Further multicentre studies with larger sample sizes and molecular confirmation using HBV DNA testing are warranted to validate these findings and determine the clinical utility of anti-HBc IgM screening in routine blood donor programmes.

Declarations

- **Ethics approval:** Approved by the Institutional Ethics Committee, College of Paramedical Sciences, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India (Ref. No. PM/ETHICAL/COPS/2023/024; dated 31 July 2023).
- **Consent to participate:** Written informed consent was obtained from all participants prior to enrolment in the study.
- **Consent for publication:** Not applicable.
- **Availability of data and materials:** The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** The authors declare that they have no competing interests.
- **Funding:** The authors received no specific funding for this study.
- **Authors' contributions:** D.S. conceptualized and conducted the study, performed data analysis, and prepared the manuscript. N.K. supervised the study, critically reviewed the manuscript, and approved the final version.

Bibliography

1. Simmonds P, Harvala H. The future of transfusion and transplant microbiology safety: can new molecular technologies improve the safety of blood and transplant? BMC Infect Dis. 2026;26(1):426. doi:10.1186/s12879-026-12678-z
2. Athalye S, Patil A, Khargekar N, Shinde S, Chavan S, Dixit A, et al. Efficacy of combined HBsAg, anti-HBc and anti-HBs screening in minimizing transfusion transmission risk of hepatitis B infection in low resource setting. Heliyon. 2024;10(3):e25805. doi:10.1016/j.heliyon.2024.e25805
3. Gish RG, Basit SA, Ryan J, Dawood A, Protzer U. Hepatitis B Core Antibody: Role in Clinical Practice in 2020. Curr Hepatology Rep. 2020;19(3):254–65. doi:10.1007/s11901-020-00522-0
4. Kumar H, Gupta P, Jaiprakash M. The Role of anti-HBc IgM in Screening of Blood Donors. Med J Armed Forces India. 2007;63(4):350–2. doi:10.1016/S0377-1237(07)80013-X PubMed PMID: 27408047; PubMed Central PMCID: PMC4922069.
5. Fu MX, Faddy HM, Candotti D, Groves J, Saa P, Styles C, et al. International review of blood donation screening for anti-HBc and occult hepatitis B virus infection. Transfusion. 2024;64(11):2144–56. doi:10.1111/trf.18018
6. Fu MX, Faddy HM, Candotti D, Groves J, Saa P, Styles C, et al. International review of blood donation screening for anti-HBc and occult hepatitis B virus infection. Transfusion. 2024;64(11):2144–56. doi:10.1111/trf.18018
7. Yano Y, Sato I, Imanishi T, Yoshida R, Matsuura T, Ueda Y, et al. Clinical Significance and Remaining Issues of Anti-HBc Antibody and HBV Core-Related Antigen. Diagnostics. 2024;14(7):728. doi:10.3390/diagnostics14070728

8. Dhawan HK, Marwaha N, Sharma RR, Chawla Y, Thakral B, Saluja K, et al. Anti-HBc screening in Indian blood donors: Still an unresolved issue. World J Gastroenterol. 2008;14(34):5327–30. doi:10.3748/wjg.14.5327 PubMed PMID: 18785287; PubMed Central PMCID: PMC2744065.

9. Cazarin-Chávez K, Béjar-Ramírez YL, Pérez-González JA, Solís-Hernández CG, Higuera-De la Tijera MF, Pérez-Hernández JL. Seroprevalence of hepatitis b and c viruses in blood donors in a third level hospital from 2019 to 2023. Annals of Hepatology. 2025;Abstracts Asociación Mexicana de Hepatología (AMH) 202430:101824. doi:10.1016/j.aohep.2025.101824

10. Kumar H, Gupta P, Jaiprakash M. The Role of anti-HBc IgM in Screening of Blood Donors. Med J Armed Forces India. 2007;63(4):350–2. doi:10.1016/S0377-1237(07)80013-X PubMed PMID: 27408047; PubMed Central PMCID: PMC4922069.

11. Dhawan HK, Marwaha N, Sharma RR, Chawla Y, Thakral B, Saluja K, et al. Anti-HBc screening in Indian blood donors: Still an unresolved issue. World J Gastroenterol. 2008;14(34):5327–30. doi:10.3748/wjg.14.5327 PubMed PMID: 18785287; PubMed Central PMCID: PMC2744065.

12. Shastry S, Bhat SS. Prevention of Post-Transfusion Hepatitis by Screening of Antibody to Hepatitis B Core Antigen in Healthy Blood Donors. Mediterr J Hematol Infect Dis. 2011;3(1):e2011062. doi:10.4084/MJHID.2011.062 PubMed PMID: 22220259; PubMed Central PMCID: PMC3248339.

13. de Almeida Pondé RA. Detection of hepatitis B virus surface antigen, IgM and IgG antibodies to hepatitis B virus core antigen in the clinical classification and epidemiological surveillance of HBV infection. Mol Biol Rep. 2025;52(1):195. doi:10.1007/s11033-025-10278-9

14. Sawke NG, Sawke G. Preventing Post-Transfusion Hepatitis by screening blood donors for IgM Antibody to Hepatitis B core antigen. J Glob Infect Dis. 2010;2(3):246–7. doi:10.4103/0974-777X.68526 PubMed PMID: 20927286; PubMed Central PMCID: PMC2946681.

15. Jindal DK, Sarmah MD, Sewlikar S, Wadia K. Health Economic Benefits of Implementing Nucleic Acid Testing in Addition To Serology for Blood Transfusion Safety in India: a Cost-Effectiveness Analysis. Indian J Hematol Blood Transfus. 2025. doi:10.1007/s12288-025-02235-0