

## Original Research

# Prevalence of antibody to hepatitis B core antigen among hepatitis B surface antigen-negative blood donors in Ilorin, Nigeria: A cross-sectional study

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## Abstract

### Background

Post-transfusion hepatitis occurs even with stringent donor selection criteria and screening for hepatitis B surface antigen (HBsAg). The objective of this study was to determine the prevalence of antibody to hepatitis B core antigen (anti-HBc) in HBsAg-negative blood donors.

### Methods

This was a cross-sectional study in which 200 HBsAg-negative blood donors were recruited. Screening for viral markers was done using both a rapid test kit and enzyme-linked immunosorbent assay (ELISA) for anti-HBc IgM. Quantitative and qualitative analysis of anti-HBc IgM was done by "capture" enzyme immunoassay using DIA.PRO HBc IgM test kits. The other viral markers were investigated using one step cassette style HBV tests. SPSS version 16 was used for data analysis. A P-value of 0.05 or less was considered significant.

### Results

There were 190 male (95%) and 10 female (5%) blood donors, with a mean age of  $31.7 \pm 7.9$  years. The prevalence of anti-HBc IgM was 4%. The other viral markers (HBsAg, anti-HBeAg, anti-HBs and total anti-HBc) had a prevalence of 1.5%, 23%, 2.5%, and 32.5%, respectively.

### Conclusions

The prevalence of anti-HBc IgM in this study was high, and this supports the fact that screening blood donors for HBsAg alone is not sufficient to prevent transmission of HBV.

## Introduction

Transfusion-associated hepatitis B virus infection (TAHBV) continues to be a major problem in developing countries, even after the adoption of mandatory screening for hepatitis B surface antigen (HBsAg) by the enzyme-linked immunosorbent assay (ELISA).<sup>1</sup> The burden of Hepatitis B virus (HBV) infection is heavy in most developing countries, particularly in rural areas; this burden is compounded by the high cost of prevention, management, and treatment.<sup>2</sup> Transmission of this virus is common through blood transfusion. Other modes of transmission include sexual contact, close interpersonal contact through blood and body fluids, mother-to-child (vertical), intravenous drug use (IVDU), and unsafe traditional practices including tattoos, ear piercing, circumcision, and traditional uvulectomy. Detection of HBsAg in the blood is a diagnostic marker for infection with HBV. In blood banks, screening for HBsAg is carried out routinely to detect current or previous HBV infection. Occult HBV infection is defined as the presence of HBV DNA in blood or liver tissues in patients negative for HBsAg but who may or may not be positive for HBV antibodies.<sup>3</sup> Thus, the absence of HBsAg in the blood of apparently healthy individuals may not indicate the absence of circulating HBV, and blood containing antibody to hepatitis B core antigen (anti-HBc) without detectable HBsAg might be infectious.<sup>1</sup> A window period exists, which represents a carrier state of the disease, and this poses a risk of TAHBV. During this window period, detection of anti-HBc serves as a useful serological marker for hepatitis B infection. The IgM class of anti-HBc is the first to appear and indicates a recent infection, while IgG anti-HBc appears

<http://dx.doi.org/10.4314/mmj.v29i1.7>

later during the infection and points to a past HBV infection.<sup>4</sup> The use of HBsAg and anti-HBc screening tests has been the basis of HBV screening in many countries, and this has significantly reduced but not eliminated TAHBV.<sup>5</sup> Anti-HBc has been found to be an excellent indicator of occult HBV infection during the window period after the disappearance of HBsAg.<sup>6-8</sup> It has been observed that a negative screening test for HBsAg does not rule out the transmission of HBV in donors, as they might be in the window period and the detection of the IgM-type anti-HBc serves as a useful serological marker during this period.<sup>9</sup>

HBV infection, with its associated sequelae, is a disease of major public health importance, being the tenth leading cause of death globally and accounting for 500,000 to 1.2 million deaths each year.<sup>10-12</sup> Approximately 15% to 40% of infected patients will develop cirrhosis, liver failure, or hepatocellular carcinoma.<sup>13,14</sup> Nigeria, the most populous nation in Africa, is considered hyperendemic for HBV infection, with a prevalence of HBsAg in the adult population ranging between 5% and 25%.<sup>15-21</sup> Nigeria, being a developing country with high level of poverty, cannot afford DNA testing of all collected units of blood, despite DNA testing serving as the only possibility of achieving a near-zero risk of TAHBV.<sup>6</sup>

The objective of this study was to help elucidate the seroprevalence of anti-HBc (IgM) among blood donors negative for HBsAg in Ilorin, north-central Nigeria, which is a possible basis for advocating for compulsory implementation of screening for anti-HBc as part of routine blood donor screening.

## Methods

This was a descriptive cross-sectional study carried out among blood donors at the University of Ilorin Teaching Hospital (UIITH), Ilorin, between May and November 2013.

The minimum sample size for this study was determined using Fisher's formula<sup>22</sup> for estimating sample size to determine the prevalence or proportion of a factor where the population is greater than 10,000.<sup>28,32</sup>

A sample size of 174 was calculated as the the minimum to estimate prevalence with 95% confidence that the result is within 5% of the true prevalence. However, a final total of 200 respondents were sampled during the study.

Inclusion criteria were age between 17 and 65 years; haemoglobin concentration (Hb) greater than 13.5 g/dL in males and greater than 12.5 g/dL in females; no blood donation in the previous 3 months for males and 4 months for females; and negative screening for HBsAg, hepatitis C virus (HCV), syphilis, and human immunodeficiency virus (HIV) using rapid test kits.

Ethical clearance was obtained from the UIITH Ethical Research Committee. Written informed consent was obtained from all participants. Questionnaires were administered and samples collected from those who met the inclusion criteria. Sociodemographic data, such as age, gender, marital status, religion, occupation, and level of education were noted. History of jaundice, surgeries, transfusion, sexually transmitted infection, tattooing, and IVDU, as well as general physical examination findings, were recorded.

Under aseptic technique, 5 mL of venous blood was collected into a plain vacuum blood collection tube (Micropoint Diagnostics, Santa Clara, CA, USA). The blood was allowed to clot and retract at room temperature. Sera were separated by centrifugation at 3000 rpm for 5 minutes. The supernatant sera were aspirated into vials and preserved at -200°C until analysed. ELISA for anti-HBc IgM quantitative and qualitative determination was done with DIA.PRO HBc IgM test kits (Diagnostics Bioprobes, Milan, Italy), following the manufacturer's instructions. According to the manufacturer's data sheet, the sensitivity and specificity for this test method were 98% and 99%, respectively.

The test for the other viral markers (anti-HBs, HBeAg, anti-HBe, and anti-HBc total) were done using one step cassette style HBV tests (Atlas Link, Manassas, VA, USA). The one step cassette style HBV test is a rapid test based on the principle of immunoassay combined with conjugated colloid gold technology. According to the manufacturer's data sheet, sensitivity is up to 1 ng/mL, while specificity is 99.8%.

Data were analysed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean  $\pm$  standard deviation (SD). Comparison of categorical data was done using the chi-square and Fisher's exact tests. A P-value of 0.05 or less was considered statistically significant.

## Results

Two hundred HBsAg-negative blood donors at the blood donor unit of UIITH were recruited for this study. The mean age of the participants was  $31.7 \pm 7.9$  years. Nearly half (46.5%) of the donors were in the 26- to 34-year-old age group. Five percent of donors were female, with a male to female ratio of 19:1. The majority (97.5%) were family replacement donors, of which most (88%) were first-time donors.

Table 1 shows that out of the 200 HBsAg-negative blood donors recruited into this study, only 5 (2.5%) were positive for anti-HBs, of which 4 (80%) were in the 17- to 34-year age group. Of the 5 anti-HBs-positives, four were as a result of immunity from natural infection, while the remaining one was from vaccination.

**Table 1: Overall prevalence of hepatitis B virus markers among blood donors**

Viral marker	Frequency (N = 200)	Percentage (%)
<b>Anti-HBs</b>		
Positive	5	2.5
Negative	195	97.5
<b>HBeAg</b>		
Positive	3	1.5
Negative	197	98.5
<b>Anti-HBe</b>		
Positive	46	23.0
Negative	154	77.0
<b>Anti-HBc total</b>		
Positive	65	32.5
Negative	135	67.5
<b>Anti-HBc (ELISA IgM)<sup>Ql</sup></b>		
Positive	1	0.5
Negative	199	99.5
<b>Anti-HBc (ELISA IgM)<sup>Qn</sup></b>		
Positive	8	4.0
Negative	192	96.0

Ql = qualitative assay; Qn = quantitative assay

Sixty-five participants (32.5%) were positive for total anti-HBc, indicating a past exposure to HBV. Positivity in first-time donors was not significant ( $P > 0.05$ ). There was also no significant association between total anti-HBc-positivity and the frequency of donation, gender, and marital status.

The quantitative analysis for HBc IgM revealed that 5 donors had antibody levels greater than 10.00 Paul Ehrlich International units per mL (PEI U/mL); these were considered positive. However, 15 donors in the grey area were traced and retested. Upon retesting, 12 had levels less than 5 PEI U/mL and were considered negative. Three still remained within the 5- to 10-PEI U/mL grey area after retesting and were therefore considered positive according to the manufacturer's instructions. The remaining 180 donors had HBc IgM levels less than 5.00 PEI U/mL and were considered negative. Overall, 8 (4.0%) of the donors were found to be positive for anti-HBc IgM alone (Table 2) and all were replacement blood donors. Only one female donor showed positivity for anti-HBc IgM. Ninety-three (46.5%) of the donors were positive for at least one of the viral markers.

There was no significant relationship between the viral marker positivity and the type of blood donors (voluntary vs family replacement); similarly, there was no significant relationship between viral marker positivity and frequency of donation, as shown in Table 4.

**Table 2: Type of donor and viral markers**

Variable	Type of donor		Odds ratio	95% Confidence interval	P-value*
	Voluntary (%)	Family replacement (%)			
<b>HBeAg</b>					
Positive	0 (0.0)	3 (100.0)	0.00	0.00 to 119.95	1.00
Negative	5 (2.5)	192 (97.5)			
<b>Anti-HBs</b>					
Positive	0 (0.0)	5 (100.0)	0.00	0.00 to 60.90	1.00
Negative	5 (2.6)	190 (74.4)			
<b>Anti-HBe</b>					
Positive	0 (0.0)	46 (100.0)	0.00	0.00 to 3.94	0.591
Negative	5 (3.2)	149 (96.8)			
<b>Total anti-HBc</b>					
Positive	3 (4.6)	62 (95.4)	3.22	0.42 to 28.33	0.331
Negative	2 (1.5)	133 (98.5)			

\*Fisher's exact test was used to determine P-values

**Table 3: Total anti-HBc status in relation to donor type and experience**

Variable	Anti-HBc total		Odds ratio	95% Confidence interval	P-value
	Positive (%)	Negative (%)			
<b>Type of donor</b>					
Voluntary	3 (60.0)	2 (40.0)	3.22	0.42 to 28.33	0.33*
Family replacement	5 (2.5)	192 (97.5)			
<b>Donor experience</b>					
First donation	58 (32.3)	118 (67.0)	1.19	0.43 to 3.38	0.71
Repeat donor	7 (29.2)	17 (70.8)			

\*Fisher's exact test was used to determine P-value

**Table 4: Prevalence of hepatitis B viral markers by age group**

Age (years)	HBeAg (%)		P-value	Anti-HBe (%)		P-value	Anti-HBs (%)		P-value	Total anti-HBc (%)		P-value
	Positive	Negative		Positive	Negative		Positive	Negative		Positive	Negative	
17 to 25	2 (4.3)	45 (95.7)	0.87	11 (23.4)	36 (76.6)	0.64	2 (4.3)	45 (95.7)	0.90	14 (7.0)	33 (16.5)	0.71
26 to 34	1 (1.1)	92 (98.9)		20 (21.5)	73 (78.5)		2 (2.2)	91 (97.8)		29 (14.5)	64 (32)	
35 to 43	0 (0.0)	44 (100.0)		13 (29.5)	31 (70.5)		1 (2.3)	43 (97.7)		16 (36.4)	28 (63.6)	
44 to 52	0 (0.0)	13 (100.0)		2 (15.4)	11 (84.6)		0 (0.0)	13 (100.0)		4 (30.8)	9 (69.2)	
≥ 53	0 (0.0)	3 (100.0)		0 (0.0)	3 (100.0)		0 (0.0)	3 (100.0)		2 (66.7)	1 (33.3)	
<b>Total</b>	<b>3 (1.5)</b>	<b>197 (98.5)</b>		<b>46 (23)</b>	<b>154 (77)</b>		<b>5 (2.5)</b>	<b>195 (97.5)</b>		<b>65 (32.5)</b>	<b>135 (67.5)</b>	



## Discussion

The safety of blood products is one of the major concerns in transfusion medicine. Although the incidence of transfusion-transmitted HBV has steadily reduced over the last four decades, HBV still remains the most frequent transfusion-transmitted viral infection.<sup>23-26</sup> HBsAg detection is presently the only diagnostic screening test for HBV infection identification in blood transfusion centres in Nigeria. Following infection by HBV, the first serological marker in the blood is HBV DNA, followed by HBsAg, DNA polymerase, and then hepatitis B e antigen (HBeAg). Thereafter, the anti-HBc antibody, anti-HBe antibody, and anti-HBs antibody appear.

From this study, 97.5% of the blood donors were family replacement donors, which is similar to the 93.2% found in the study by Gulia et al. in Andhra Pradesh, India.<sup>27</sup> This is in contrast to the study by Lavanya et al., where replacement donors made up only 14% the donor population.<sup>28</sup> This observed difference could be a result of differences in awareness levels regarding voluntary blood donation.

The transmission of HBV infection following transfusion of HBsAg-negative blood containing anti-HBc was first described in the Western world. Cases have also been reported of some blood derivatives negative for HBsAg but positive for anti-HBc, which were transmitted to recipients both after transfusion and after transplantation of organs.<sup>29,30</sup> In a study carried out by Sodhi et al., 6% of cancer patients were shown to develop post-transfusion hepatitis and their corresponding blood donors, on retesting their stored samples, were positive for anti-HBc and HBV DNA.<sup>31</sup>

In a study by Salawu et al. in Ile Ife, Nigeria, a prevalence of 4.4% was reported for anti-HBc.<sup>32</sup> Ramezani et al.<sup>33</sup> and Sofian et al.<sup>34</sup> both reported prevalence rates of 2.1%, but without detection of HBV DNA. A serological pattern of anti-HBc as a sole marker is not infrequent. In another study among 545 Iranian blood donors, 8% of donors were found to be positive for isolated anti-HBc.<sup>35</sup> However, despite non-detection of HBV DNA in the serum, there have been reports of post-transfusion HBV infection in recipients of blood positive for anti-HBc alone.<sup>36</sup>

The overall prevalence of anti-HBc in this study was found to be 32.5% for total anti-HBc. This is higher than the 10.5% reported by Lavanya et al.<sup>28</sup> in India, and the 16.6% reported by Said et al.<sup>37</sup> These differences could be the result of the endemicity of HBV infection in Nigeria.<sup>17</sup> This, however, is in contrast to the reports on total anti-HBc in Europe and North America, with prevalence rates of 0.07% and 1.5%, respectively.<sup>38</sup> There is low endemicity in these regions and the stringent donor selection criteria, high literacy rates, greater availability of voluntary donors, and differences in sociocultural practices could account for this.

In Nigeria, the seroprevalence of anti-HBc antibody is quite high when compared to Western countries, and hence screening of donor blood for total anti-HBc may not be practically important and cannot be a criterion to discard blood units. In this study, just 4 (6.1%) of the donors who tested positive for total anti-HBc had evidence of immunity from natural infection with HBV, thus giving a prevalence of 6.1% of anti-HBs in donors positive for total anti-HBc.

This study shows that 4.6% of the blood donors had positive anti-HBc and anti-HBs. This is, however, lower than the 6.3% found in a study by Ashshi in Saudi Arabia.<sup>39</sup> In that study, donors were further tested for HBV DNA and 3.2% were found to be positive. Lavanya et al.<sup>28</sup> found a prevalence of 3.0% anti-HBs in total anti-HBc-positive donors. This was in contrast with a study conducted in Iran (which showed a higher prevalence rate of 37.5% for both anti-HBc and anti-HBs) and the 40.3% prevalence found in a study by Said et al.<sup>30</sup> These higher rates could be the result of past infections or carrier states. Anti-HBc IgG may remain positive for life in an affected individual, although the individual has a protective level of anti-HBs, and therefore positivity of anti-HBc IgG does not necessarily mean that the blood of such donors is infectious.

In the present study, the prevalence of anti-HBc IgM alone was found to be 4.0%. This was similar to findings of 4.3% and 4.8% in Ecuador and Brazil among potential blood donors,<sup>9</sup> but slightly lower than the findings of Japhet et al.<sup>8</sup> and Lavanya et al.,<sup>28</sup> who found prevalence rates of 5.4% and 5.5%, respectively. Furthermore, our finding of 4.0% is lower than the 18.1% that was reported for isolated anti-HBc IgM by Jeremiah et al.<sup>40</sup> in the semi-arid region of Nigeria. A Lebanese study also demonstrated that 22.0% of blood donors screened for HBV markers were positive for anti-HBc alone.<sup>41</sup> This marked difference could be a result of geographical location, different sociocultural practices, marital practices, and the sensitivity of the test kits used. The finding of anti-HBc IgM alone may be a result of the presence of anti-HBc IgM during the window period following acute HBV infection, infection with HBV without persistent viraemia, remote infection with persistent occult infection, or the presence of a vaccine escape mutant not detected by most of the currently available HBsAg detection tests. Considering the above factors, blood from such donors might be infectious. Previous studies have also demonstrated the presence of HBV DNA in blood positive for only anti-HBc among the other viral markers. It has been observed that the screening tests for detection of HBsAg do not rule out the possibility of HBV transmission, as the donor might be in the window period, and detection of anti-HBc would serve as a useful serologic marker during this period.

The major limitation to this study is the lack of facilities for HBV DNA testing to confirm the presence of occult HBV infection in those blood donors who were positive for anti-HBc IgM alone. This is due to the high cost and the expertise required to carry out this test. Additionally, anti-HBs titre quantification was not carried out to detect if those who developed the antibody from natural infection were indeed immune.

## Conclusions

There is potentially a substantial risk of HBV transmission despite HBsAg testing, and this is an important message for clinicians deciding to transfuse blood. The usefulness of screening for anti-HBc as an additional screening test to improve the safety of the blood supply in Nigeria needs further studies. Though there is a paucity of similar studies in the country, a nationwide multicentre study should determine whether screening for anti-HBc in addition to HBsAg detection, and introduction of PCR, are necessary.

## Acknowledgements

We want to appreciate the efforts of Dr R.O. Olaosebikan, Dr K.W. Wahab, and Mr Temim, all of UIITH, Ilorin, towards the successful completion of this work.

## Financial support

This research was supported by the University of Ilorin Teaching Hospital, Ilorin.

## Competing interests

The authors declare that they have no conflicts of interest.

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