

Immunity Status against Hepatitis B Virus in Regular Blood Donors at the Yaoundé Central Hospital Blood Bank

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Abstract

Background: Blood donation is an essential component of healthcare worldwide. Given its importance, ensuring the safety of these donations requires effective diagnosis of transfusion-transmitted pathogens such as HIV, HCV, TP, and especially HBV, which is currently a global public health concern. One of the best methods for ensuring this safety is verifying donor protection against the hepatitis B virus after vaccination. The more immunized donors are the safer blood donations will be. Thus, this study aimed to assess the humoral immunity status against hepatitis B in regular blood donors. **Methods:** A descriptive cross-sectional study was conducted on regular blood donors at the Yaoundé Central Hospital (HCY) blood bank, in which total HBcAg antibodies, HBs antibodies, and HBsAg were detected using the ELISA method (Fortress®). **Results:** Among 128 seronegative blood donors screened for HBsAg, we obtained a vaccination coverage rate of 13.28% (17/128) with a humoral immunity rate of 33.59% (43/128) for immunizing HBsAg and an HBsAg-mediated humoral response significantly associated with receiving three doses of vaccine (OR = 4.52, 95% CI: 1.069 - 19.18, p = 0.040). **Conclusion:** This study shows that vaccination coverage among blood donors is very low and that humoral immunity against HBV is primarily due to prior expo-

sure to the virus. However, it is recommended that all blood donors be systematically screened for HBsAg.

Keywords

Blood Donor, Humoral Immunity, Hepatitis B Virus

1. Background

In 1997, Titmuss considered blood donation to be the most beautiful symbol of giving to strangers, in that it is a gift of life, a gift of oneself [1]. Blood donation is therefore a voluntary, anonymous, and altruistic act that consists of authorizing the removal of a certain quantity of one's blood (whole blood donation) or certain of its components (plasma and platelet donation) for use in blood transfusions to save lives or improve the quality of life of patients. These blood transfusions can transmit infectious agents. [2] and [3] are present in the donor's blood, and therefore all blood donations must undergo rigorous testing. Among these infections, hepatitis B, given its numerous routes of transmission, is one of the most dangerous infections that can affect the quality of blood donations. Infection with the hepatitis B virus (HBV) is a major public health problem worldwide. Viral hepatitis B is an infectious, contagious disease caused by a hepatotropic virus and is extremely widespread. The hepatitis B virus (HBV) causes inflammation of the liver. The infection can be acute (severe and short-lived) or chronic (long-term). Lead to a significant risk of death from cirrhosis or liver cancer. HBV belongs to the *Hepadnaviridae* family. This family includes all viruses whose genome consists of circular double-stranded or partially double-stranded DNA and possesses reverse transcriptase. Ten HBV genotypes have been identified and are labeled A to J. In Cameroon, it has been reported that genotypes A and E circulate in a study population [4]. According to the World Health Organization (WHO), approximately 2 billion people worldwide carry serological markers for the hepatitis B virus. In 2015, the WHO estimated that 257 million people were living with chronic hepatitis B. It is particularly prevalent in sub-Saharan Africa and East Asia, affecting 5% to 10% of all adults, while in North America, less than 1% are infected. The estimated prevalence is less than 5% in Eastern Europe, 1.5% in Northern Europe, 2% in Southern Europe, and 1% in Western Europe. In Cameroon, its prevalence was estimated at 11.2% in a meta-analysis [5]. However, this is a preventable infection thanks to vaccination, which has been available since 1981 [6]. In a context where blood donations are steadily declining in blood banks, it seems appropriate to secure and retain blood donors through appropriate and complete vaccination to provide them with protective humoral immunity against the hepatitis B virus (HBV). An individual is considered immune when they have a sufficient level of antibodies directed against the surface antigen (anti-HBs antibodies). These antibodies can develop through vaccination or after exposure to

the hepatitis B virus. However, certain factors such as age, weight, and lifestyle can influence an individual's immunity. Hepatitis B virus infection remains a significant threat to our blood banks despite the preventive measures implemented over the past three decades. Similarly, despite the high prevalence of HBV in Cameroon, very few studies have been conducted on the immunity status of blood donors. Therefore, in a context where blood donations are scarce in our blood banks, ensuring immunity against the hepatitis B virus is of particular importance for the protection of donors. This study was thus conducted to evaluate the humoral immunity status against the hepatitis B virus in regular blood donors at the Yaoundé Central Hospital blood bank in Cameroon.

2. Methods

2.1. Type and Setting of the Study

This was a cross-sectional descriptive study with an analytical purpose, conducted at the Yaoundé Central Hospital (YCH), precisely in the blood bank service. Patient recruitment took place in the blood bank service of the hospital. The YCH, a second-category hospital, has a high demand for Hepatitis B screening in Yaoundé. The screening of Hepatitis B was performed in the laboratory of the YCH.

2.2. Study Period and Duration

The study was conducted over a period of four months, from February to May 2024, with a one-month data and biological sample collection.

2.3. Study Population

The study population consisted of regular blood donors who came to give blood at the blood bank service of the study sites. Included were all blood donors who respected the criteria for blood donation, who at least gave blood twice, gave informed consent and were tested negative for Hepatitis B antigen screening. Excluded were patients who voluntarily withdrew from the study and those screened positive for Hepatitis B antigen after their screening.

2.4. Sampling and Sample Size

Recruitment was carried out using a non-probabilistic consecutive sampling method. The minimum sample size was estimated using the Cochran formula ($N = \frac{Z^2(P)(1-P)}{d^2}$) where N represented the study population; Z Confident interval at 95% which was 1.96; P represented the immunization prevalence against Hepatitis B which was 6.5% (based on a study done in the South-west region of Cameroon [7]); and d represented the study precision with interval of 5% (0.05). This calculation permitted to obtain the minimal sample size of 94 donors.

In total, 155 blood donors were initially enrolled at the HCY blood bank. Of these 155 donors, sixteen did not consent (10.32%). Blood was collected from 139

donors; among them, eleven (7.91%) were excluded from the study, including four (36.36%) who were HBsAg positive and 7 (63.63%) whose samples were hemolyzed. In the end, 128 potential donors were selected, thus exceeding the minimal sample size previously calculated.

2.5. Operational Variables

The dependent variable was the humoral immunity status against hepatitis B virus (HBV), measured through the presence of anti-HBs antibodies detected by ELISA, and categorized as immune or non-immune. The independent variables included vaccination status (number of doses received), total HBc antibodies indicating prior exposure to HBV, and sociodemographic factors such as age and sex. The control variables were the eligibility criteria for inclusion, namely regular blood donors with at least two donations, negative HBsAg screening, and informed consent. The exclusion variables comprised donors who tested positive for HBsAg, those with hemolyzed samples, and individuals who withdrew consent. Finally, the measurement variables were based on laboratory assays (ELISA Fortress®) with manufacturer-defined cut-off values, and statistical analysis was performed using odds ratios, confidence intervals, and p-values to determine associations between vaccination and humoral immunity. This operational framework allowed the study to systematically evaluate the relationship between vaccination coverage and immunity among blood donors.

2.6. Ethical Considerations

The study received research authorization from the Yaoundé Central Hospital (No. 046/24/AP/MINSANTE/SG/DHCY/CM/SM) on February 16, 2024, as well as the ethical clearances from Yaoundé Regional Ethics Committee (No. 00070/CRERSHC/2024) on January 30, 2024, obtained prior to the start of data collection. As this was an observational study, no registration in a clinical trials registry was required. Written informed consent was obtained from each participant; anonymity was ensured by a coding system, and access to the data was restricted to the research team.

2.7. Sample Collection

2.7.1. Serological Tests

All the reagents and samples needed to carry out the different tests were placed on the work surface so that they could return to room temperature.

2.7.2. Anti-HBs Antibody Assay

For this assay, we used the Fortress Diagnostics Limited (UK) kit. The washing solution was diluted 1/20 with distilled water in bottles connected to the ELISA washer. Next, the blank well (A1), the wells containing the standard solutions (B1 to E2) corresponding to the standard solutions (1 - 6), and the wells containing the various samples (F2 to H12) were numbered. We added 50 µL of each standard and sample to their respective wells, and then 50 µL of Horseradish Peroxidase

(HRP-conjugate) was added to each of the wells except the blank. The solution was gently stirred to mix well, and the plate was then covered. The covered plate was incubated at 37°C in a water bath for one hour. The wash solution was already introduced into the bottles connected to the ELISA washer. Five (5) washes were performed per well for each wash cycle. Once the wash was complete, the plate was dried by inverting it several times on absorbent paper. Then, 50 µL of Chromogen A solution and 50 µL of Chromogen B solution were added to all wells, including the blank, and the covered plate was incubated at 37°C for 15 minutes. A blue color was observed in the positive standard wells and the positive sample wells. Adding 50 µL of the stop solution (sulfuric acid) to each well, followed by gentle agitation of the plate for five (5) minutes, stopped the reaction. Finally, the optical density of each well was measured using an ELISA reader at 450 nm.

Run validity criteria: The OD of the blank, which contained only the chromogens and the stop solution, had to be less than 0.080 at 450 nm. The OD of the 0 mIU/ml standard had to be less than 0.100 at 450/630nm or 450 nm. The OD of the 160 mIU/ml standard had to be greater than 1.500 at 450/630nm or 450 nm.

Each concentration was obtained by plotting the logarithm of the absorbance (log-OD) of each duplicate standard on the ordinate axis (Y) against the corresponding anti-HBs antibody concentration (log-mIU/ml) on the abscissa axis (X), on graph paper, without averaging the standard duplicates before plotting the curve. Next, we drew the calibration curve passing through the maximum number of points. Finally, to determine the anti-HBs antibody concentration of each sample, we located the sample's absorbance on the ordinate axis (Y), found the point of intersection on the calibration curve, and read the concentration (log-mIU/ml) from the abscissa axis (X). The anti-HBs antibody titer was obtained by applying the function $10^{\log}$. Following this calculation, any donor with an anti-HBs antibody concentration ≥ 10 mIU/ml was considered to have an immunizing titer, and any donor with an anti-HBs antibody concentration < 10 mIU/ml was considered to have a non-immunizing titer.

2.7.3. Anti-HBc Antibody Assay

For this assay, we used the Fortress Diagnostics Limited (UK) kit. The wash solution was diluted 1/20 with distilled water in bottles connected to the ELISA washer. Next, the wells for the blank (A1), the negative controls (B1 - D1), the positive controls (E1 - F1), and the various samples (G1 to H12) were numbered. We then added 50 µL of each control and sample to their respective wells, followed by 50 µL of Horseradish Peroxidase (HRP-conjugate) to each of the wells except the blank. The solution was gently swirled to mix and the plate was covered. The covered plate was incubated at 37°C in a water bath for one hour. The wash solution was already in the bottles connected to the ELISA washer. Five washes were performed per well. Once the wash was complete, the plate was dried by inverting it several times on absorbent paper. We then added 50 µL of Chromogen A solution and 50 µL of Chromogen B solution to all wells, including the blank, and incubated the covered plate at 37°C for 10 minutes. A blue color was observed in

the negative standard wells and the negative sample wells. Adding 50 μ L of the stop solution (sulfuric acid) to each well, followed by gentle agitation of the plate for five (5) minutes, stopped the reaction. The optical density of each well was measured using an ELISA reader at 450 nm.

Run validity criteria: The OD of the negative control had to be ≥ 0.800 at 450/630nm. The OD of the positive control had to be < 0.100 . The OD of the blank had to be < 0.08 at 450 nm.

For positive results ($S/Cut-off \leq 1$): samples that gave an absorbance less than or equal to the cut-off value. For negative results ($S/Cut-off > 1$): samples that gave an absorbance greater than the cut-off value.

2.8. Statistics

The database was designed using Excel 2019 spreadsheets, statistical analysis was performed to describe donor characteristics and evaluate associations between vaccination and humoral immunity. First, descriptive statistics summarized the recruitment process and demographics: of 155 donors initially enrolled, 128 were retained after exclusions, with a male-to-female ratio of 4.12 and a mean age of 31.44 ± 7.44 years. Frequencies and percentages were calculated for serological markers, showing that 25% of donors were negative for HBV markers, 15.6% were positive for HBsAg, 41.4% for HBcAg, and 18% for both. Vaccination coverage was 13.28%, with 11.72% completing the full schedule. Inferential statistics were then applied to assess factors associated with humoral immunity. Odds ratios (OR) with 95% confidence intervals (CI) were computed, and chi-square tests with p-values determined statistical significance. The analysis revealed that completing three doses of the vaccine was significantly associated with the presence of protective humoral immunity (OR = 4.52, 95% CI: 1.069 - 19.18, $p = 0.040$). This statistical approach combined descriptive measures to profile the donor population with inferential tests to establish meaningful associations between vaccination and immunity outcomes.

3. Results

During the study period, 155 blood donors were initially enrolled at the HCY blood bank. Of these 155 donors, sixteen did not consent (10.32%). Blood was collected from 139 donors; among them, eleven (7.91%) were excluded from the study, including four (36.36%) who were HBsAg positive and 7 (63.63%) whose samples were hemolyzed.

In the end, 128 potential donors were selected. The male-to-female ratio was 4.12 (103 men and 25 women). The mean age of the participants was 31.44 ± 7.44 years, with a range from 20 to 63 years (**Table 1**).

Table 1. Distribution of blood donors by age group.

Age range	Number (n)	Percentage (%)
[20 - 29]	65	50.78

Continued

[30 - 39]	43	33.59
[40+]	20	15.63
Total	128	100

3.1. Distribution of Serological Markers in the Study Population

The number of blood donations previously made by the donors ranged from two to five. The detection of the two serological markers of HBV (Anti-HBs and Anti-HBc) using the ELISA method yielded 32/128 (25%) negative results, 20 (15.6%) positive for HBsAg, 53 (41.4%) positive for Anti-HBc and 23 (18%) positive for both Anti-HBs and Anti-HBc (**Table 2**). Among the 128 donors interviewed, 17 (13.28%) had been exposed to the vaccine, with only 9 (7.03%) having completed their vaccination schedule (**Table 3**).

Table 2. Distribution of participants according to serological markers.

Serological marker	Number (n)	Percentage (%)
AcHBs only	20	15.6
AcHBc only	53	41.4
AcHBs and AcHBc	23	18
None (negatives)	32	25
Total	128	100

Table 3. Vaccination status against the hepatitis B virus.

Vaccination status	Number (n)	Percentage (%)
Vaccinated	17	13.28
Unvaccinated	111	86.72
Total	128	100

3.2. Factors Associated with Humoral Immunity against HBV

We obtained a proportion of 43/128 (33.59%) immunized donors versus 85 (66.41%) non-immunized donors. The study of factors associated with immunity against HBV allowed us to observe that vaccination completion (3 doses) was significantly associated with the development of humoral immunity with a p-value of 0.040 (**Table 4**).

Table 4. Univariate analysis of donor history that may influence the HBs-mediated humoral response.

Variables	Staff (N = 128)	AcHBs		OR (95% CI)	p-value
		Yes (n = 43)	No (n = 85)		
Type of donation					
Volunteer	17	8	9	1.930 (0.687 - 5.423)	0.212
Family	111	35	76	1	1

Continued

Number of donations					
2	79	29	50	1	1
3	37	12	25	0.828 (0.362 - 1.891)	0.654
4+	9	2	7	0.493 (0.096 - 2.531)	0.397
NR*	3	0	3		
Vaccination					
Yes	17	9	8	1	1
No	111	34	77	2.548 (0.906 - 7.168)	0.070
Number of vaccine doses received					
None	111	34	77	1	1
1 dose	2	1	1	2.265 (0.138 - 37.28)	0.567
2 doses	6	2	4	1.132 (0.198 - 6.48)	0.889
3 doses	9	6	3	4.52 (1.069 - 19.18)	0.040
Number of years since the last dose					
≤2	5	3	2	1.100 (0.097 - 12.538)	0.939
[3 - 5]	3	1	2	2.200 (0.520 - 9.307)	0.284
[5+]	8	4	4	3.300 (0.528 - 20.639)	0.202
NR*	112	35	77		
AcHBc					
Yes	76	23	53	1.440 (0.685 - 3.027)	0.336
No	52	20	32	1	1

4. Discussion

The objective of this study was to evaluate the status of humoral immunity against the hepatitis B virus in regular blood donors. More specifically, it aimed to assess the vaccination coverage of regular blood donors at the HCY blood bank, determine the rate of humoral immunity, and identify any factors that could influence the development of humoral immunity. Overall, regular blood donors were predominantly male (80.47%), with a male-to-female ratio of 4.12. The minority of female donors could be explained by certain physiological conditions specific to women, such as breastfeeding, menstruation, and pregnancy. Our population comprised two types of donors: voluntary donors (17) and family donors (111). The type of donation is an important characteristic due to its direct impact on transfusion safety [8]. It can be deduced that the population does not have a culture of voluntary blood donation, hence the high number of family donations. Patients requesting blood are at risk of contracting parenterally transmitted infections. In our study, we determined vaccination coverage among regular blood do-

nors and found a vaccine exposure rate of 13.28% with a completion rate of 11.72%. This vaccination coverage remains low. Given that more than half the population was aware of the vaccine's existence and that our study took place in an urban setting, we would have expected higher coverage. This could be explained by a lack of awareness about the importance of vaccination, as Cameroon does not yet have a national policy for vaccinating regular blood donors, but also by some people's ignorance of the existence of a vaccine against HBV. Similar rates have been reported in Cameroon among medical students, such as 17.6% by Bagnaka *et al.* in 2011 and 18% by Noubiap *et al.* in 2012 [9] [10]. Given that these surveys were also conducted in urban areas, this slight difference could be explained by the fact that medical students have a good knowledge of the hepatitis B vaccine due to their training. However, in 2018 in Cameroon, a study by Aroke *et al.* found a coverage rate of 26.05% among medical students, while another survey conducted in 2017 by Meriki *et al.* in Cameroon reported 30.2% [7] [11].

Determining humoral immunity against the Hepatitis B virus in regular blood donors revealed that 33.59% of the population had an immunizing level against HBV. This prevalence could be explained by two factors: First, our study showed that among the 15 donors with complete vaccination, only 8 (53.33%) had an immunizing humoral response, representing half of the total. This low prevalence among vaccinated individuals could be explained by several factors, such as the fact that the absence of a protective Anti-HBs titer in vaccinated patients does not preclude the presence of memory immunity against HBV [12]. Age influences the vaccine response and this low response among donors exposed to the vaccine could be due to cases of non-responders. However, these frequencies are significantly higher than those observed in a study conducted by researchers Al Mutairi and colleagues in Saudi Arabia in 2011 (3.34%), and Japhet *et al.* in Nigeria, who reported 15.2% in 2011 [13] [14]. These discrepancies could be attributed to hepatitis B vaccination coverage, which varies from one country to another. Donor lifestyles could also contribute to these differences.

Secondly, most of these subjects appear to have humoral immunity resulting from HBV exposure, since all our donors were HBsAg negative. This is confirmed by at least two facts: a vaccine exposure rate of 7.03%, a humoral response rate of 33.59% (which is higher than the exposure rate), and an overall HBV exposure prevalence of 59.4%.

These results demonstrate considerable exposure of the donors to HBV. However, the presence of anti-HBcAg alone, in the absence of HBV DNA testing, does not allow for a diagnosis of occult hepatitis B, and this reactivity cannot, by itself, be interpreted as evidence of occult HBV infection in these donors; the prevalence of occult HBV infection was therefore not assessed in the present study. A more recent study conducted between February and June 2025 at the same blood bank confirmed occult HBV infection in 9 of 269 donors (3.34%) through detection of HBV DNA, with co-circulation of genotypes A and E [15]. This high exposure to HBV could be explained by the fact that Cameroon is located in a high-endemicity zone, with a seroprevalence of 11.2%. A similar study conducted by Etheline *et al.* in Cam-

eroon in 2017 reported an exposure prevalence of 47.3% [16]. Our exposure rates are higher than the 19% reported by Tatsilong *et al.* in 2016 in Cameroon [17]. This difference in prevalence could be due to the difference in the diagnostic technique used, as Tatsilong *et al.* worked with the rapid strip test, which has relatively lower sensitivity and specificity compared to the ELISA technique used in our study.

The identification of factors that could influence humoral immunity in blood donors revealed no significant differences for sociodemographic factors, including age and sex. Yihua Zhou *et al.*, in their 2019 study conducted in China, also found no significant differences for these two factors [18]. However, in univariate analysis, we were able to establish that receiving at least three (3) doses of vaccine was significantly associated with the development of protective humoral immunity. Mwangui *et al.*, in Kenya, also obtained a similar result [19]. This result is explained by the fact that the HBV vaccine provides immunity in at least 90% of cases, and that taking three (3) doses of the vaccine ensured complete immunization. Furthermore, taking a single dose of the vaccine does not provide immunity. This demonstrates in our study that vaccination was the sole factor in the development of humoral immunity.

5. Conclusion

This study shows that vaccination coverage among blood donors is low and that vaccination completeness is the factor that effectively influences the occurrence of protective humoral immunity. These data sufficiently prove that there is an urgent need to put in place vaccination strategies whose main purpose will be to demonstrate the importance of protecting oneself against HBV through vaccination.

6. Study Limitations

One limitation of this study is the absence of objective verification of the number of vaccine doses received, as the data were collected based on participants' self-reported recall in the absence of an available vaccination booklet at the time of data collection; these data are therefore subject to recall bias, which may lead to an underestimation or overestimation of the true vaccination coverage.

State of Knowledge on the Subject

- Transfusion-transmitted HBV is a reality in countries where blood donations are not tested for HBc antibodies and the viral genome.

Contribution of Our Study to Knowledge

- The vaccination coverage against HBV is 13.28% in the HCY blood bank.
- The immunization rate against HBV is 33.59%, mainly resulting from possible exposure to the virus.

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hospital management.

Author Contributions

Conceptualization of the study: Symphorien Ewodo, Guy Roger Nsenga;

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Informed Consent

All participants signed a handwritten consent form to participate in our study.

Data Availability Statement

The data supporting the conclusions of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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