Vol. 13(1), pp. 5-12, January-June 2021
DOI: 10.5897/IJMMS2021.1455
Article Number: E4B084966945
ISSN: 2006-9723

http://www.academicjournals.org/IJMMS

ACADEMIC JOURNALS

International Journal of Medicine and Medical Sciences

Full Length Research Paper

Prevalence and risk factors of Hepatitis B virus infection among blood donors in Gabonese National Blood Transfusion Center: A cross-sectional and analytic study

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Received 26 March, 2021; Accepted 25 May, 2021

Hepatitis B virus (HBV) infection is prevalent in Gabon and poses a potential risk of transmission by blood transfusion. However, few studies have examined epidemiological data regarding HBV infection of Gabonese blood donors. This article reports on research conducted to estimate the seroprevalence of HBV and associated risk factors in the urban population of Gabon. A cross-sectional and analytic study survey of blood donors attending at the Gabonese National Blood Transfusion Center, was carried out between June and August 2020. The ELISA technique (Evolis®, BioRad) and the chemiluminescence technique (Cobas® e601, Roche) had been used for the detection of hepatitis B surface antigens in the plasma of donors. Repeatedly reactive hepatitis B surface antigen levels among first-time and repeat donors were used to assess prevalence and risk factors using multivariable logistic regression. Results revealed that a total of 3665 donations were collected at the Gabonese National Blood Transfusion Center, of which 100 were confirmed HBV positive. The seroprevalence of HBsAg among total blood donors was 5.5% (95% confidence interval [CI] = 4.4 - 6.7) indicating a moderate burden. In our multivariate analysis controlling for age, HBsAg positivity was associated with first-time donor status (aOR = 6.5) and residence outside of Libreville (aOR = 1). The prevalence of HBsAg among Gabonese blood donors is at a moderate-level endemicity among first-time donors, indicating the need to further limit the burden. In this Gabonese context, status of first-time blood donor and living in rural settings are primary risk factors of HBV-infection, and henceforth considered as exclusionary criteria for blood donation in Gabon.

Key words: Hepatitis B, prevalence, risk factors, blood donor

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global public health problem. It is estimated that there are around 400 million chronic carriers of the hepatitis B virus (HBV) and more than one million deaths per year worldwide (WHO, 2016; Atipo-Ibara et al., 2015). Estimates of hepatitis B antiginaemia seroprevalence of 5-20% have been reported (Te and Jensen, 2010), making sub-Saharan Africa a hyper-endemic region. The lifetime and mortality

risks from hepatitis B infection in this region are estimated to be around 25% (Matthews et al., 2014). In countries in sub-Saharan Africa, hepatitis B virus seroprevalence levels ranging from 8 to 15% have been reported in blood donors (Tagny et al., 2010).

Gabon, a middle-income country of approximately 2 million inhabitants, with approximately 241,080 individuals with hepatitis B (Makuwa et al., 2009; Bivigou-Mboumba et al., 2016; Schweitzer et al., 2015), is among the countries of 'High endemicity with marked heterogeneity of HBsAg seropositivity among population subgroups.

In Gabon, some studies have reported the prevalence of HBsAg in first time blood donors, with estimated rates ranging from 5.63 to 7.28% (Kounegnigan Rerambiah et al., 2014; Eko Mba et al., 2018). However, there is little literature on assessment of the prevalence of HBsAg among blood donors in the Estuary region, Libreville in Gabon.

Determinants and markers of hepatitis B virus disease outcome pose challenges in understanding the routes of transmission of infection and characterizing actively infected individuals. While transfusion of infected blood and unsterile needles played a role (Jayaraman et al., 2010; Eke et al., 2015), host factors (exposure to alcohol, traditional medicine, sex, age, presence of co-infections) and environmental factors do not support transmission via a singular mode (Ragheb et al., 2012; Hoffmann et al., 2012). An earlier study in Gabon suggested that certain demographic and behavioural factors were potentially relevant modes of HBV transmission (Groc et al., 2019). These data mainly concern the general population with low socio-economic status and high risk groups. However, blood donors including those mono-infected with HBV were not taken into account. We thus sought to assess the seroprevalence and risk factors associated with HBV infection among blood donors in Gabon.

MATERIALS AND METHODS

Ethics statement

The study protocol was reviewed and approved by the National Ethics Committee for Research (Authorization n° 0088/2019/PR/SG/CNER) and the National Blood Transfusion Center (Authorization n° 527 /MS/SG/CNTS/DG) of the city of Libreville, Gabon. After obtaining verbal informed consent, their answers to 12 questions were recorded.

Study design, setting and population

This cross-sectional and analytic study was carried out on data from blood donors recorded by the blood bank in the Gabon Estuary area, from June 2020 to August 2020 (Figure 1). The Gabonese National Blood Transfusion Center is the largest specialized institution for the collection and supply of blood in the Gabon Estuary. Currently, the amount of blood collected by the Blood Center is approximately 22, 200 units each year. There are two collection methods in this transfusion center, in particular a collection on a fixed site and on a mobile site from which a mobile vehicle ensures the collection.

Study design and settings

A total of 3665 blood donors were recruited by random sampling in multistage clusters. between June and August, 2020. One collection methos (fixed site) was randomly selected for the study. From June to August 2020, all blood donors who presented themselves at this fixed collection site were enrolled in the prevalence estimation study. The data, including post-donation testing, were used to estimate the seroprevalence of HBV among blood donation.

Sample size determination and sampling techniques

The sample size for sero-prevalence of HBV infection among blood donors was determined by considering the seroprevalence of HBsAg (5,63%) among blood donors from the study conducted at the Gabonese National Blood Transfusion Center (Kouegnigan Rerambiah et al., 2014). All blood donors who donated the blood at the Gabonese National Blood Transfusion Center were selected consecutively until the required sample size was obtained.

The risk factors associated with hepatitis B in the blood donor population have been assessed using case-cohort data according to Jagannathan et al. (2010). Eligible cases were defined as all people who donated blood between June and August 2020 and were repeatedly reactive for HBsAg. HBsAg seropositive donors were contacted by telephone and notified of their HBsAg status. These individuals were asked to come to the blood bank for test result disclosure counselling and follow-up, and if they presented, were asked to participate in the study. Donors who gave informed consent to participate in the study were asked to complete a self-administered questionnaire on potential demographic and behavioral risk factors.

A comparison group of donors whose HBsAg status was unknown was sampled at fixed blood donation site organized at the Gabonese National Blood Transfusion Center.

We aimed for a ratio of three comparison subjects for each positive HBsAg in order to maximize statistical power. Data from the comparison donor group were collected with an anonymous, self-administered questionnaire at the time of blood donation at the fixed site, so the questionnaire data could not be linked to the HBsAg test data performed. later in the Viral Serology Department of the Blood Bank.

Laboratory methods

Vacuum samples were used for approximately 5 ml x 2 of blood from each donor, then transferred to the Viral Serology Department and centrifuged at 1500 rpm for 3 min to obtain plasma. All the plasmas were separated into an aliquot and stored in a -20°C freezer until further testing. Plasma samples were tested for four

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Figure 1. Gabon: Towns and villages.

infectious markers including HCV, HIV, syphilis and HBsAg in accordance with blood bank operational policy using an alternative strategy of enzyme immunoassay and/or chemiluminescence. Due to cost and availability, neutralization of HBsAg, hepatitis B (anti-HBc) and HBV DNA testing were not performed during the study period. Although confirmatory testing could not be performed, the specificity of the alternative EIA strategy is expected to be greater than the 95-99% specificity of a single HBsAg EIA test (Scheiblauer et al., 2010).

Statistical analysis and definition of variables

The burden of HBsAg was defined as low (<2%), moderate (2-8%), and high (>8%). Statistical analyses were performed using Epi-Data 3.1 and SPSS software version 22.0. The overall prevalence of HBsAg among blood donors was calculated with a 95%CI. The study population was described by tabulating univariate frequencies of demographic variables. Pearson chi-square tests were performed for each of the demographic and behavioral characteristics to assess significant differences between $HBsAg^+$ and $HBsAg^-$ donors. Fisher's exact chi-square tests were used when cell sizes were less than five. We first analyzed the major risk factor variables using the case-cohort adjustment of Miettinen and found that the estimated relative risk approximated the odds ratio derived from a traditional case-control analysis, with the odds ratio being the more conservative estimate. Thereafter, we used logistic regression models to examine univariate and multivariate associations between HBsAg status as the dependent variable and demographic and behavioral risk factors as independent variables. All demographic and behavioral risk factors with p values less than 0.1 in univariate logistic regression models were included in the "first" logistic regression model. Variables not significant in the multivariate model (p>.1) were removed to produce the most parsimonious model, as indicated by using the likelihood ratio test. All behavioral risk factor variables in this model (blood exposure) were tested for interaction with age, but the addition of these interaction terms did not improve the model fit, and so they were omitted from the final model. The results of the univariate and multivariate logistic models were expressed as odd ratios (OR) or adjusted odds ratios (aOR) with 95% confidence intervals (CI).

Table 1. Distribution of blood donors by age stratified by sex, at the Gabonese National Blood Transfusion Center, 2020.

Age group (years)	Male [No. (%)]	Female [No. (%)]	Total [No. (%)]
<20	43(74.1)	15 (25.9)	58 (3.6)
20-29	295 (55.7)	235 (44.3)	530 (32.9)
30-39	385 (77.6)	111 (22.4)	496 (30.8)
≥40	304 (58)	222 (42.4)	524 (32.5)
Total	1048 (65)	562 (35)	1610 (100)

Table 2. Prevalence of hepatitis B in donor blood stratified by age group and sex.

Age group (years)	Male [No. (%)]	Female [No. (%)]
<20	0/43 (0)	0/15 (0)
20-29	35/295 (11.9)	8/235 (3.4)
30-39	29/385 (7.5)	3/111(2.7)
≥40	11/304 (3.6)	2/222 (0.9)

Table 3. Prevalence of hepatitis B in blood donors by demographic characteristics.

Variable	Total donors	Overall positive HBsAg	Prevalence	95% CI
Age group (years)				
<20	58	0	0	0-0
20-29	530	43	8.1	5.9-10.8
30-39	496	32	6.5	4.5-9
≥40	526	13	2.5	1.3-4.2
Sex				
Male	1048	75	7.2	5.7-8.9
Female	562	13	2.3	1.2-3.9
Total	1610	88	5.5	4.4-6.7

CI: 95% Confidence Interval ; HBsAg: Hepatitis B antigen surface.

RESULTS

From June to August 2020, a total of 3665 donations were collected on fixed site. Out of 3665 blood donors, 1610 (43.9%) were first-time blood donors that were screened at the Gabonese National Blood Transfusion Center. The age distribution of blood donors is shown in Table 1. The proportions of male and female donors were 65 and 35% respectively. The majority (32.9%) of donors were between 20 and 29 years old, while the younger group (<20 years) comprised the smallest blood donor population (3.6%).

Prevalence of HBV among first time blood donors

In Gabon, the overall prevalence of hepatitis B surface antigen among the total number of first-time donations in Gabonese National Blood Transfusion Center during this study period was 5.5% (95% confidence interval [CI] = 4.4 - 6.7) or 88 reactive ELISA tests out of 1610 first time blood donors (Table 3). The highest prevalence was recorded among the 20-29 years age group (8.1%) (95% confidence interval [CI] = 5.9-10.8), higher than that of 30 years and over age group, and the least prevalence of 0% was observed among younger groups (<20 years) donors. Seventy-five (7.2%) (95% confidence interval [CI] = 5.7-8.9) males were tested HBsAg positive as compared to thirteen (2.3%) (95% confidence interval [CI] = 1.2-3, 9) females. The prevalence was highest among males in all the age groups (Table 2) with the risk of being HBsAg positive found to be 3-fold higher in males than in females (Table 3).

Table 4. Demographic characteristics of the study population according to HBsAg status.

Demographic characteristics	HBsAg negative [No. (%)]	HBsAg positive p-value [No. (%)]
All participants	212	71
Age (years)		**0.0001**[a]
20-29	97 (45.7)	35 (49.3)
30-39	83 (39.2)	22 (31)
40-49	32 (15.1)	14 (19.7)
Education		0.074[b]
Graduates	111 (52.4)	28 (40)
Less diploma	101 (47.6)	43 (60)
Residence		**0.0001**[a]
Libreville	169 (79.7)	58 (84.3)
Akanda	7 (4.1)	4 (5.6)
Owendo	20 (9.4)	4 (4.3)
Ntoum	16 (7.5)	5 (7.1)
Civil status		**0.057**[a]
Single	110 (51.9)	39 (55)
Live together	86 (40.6)	32 (45)
Married	16 (7.5)	0 (0)
Donor status		**0.0001**[a]
First time	72 (34)	63 (88.7)
Repeated	140 (66)	8 (11.3)
Donor type		**0.0001**[b]
Voluntary	4 (1.9)	23 (32.4)
Replacement	208 (98.1)	48 (67.6)

HBsAg : Hepatitis B antigen surface. Bold was used for significant ($P < 0.05$) results. [a]Pearson χ^2 test. used. [b]Fisher's exact test used.

Risk factors of HBV infection

From 100 blood donors who tested repeatedly reactive for HBsAg between June and August 2020, 29 declined to participate in the study and 71 (71% of all HBsAg positives) consented to participate in the study. A total of 212 comparison donors of unknown HBsAg status were recruited during the same time period from among 284 randomly selected donors at blood drives approached at the same locations from which the HBsAg positives were drawn. The case-cohort study included 71 cases and 212 comparison subjects. Demographic characteristics are presented in Table 4.

90.4% of the participants were replacement donors, 72.4% resided in Libreville and 43.1% were between 20 and 29 years old against 47.3% for all donors. There were differences according to HBsAg status (Case and control group) on several socio-demographic and blood donation characteristics, including age, place of residence, voluntary donor versus replacement donors and first-time donor status versus repeat donors. Data on behavioral risk factors according to HBsAg status, with unadjusted ORs, showed that HBsAg positive cases had significantly higher uncorrected odds of having contact with blood exposure (OR= 2.98) (Table 5).

In the final multivariate model (Table 6), adjusting for age, first time donor status (aOR=6.5) and residence outside of Libreville (aOR= 1) were significantly associated with HBsAg status. Other behavioral risk factors associated with HBsAg in the univariate analysis were no longer significant after controlling for other covariates and were excluded from the final model. Interactions between age and behavioral risk factors were not significant and therefore were not included in

Table 5. Behavioral risk factors according to HBsAg status.

Risk factor	HBsAg negative (N=212)	HBsAg positive (N=71)	OR 95% CI
Blood exposure			
Ever	1(0.5)	1(1.4)	2.98 0.18 - 48.4
Traditional treatment			
Yes	34 (16)	1 (1.4)	0.08 0.01-0.65
Scarification / tattoo			
Ever	56 (26.4)	2 (2.9)	0.1 0.02- 0.44
Consumes alcohol			
Yes	123 (58)	3 (4.3)	0.07 0.02-0.24
Dental care			
Ever	42 (19.8)	2 (2.8)	0.14 0.03-0.6
Multipartner sex			
Ever	55 (25.9)	3 (4.2)	0.16 0.05-0.54

Number (%) admitting risk behaviour, with unadjusted OR and 95% CI.

Table 6. Results of the final logistic regression model.

Explanatory variable	Adjusted odds ratios	95% CI
Age (20-29 vs 30-49)	3.2	0.0-7.8
First time donor (vs repeat)	6.5	3.0-74.0
Residence (outside vs Libreville)	1	0.0-3.0

AOR: Adjusted Odds Ratio; CI: 95% Confidence Interval.

the final multivariate model.

DISCUSSION

This study documented a prevalence of 5.5% of HBsAg-positive among overall blood donor tested by two rounds of screening with different enzyme-linked immunosorbent assays in post-donation, between June and August 2020.

This was higher than in Marocco in previous studies (1.34%) (Adouani et al., 2013) and in other developing countries such as Burundi (2.5%), Egypt (1.4%), Rwanda (4.1%), Burundi (2.5%), Iran (0.15%) and Botswana (1.02%) (Tagny et al., 2010; Wasfi and Sadek, 2011; Twagirumugabe et al., 2017; Farshadpour et al., 2016; Choga et al., 2019) and some blood bank from industrialized countries like China (0.87%); France (0.65%) and the United States (0.76%) (Song et al., 2014; Meffre et al., 2010; Dodd et al., 2016).

The high rate among blood donors, which represents a large part of the population, objectively reflects the prevalence of HBV in Sub-Saharan Africa (Te and Jensen, 2010). More attention should be paid to blood safety to promote the need for strict donor selection criteria.

Several plausible demographic and behavioural risk factors were found to be associated with HBsAg status among blood donors in Gabon. First-time donor status is previously recognised as risk factors for HBV infection. In addition, association with place of residence is a novel finding for Gabon and may have relevance for blood safety and public health.

It was amazing to find that donors living outside Libreville had more than 1 time the odds of being $HBsAg^+$ than donors from Libreville while adjusting for other factors. The researchers are also aware of recognised high-prevalence areas for HBV in the Libreville region, although a higher frequency of percutaneous exposure could be more common in rural areas. Further investigation of Geography-specific lifestyle issues is needed to understand what might explain this increased risk for HBV.

Although not significant in the logistic regression model, having contact with blood exposure were significant in the univariate analysis. This parenteral risk factors is biologically plausible and have been found to be associated with HBsAg status in previous studies (Jayaraman et al., 2010; Eke et al., 2015). It is also

conceivable that previous reports may have been due to confounding, as we did not detect an association between these variables and HBV after controlling for likely confounders such as age and gender by exclusion and multivariate analysis. Donor deferral based on risk behaviour assessment is a routine part of blood donor screening in the Europe and United States; yet in Gabon, donor screening procedures are not uniform across blood centers. Few centers in Gabon use individual risk behaviour questionnaires.

Local blood bank personnel realise the need for an individual donor screening questionnaire. However, behavioural risk factors for HBV infection and other TTIs in blood donors in Gabon are not well studied.

Volunteer blood donors, especially young volunteer donors representative of demographics currently being recruited to replace family replacement donors in Gabon, are a healthy group of people from low-risk populations (WHO, 2008). They were screened for potential risk factors such as gay men, drug use, and a history of diabetes. These high-risk behaviors were not associated with HBV infection in this study because subjects were excluded from donation. However, this does not mean that these risk factors are not potential factors for infection with HBV. Some blood donors may conceal exposure to high risk factors on the medical history questionnaire. For a complete and accurate investigation, further study is warranted.

Our study had some limitations. Of note, the analysis with HBsAg does not cover occult hepatitis B infection, suggesting an underestimate of the real burden. The sample size was limited and the completeness of the self-reported risk factor questionnaire was not always effective. The study period was semingly short, which restricted time-dependent variation analysis.

Conclusion

Our findings suggest that the prevalence of overt HBV infection is at a moderate-level endemicity among blood donors. Overall, first time blood donation and living in peripheral areas are driven factors of HBV serpositivity. These provide useful information in the development of individual pre-donation questionnaires to defer those at risk from donating blood. However, our study also highlights the need to continue throrough epidemiological surveillance for blood safety in Gabon.

ABBREVIATIONS

Ab, antibody; **Ag,** Antigen; **AOR,** Adjusted Odds Ratio; **ELISA,** Enzyme Linked ImmunoSorbent Assay; **HBsAg,** Hepatitis B surface antigen; **HBV,** Hepatitis B virus; **HCV,** Hepatitis C virus; **LBP,** Labile blood product; **NREC,** National Research Ethics Committee; **NBTC,** National Blood Transfusion Center; **OR,** Odds Ratio; **SSA,** Sub-Saharan African; **TTI,** Transfusion Transmitted Infections.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Content is the sole responsibility of the authors and the National Blood Transfusion Center.

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