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Anti-HBc antibodies and occult hepatitis B infection (OBI) among blood donors in Lomé, Togo

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ABSTRACT

Aims: Among the progressive forms of hepatitis B virus (HBV) infections, the HBs antigen (HBsAg) remains undetectable in 25% of cases. There is also a form of HBV infection called occult HBV infection (OBI) characterized by the presence of viral DNA in the blood and tissues with no detectable levels of HBsAg associated with or without anti-HBc Ab, or anti-HBs Ab out of the pre-seroconversion period, which constitutes an infectious risk in blood transfusion.

Methods: To determine the prevalence of anti-HBc Abs and the HBV DNA in the blood of donors recruited at the blood transfusion center (CNTS) of Lomé. This is a cross-sectional study including 967 blood donations which were seronegative to HBs antigen during the biological qualification of the donation (BQD) from July 28 to August 19, 2022. The detection of total anti-HBc Abs (IgM and IgG) was performed by BIORAD enzyme-linked immunosorbent assay (ELISA) method; the viral load measurement was carried out according to the polymerase chain reaction method by amplification of COBAS AmpliPrep/TaqMan 4800 (Roche diagnostics). Data processing and analysis were performed using EXCEL 2013 software and SPSS software. Concerning the ethical aspect, we have obtained written consent from the donors and the agreement of the bioethics committee for health research No. 052/2022/CBRS.

Results: The prevalence of anti-HBc Abs was 33.82% (CI: 30.83–36.80). Viral DNA was detected in 10 cases. The prevalence of occult hepatitis B was 3.05% (10/327) in blood donors carrying anti-HBc antibodies, and 1.03% (10/967) in the blood donors population that does not carry HBsAg.

Conclusion: The presence of occult hepatitis B within the population of blood donors at the blood transfusion center of Lomé constitutes a real threat to transfusion safety. The association of the viral DNA detection by PCR to the serological tests that are currently carried out during the biological qualification of the donations could be a better approach to reduce the risk of transmission of HBV by blood transfusion at the CNTS of Lomé.

Keywords: Blood donors, Lomé, OBI

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INTRODUCTION

The prevalence of viral hepatitis B is higher in sub-Saharan Africa and eastern Asia, with a proportion of the adult population chronically infected between 5% and 10% [1]. The hepatitis B virus (HBV) is transmissible by contact with blood or other biological fluids of an infected person [2] and therefore potentially transmissible by blood transfusion [3]. Among the progressive forms of HBV infections, the HBs antigen (HBsAg) remains undetectable in 25% of cases [4, 5], and the anti-HBc antibody (Ab) IgM is synonymous of recent or previous infection. There is also a form of HBV infection called occult HBV infection (OBI) characterized by the presence of viral DNA in the blood and tissues with no detectable levels of HBsAg associated with or without anti-HBc Ab, or Anti-HBs Ab out of the pre-seroconversion period [6]. For more than four decades, screening for HBV in blood donors has mainly been done by detecting HBsAg and the most commonly used HBsAg screening tests are based on enzyme linked immunosorbent assay (ELISA) technique [3, 7]. The WHO recommends the choice of tests capable of detecting a minimum level of 0.5 ng/mL of HBsAg in the blood. This level of 0.5 ng/mL poses a problem for donors with a level of HBsAg that cannot be detected by standard tests, such as in cases of occult viral hepatitis B [3]. Anti-HBc Ab is sometimes the only detectable serological marker after the disappearance of HBs Ag [3] and screening for anti-HBc Abs remains the way to fight the transmission of occult viral hepatitis B [8]. Finally, there are mutations in the HBV genome [9, 10] that can lead to false negative tests during screening for HBsAg when using common tests [11]. Thus, such variants can escape screening in blood transfusion and be a source of a low risk of post-transfusion hepatitis B (PTHB), threatening transfusion safety [12]. In Togo, a study carried out by Teko et al. in 2012 revealed that the prevalence of anti-HBc antibodies in blood donors was 53.9% [13] and since then no study has been conducted to assess the evolution of this serological marker. Furthermore, there are no data concerning the detection of the viral DNA among occult HBV infections in these blood donors. This study aims to determine the evolution of the prevalence of anti-HBc Abs and to estimate the prevalence of occult viral hepatitis B among blood donors at the CNTS of Lomé.

MATERIAL AND METHODS

This is a cross-sectional study carried out at the CNTS of Lomé on HBs Ag seronegative blood donations found during the biological qualification of the donation from July 28 to August 19, 2022. We excluded the donations that were found positive for at least one of the serological markers tested at the CNTS: human immunodeficiency virus (HIV) screened by Genscreen ULTRA HIV Ag-Ab from BIORAD (REF 72561), hepatitis C virus (HCV) screened by Monolisa HCV Ag-Ab ULTRA V2 from BIORAD (REF 72346), HBV screened by Monolisa HBsAg ULTRA (REF 72348), syphilis screened by RPR CARBON Slide agglutination of LABKIT (REF: 40130). The screening for total anti-HBc Abs (IgM and IgG) was carried out with Anti-HBc Total Elisa (KAPG4CBE3) from DIASOURCE; the hepatitis B viral load was carried out by PCR using the COBAS AmpliPrep/TaqMan 4800 amplification method (Roche diagnostics).

Course of laboratory tests

After the screenings for HIV, HBV, HCV and syphilis, only the negative samples were selected for qualitative screening of anti-HBc Abs (IgM and IgG) at the CNTS serology laboratory. We made two aliquots of 10 mL for each sample that were negative for HbsAg and positive for anti HBc Abs. These aliquots were stored at minus 30°C for the viral load measurement by PCR done in the molecular laboratory of the National Institute of Hygiene (INH) of Lomé. Validation of the results of the anti-HBc Ab serology and of the viral load was carried out according to the recommendations of the manufacturers of the reagents.

Data were processed by EXCEL 2013 software. The variables studied were: age, sex, regularity of donations, anti-HBc Ab serology, and HBV viral load. SPSS software was used for data analysis.

Ethical aspect: we subsequently obtained the agreement of the bioethics committee for health research No. 052/2022/CBRS of December 16, 2022. We have also obtained written consent from the donors.

RESULTS

Occult hepatitis B prevalence

A total of 1015 donors were recruited for this study and 967 (95.27%) were negative for the HBsAg. Among the HBsAg negative individuals, 327 (33.82%) were positive for HBc Abs. The molecular detection of the viral DNA on HBc Ab positive donors revealed that 10 (3.05%) of them were positive for HBV DNA. The general prevalence of the OBI in our study was then 0.99% (10/1015) (CI 95% [0.38–1.59]) (Table 1).

Sociodemographic characteristics of blood donors' positives for anti-HBc Ab and viral DNA

In this study, at least 90% of donors were male and they were not at their first blood donation. So, most of them were regular donors. The age of donors who carried occult hepatitis B ranges from 29 to 39 years while those who did not carry occult hepatitis B donors were 18–28 years old. Fifty percent of those who carry occult hepatitis B work as office manager and 30% were students. In non-

occult hepatitis B donors, 39% work as office manager and 33.1% were in the informal sector (Table 2).

*Men were more represented than women: sex-ratio M/F= 16

**The average age of anti-HBc Ab positive blood donors was 33.41±8.63 years.

Table 1: Prevalence of occult hepatitis B

Statuts	HBs Ag		Anti-HBc Ab (IgM+IgG)		HBV ADN	
	N (%)	CI 95%	N (%)	CI 95%	N (%)	CI 95%
Negative	967 (95.27)	(93.97–96.58)	640 (66.18)	(63.20–69.17)	317 (96.94)	(95.08–98.81)
Positive	48 (4.73)	(03.42–06.02)	327 (33.82)	(30.83–36.80)	10 (3.05)	(01.19–04.2)
Total	1015 (100)	(100.00–100.00)	967 (100)	(100.00–100.00)	327 (100)	(100.00–100.00)

Table 2: Sociodemographic characteristics of blood donors' positives for anti-HBc Abs and viral DNA

Characteristics		HBV DNA		p-value
		Negative (n=317)	Positive (n=10)	
Gender				1
	Male	**299 (94.3)**	**9 (90.0)**	
	Female	18 (5.7)	1 (10.0)	
Type of donor				0.293
	Regular	**307 (96.8)**	**9 (90.0)**	
	New	10 (3.2)	1 (10.0)	
Age				0.868
	18–28	**96 (30.3)**	2 (20.0)	
	29–32	73 (23.0)	**3 (30.0)**	
	33–39	76 (24.0)	**3 (30.0)**	
	40–59	72 (22.7)	2 (20.0)	
Profession				0.672
	Student	86 (27.1)	3 (30.0)	
	Manager	**126 (39.7)**	**5 (50.0)**	
	Informal	105 (33.1)	2 (20.0)	

DISCUSSION

Socio-demographic characteristics of the study population

In this study, men were more represented than women among the blood donors; the sex-ratio M/F was 16. We found this male predominance at the CNTS of Lomé in previous studies done in 2012 [14] and 2015 [15]. Other studies found this male predominance with 94.36% in Mali by Goita et al. [16], 80% in Sierra Leone by Yambasu et al. [17]. Buseri et al. in Nigeria also described this strong male representation with a ratio of 6 men for 1 woman [18]. The male predominance among blood donors is a general trend. This is partly related to the fact that women physiologically have lower hemoglobin concentrations due to regular menstrual bleeding. The average age of anti-HBc Ab positive blood donors was 33.41±8.63 years, and the most anti-HBc Ab seropositive age group is 18–28 years without significant difference with the other age groups (p = 0.868). This result is similar to that of Fopa et al. in Cameroon [19]. Teko et al. in 2012 at the CNTS of Lomé found a greater representation of the age group of 38–48 years [13]. Young adults who do not have chronic diseases are generally the most willing to donate blood.

Prevalence of anti-HBc antibodies

The prevalence of anti-HBc Abs was 33.82% (CI: 30.83–36.80). Teko et al. in 2012 did the same investigation and found a higher prevalence of anti-HBc Abs with 53.9% [13]. This decrease in the prevalence of anti-HBc antibodies after ten years could be due to the establishment and application since 2018 of the national blood transfusion policy and the practical guide of good transfusion practices [14]. An efficient quality management system (QMS) based on the standards of the African Blood Transfusion Society (SATS) has also been put in place, with rigorous selection criteria for blood donors at the CNTS of Lomé, in particular the systematic elimination of donors presenting any risk of infection

such as risky sexual behavior, notions of fever or jaundice during the last three months, tattoos, etc.

Data in other African countries have also reported similar prevalence. In Cameroon, it was 57% among blood donors at Laquintinie Hospital of Douala according to Sida et al. in 2014 [20] and 48.7% at the University Teaching Hospital Blood Service of Yaoundé in 2017 according to Fopa et al. [19]. Said et al. reported in 2011 [21] in Egyptian blood donors, Behzad-Bedbahani et al. at Shiraz in Iran [22] and van de Laar in the Netherlands [23] found lower prevalence, respectively 14.2% and 6.55% and 0.71%.

Prevalence of OBI

In blood donors carrying total anti-HBc Abs, we detected HBV DNA in 10 cases, corresponding to a prevalence of 3.05% (10/327). In Cameroon, Fopa et al. reported a prevalence of 1.1% in 2017 [19], and Kengne et al. reported it at 0.52% in 2019 [24]. In Egypt, Said et al. found a higher prevalence of 17.2% among blood donors in 2011 [21]. In Iran, the prevalence of total anti-HBc Abs was relatively low compared to that found in our study; on the other hand the HBV DNA was more isolated there with 12.2% of OBI. Among all blood donors who do not carry HBsAg in our study, we found a prevalence of OBI at 1.03% (10/967). Fopa et al. in 2017 found a prevalence of 0.56% in Cameroon [19]. Oluyinka et al. in Nigeria found a higher prevalence of 17% in blood donors who do not carry HBsAg [25]. According to Makvandi [26], OBI is identified in individuals who are seropositive for anti-HBc Abs at more than 80%, but there are also OBI in individuals who are seronegative for anti-HBc Abs in a lower frequency. Our study did not take into account OBI in blood donors who were seronegative for anti-HBc Abs. A future investigation will be carried out to estimate the prevalence of OBI in this group of blood donors in order to have an overview of OBI in all blood donors at the CNTS of Lomé.

CONCLUSION

The presence of occult hepatitis B (OBI) within the population of blood donors at the CNTS of Lomé is a real threat to infectious transfusion safety despite the mechanisms put in place for rigorous selection of donors and biological qualification of blood donations. The association of the detection of viral HBV DNA using PCR with the serological tests which are already carried out during the biological qualification of the donations could be a better approach to reduce the risk of transmission of the hepatitis B virus by blood transfusion at the CNTS of Lomé.

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RAFTS: Réseau d'Afrique francophone de transfusion sanguine (French-speaking African blood transfusion network).

CARESP: Centre Africain de Recherche en Epidémiologie et en Santé Publique (African Center for Research in Epidemiology and Public Health).

OMS/WHO: Organisation Mondiale de la Santé/World Health Organistion

Author Contributions

Liza Koboyo Nadjir – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

Gnatoulma Katawa – Conception of the work, Design of the work, Acquisition of data, Analysis of data, Interpretation of data, Drafting the work, Revising the work critically for important intellectual content, Final approval of the version to be published, Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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Data Availability
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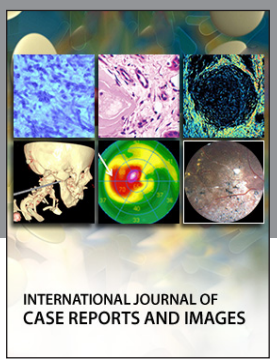

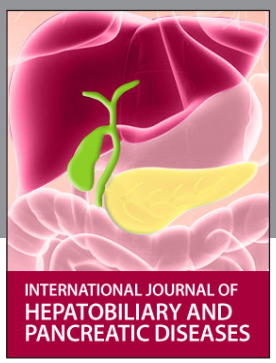

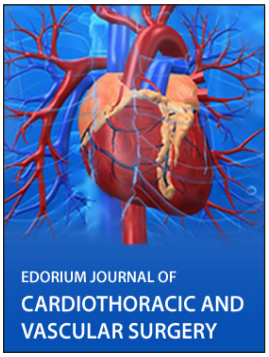

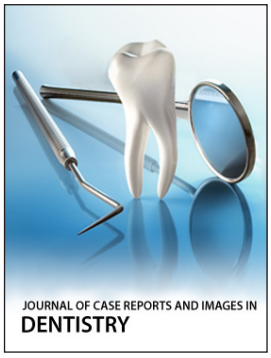

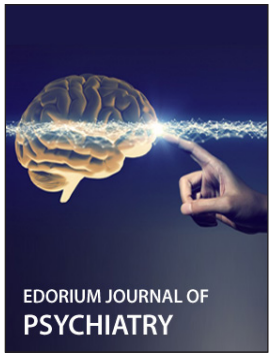

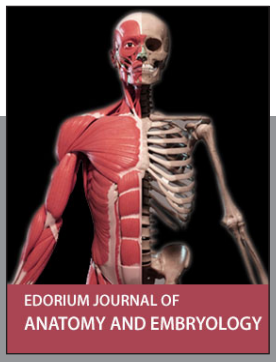

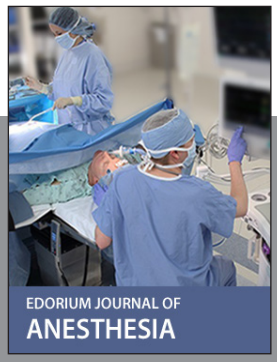

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