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Occult Hepatitis B Infection among Blood Donors in Pointe Noire, Republic of the Congo

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Abstract **Background:** Occult hepatitis B infection (OBI) is one of the most important transfusion safety issues and is considered a potential risk for hepatitis B virus (HBV) transmission. The aim of this study was to evaluate the prevalence and characteristics of OBI among blood donors in Pointe Noire. **Methods**: A cross-sectional study conducted among blood donors at Pointe Noire between October 2019 and May 2020. Blood samples from blood donors negative for hepatitis B surface antigen (HBsAg) were included. HBsAg and hepatitis B core antibodies (HBcAb) were done by ELISA. HBV DNA was detected by nucleic acid testing (NAT) and sequencing of preS1 region was done to determine HBV genotypes. **Results:** Out of 350 samples tested, 283 (80.9%) were males and 67 (19.1%) females. The mean age was 32.4 ± 12.3 years (range 18–60 years). The rate of anti-HBc was 33.43% (117/350) and the OBI prevalence was 2.3% (8/350). All samples were identified HBV genotype E and multiple mutations in preS1 region were observed in 75% of samples. Majority substitutions were: sA35E in 5 cases, sH44L and T52R in 4 cases, sR38G in 3 cases, S89T and Q92V in 2 cases, sN97R and I108L in 1 case. OBI rate and mutations were unrelated to gender, age, donor group and genotypes. **Conclusions:** This study highlights the significant prevalence of occult HBV in blood donors, indicating that HBsAg screening of blood donors is not sufficient and should be improved by anti-HBc and HBV DNA screening.

Keywords: *Hepatitis B virus, occult, genotype, blood donors, transfusion, Congo*

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1. Introduction

Hepatitis B virus (HBV) is a partially double-stranded circular DNA genome belonging to the Hepadnaviridae family [1]. HBV genome contains four open reading frames (ORF) for the polymerase (P), envelope (S/Pre-S), Core (C/pre-C), and the X proteins [2]. HBV variants have been classified into ten genotypes designated A to J, which show distinct geographical distribution pattern [3]. This virus causes a chronic infection of more than 350 million people worldwide, of which 65 million reside in Africa [3]. HBV constitute one of the threats to the blood supply safety and a major risk for patients transfused [4]. The prevention of HBV infection is based by screening of hepatitis B surface antigen (HBsAg) among blood donors [5]. Occult HBV infection (OBI) has been described as the presence of HBV-DNA without detectable HBsAg [6]. The OBI constitutes a main cause of the residual cases of HBV transmission by blood transfusion and may be associated with more severe liver damage [4,7]. Thus, for better prevention of HBV transmission by transfusion in developed countries, nucleic acid testing (NAT) has been introduced as a marker of HBV infection for qualification of blood units [7,8]. The Republic of Congo is classified among the countries with a high HBV prevalence. The positive rate of HBsAg varies between 9 to 11% among hospitalized patients, pregnant women and blood donors [9,10,11]. However, the NAT are not used for screening HBV infection in Republic of Congo and no study has performed among blood donors with occult HBV infection. Thus, this study was aimed to evaluate the prevalence and characteristics of OBI among blood donors in Pointe Noire, Republic of the Congo.

2. Materials and Methods

2.1. Study Design and Data Collection

This cross-sectional study was carried out among blood donors at the National Blood Transfusion Centre in Pointe Noire, Republic of Congo between October 2019 and May 2020. A total of 350 donors negative for hepatitis B surface antigen (HBsAg) were included in this study. Samples were anonymous and identified only by alphanumerical codes. All samples were hepatitis C and human immunodeficiency virus negative.

2.2. HBV Serological Tests

All serum samples were reanalyzed to reconfirm their HBsAg seronegative status using an enzyme enzyme-linked immunosorbent assay (ELISA) (Monolisa HBsAg PLUS) and negative samples were tested for the presence of anti-HBc (Monolisa anti-HBc PLUS) according to the manufacturer's instructions (Bio-Rad Laboratories, Marnes La Coquette, France). Serum samples were aliquoted and stored at -80°C until use.

2.3. HBV DNA Amplification

DNA was extracted from 200 ml of serum using the QIAamp viral DNA mini kit (Qiagen Inc., Valencia, California, USA) following the manufacturer's instructions. HBV preS1 region was amplified by nested PCR with HBPr1 (nt 2850–2868, 5'GGGTCACCATATTCTTGGG-3') and HBPr135 (nt 803-822, 5'-CAAGACAAAAGAAAATTGG-3') as primers for the first PCR and the HBPr2 (nt 2867–2888, 5'-GAACAAGAGCTACAGCATGGG-3') and HBPr3 (nt 1547–1569, 5'-CCACTGCATGGCCTGAGGATG-3') for the second PCR as previously described [12]. The first-round PCR was performed with Taq DNA polymerase (Promega, Madinson, USA) in a total volume of 25µl, with the following reactions: predenaturation at 95°C for 5 minutes, followed by 35 cycles of 30 seconds of denaturation (95°C), 30 seconds of annealing (50°C), and 30 seconds of extension (72°C), with a final extension at 72°C for 7 minutes. The cycling conditions of the second-round PCR were the same as the first-round PCR but using 2µl of the first-round PCR product as template. PCR cycling was performed on Perkin Elmer 2400 GeneAmpR® PCR thermal Cycler (Scientific Support, Inc, Hayward, CA). The PCR products amplified were subjected to electrophoresis on a 2% agarose gel stained with ethidium bromide-stained and visualized by using an ultraviolet transalluminator.

2.4. HBV Sequencing and Phylogenetic Analysis

The amplified PCR products were purified using the ExoSaP-IT clean up system (USB, USA) and the sequencing reaction was performed using HBPr2 primer with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were analyzed on an ABI 3130 XL DNA analyzer according to manufacturer's protocol. Phylogenetic trees were constructed using neighbor-joining method with Kimura's two-parameter and 1000 replicates of bootstrap resampling as implemented in MEGA software V 6.0 [13].

2.5. Statistical Analysis

The Epi-Info software version 7.0 and chi-square test were used for data analysis. A p-value of less than 0.05 was statistically significant.

3. Results

3.1. Baseline Characteristics of the Study Subjects

A total of 350 donors were included in this study, 283 (80.9%) were males and 67 (19.1%) females. The mean age was 32.4 ± 12.3 years (range 18–60 years). The rate of anti-HBc was 33.43% (117/350). The percentage of OBI was 2.3% (8/350) among HBV negative donors tested. This prevalence was higher in male donors than female (2.47% vs. 1.49%). Regarding age, donors carrying OBI was high in the age group of 31-45 years than in the 18-30 and 46-60. The population of replacement donors had higher prevalence of HBV DNA (3.37%). Furthermore, no significant difference in OBI was observed according gender, age group and categories of blood donors (Table 1).

Table 1. Characteristics of occult hepatitis B infection among the blood donors in Republic of Congo

Characteristics	Tested	HBV DNA positive		
		Prevalence (%)	OR (95% CI)	*p*-value
Gender				
Female	67(19.1)	1(1.49)	0.597(0.072-4.93)	0.632
Male	283(80.9)	7(2.47)	1	
Age group (years)				
18-30	188(53.7)	2(1.06)	0.22(0.042-1.16)	0.075
31-45	108(30.9)	5(4.63)	1	
46-60	54(15.4)	1(1.85)	0.39(0.04-3.41)	0.394
Blood donor type				
Family/replacement	178(50.9)	6(3.37)	1.73(0.34-8.71)	0.508
Voluntary	101(28.9)	2(1.98)	1	
Regular	71(20.3)	0	NA	

%: Percentage; CI: confidence interval

3.2. OBI Genotyping and Characterization

Among the 318 OBI samples, phylogenetic analysis of the preS1 region indicated that all sequences clustered with HBV genotypes E (Figure 1).

Multiple nucleotide mutations were identified, using sequence AB091255 from HBV as the reference. The alignment shows that seventy-five (75%) OBI cases showed multiple amino acid (aa) substitutions in the preS1 gene. The most common mutations detected were as follows: sA35E in 5 cases, sH44L and T52R in 4 cases, sR38G in 3 cases, S89T and Q92V in 2 cases, sN97R and I108L in 1

case. However, the average amino acid diversity was high in OBI strains independently of sex, age group, category of blood donors and genotypes (Table 2).

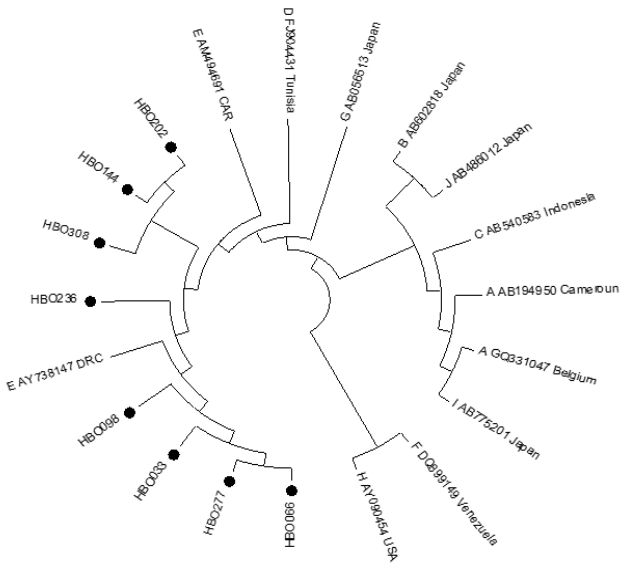

Figure 1. Phylogenetic analysis of HBV genotypes. This phylogenetic tree is derived from an alignment of a set of 08 HBV sequences. Sequences from OBI samples are identified with the initials "HBO", while the reference sequences are identified by NCBI accession number

Table 2. Characteristics and Location of Pre-S substitutions of HBV preS1 region among occult hepatitis B samples from blood donors

Donor ID	Gender	Age	PreS1-region Escape Mutations S Protein Amino Acid Substitutions
HBO236	M	36	N19T, P25L, D26H, R34I, W42G, T52R, W76T, Q81S
HBO098	M	28	K13E, A35E, H44L, D49L
HBO308	M	44	A35R, N36S, R38G, H44L, T52R, G71S, W76T, Q81S, N97R, I108L
HBO202	M	51	K13E, A35R, N36S, R38G, H44L, S78T, S89T, Q92V
HBO277	F	49	F24S, A35R, N36G, T37A
HBO066	M	31	F24S, Q28H, H44L, T52R
HBO033	M	29	D41K, P64S, R38G, D49L, T52R, S78T, S89T, Q92V
HBO236	M	54	K13E, A35E, R38K

4. Discussion

Occult HBV infection (OBI) could be transmitted by several routes, including blood transfusion and may progress to liver fibrosis and HCC [5]. The OBI prevalence varies according to HBV endemicity of different geographic areas [14]. In this study, we found 2.3% of OBI cases among blood donors in Pointe Noire. Additionally, high prevalence of OBI was reported in several studies from Mexico with 6.4% [15] , 7.9% in Sudan [16] and 17% in Nigeria [17] . In other countries where HBV is not endemic, the prevalence of OBI was low, 0.016% in Korea [18], 0.2% in Saudi Arabia [19], 1.26% in Egypt and 1.98% in Colombia [20,21]. The OBI are generally chronic, asymptomatic with low level of viral replication and may contribute to the development of HBV-associated diseases such as hepatic inflammation, cirrhosis, and HCC [22]. Our study, found a high frequency of OBI cases among subjects aged between 31-45 years (4.63%). This prevalence among this age group could be attributed to greater exposure to occupational risk factors for HBV, mainly to those who engage in risky sexual behavior [3,17]. In the current study, the majority of OBI sequences were identified as genotype E. However, previous studies have shown that the majority of HBV infections in our country are genotype E and A [19,21]. In the study performed among blood donors in Europa, high rate of major hydrophilic loop (MHL) region mutation was reported in OBI genotypes D and A2 strains [23]. Another report from Saudi arabia and Colombia concluded that HBV genotype D and F are the most prevalent OBI among blood donors [19,21]. In the present study, 75% of preS1 gene mutations were observed in B-cell and T-cell and hepatocytes binding epitope. On the other hand, studies performed in study, has found 86% among OBI strains of blood donors [24]. The mutations over these regions affect the expression and secretion of surface proteins (HBsAg) [25]. Furthermore, the study carried out among blood donors in Nigeria, also suggest that mutation in the MHR of the S gene may explain the occult nature of the HBV infection [17]. Several studies report that, the pre-S1 mutation cause the accumulation of large HBV surface antigen in endoplasmic reticulum (ER), who induce strong ER stress and DNA damage, which are essential factors for carcinogenesis [26].

5. Conclusion

This current study shows that occult HBV infection remains a major concern and indicated that HBsAg screening among blood donors is not sufficient and should be improved with anti-HBc and HBV DNA testing. The clustering of mutations in the preS1 region observed may have played a crucial role in the diagnostic failure of HBsAg and could possibly explain the occurrence of OBI. Thus, the introduction of anti-HBc and HBV DNA as markers could result in discarding a significant amount of infected blood units and would prevent the occurrence of post-transfusion hepatitis B.

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Author Contributions

Conceptualization, Methodology, Investigation, Analysis, and Writing of the manuscript- Brunel Monic Angounda, Serge Oscar Mokono, Fabien Roch Niama, Gabriel Ahombo. Methodology, Data curation, Drafting, Interpretation, and Supervision and edition of the manuscript- Anicet Luc Magloire Boumba, Viny Andzi Elenga, Edwige Paola Chancelle Nanitelamio, Renée Sorine Akouala, Geneviève Boukatou, Arsène Bikoue. All authors revised the manuscript and have approved the final version of the manuscript.

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Conflicts of Interest

The authors declare that there are no conflicts of interest associated with this manuscript.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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