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Hepatitis B Virus Prevalence and Genotype Distribution in Human Immunodeficiency Virus Infected Patients from Brazzaville, Republic of the Congo

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Hepatitis B virus infection is a major global health problem, particularly in HIV-infected patients and increases the risk of liver damage. The purpose of this study was to assess the HBV prevalence and genotypes among HIV-infected patients in Brazzaville, Republic of the Congo. This cross-sectional study was conducted among HIV-positive patients attending an outpatient treatment center for HIV/AIDS in Brazzaville, from June 2018 to February 2020. Blood samples were screened for HBsAg by ELISA and HBV genotypes were determined by phylogenetic inference. Data analysis was done using SPSS V.21.0 and P value less than 0.05 was considered as statistically significant. A total of 275 patients HIV-infected were included. The prevalence of HIV-HBV coinfection was 6.9% (95%CI:4.21-10.58). The majority of the co-infected with HBV were mainly male patients (7.7%). HIV-positive individuals aged 28-38 (8.4%) were mostly affected. None of the risk's factors tested was significantly associated with HBV in HIV-infected patients. Phylogenetic analysis revealed that 9/15 (60%) belonged to genotype E while 6/15 (40%) belonged to genotype A including subgenotypes A3 and A5. In conclusion, these data underline the importance of HBV genotype identification as an integrated measure of HIV routine care to improve the treatment of HIV/HBV co-infected patients.

Keywords: HBV; HIV; coinfection; genotypes; Brazzaville; Congo.

1. INTRODUCTION

"Hepatitis B virus (HBV) is a member of the *hepadnaviridae* family and it causes acute and chronic liver disease" (Liang, 2009). "HBV is endemic in many parts of the world and more than 360 million people are chronic carriers" (Lozano et al.,2010). "HBV was estimated to have resulted in 786,000 deaths, the vast majority being attributable to liver cancer and cirrhosis" (Lozano et al., 2010). "Human immunodeficiency virus (HIV) is an enveloped RNA virus that replicates by reverse transcription. Of the estimated 29.2 million individuals living with HIV globally" (Murray et al.,2014) between 5% and 10% are thought to be living with HBV coinfection (Kourtis et al.,2014). HBV and HIV share the same transmission routes, but differ in their geographic distribution and transmission efficiency (Liang, 2009). "It is estimated that 10% of HIV-infected patients worldwide are coinfected with HBV (Sun et al., 2014). HBV and HIV have a mutually detrimental impact in that HIV infection accelerates HBV-related liver damage and the presence of HBV infection complicates the management of HIV infection"(Rockstroh, 2006). "In the Republic of Congo, the prevalence of HBV infection varies from 6.5% to 10% and HIV prevalence is estimated at 3.2%" (Elira-Dokekias, 2002, Bossali et al.,2012, Atipo-Ibara et al.,2015, Angounda et al., 2016, ESISC-I, 2009). "HBV has a wide genetic heterogeneity which has classified into 10 genotypes with various subgenotypes which are labelled alphabetically from A to J" (Kramvis and Kew, 2007). "The diversity of HBV genotypes may influence disease progression and antiviral response" (Koziel and Peters, 2007). "In Republic of the Congo, HBV genotype E is the most frequently detected followed by genotypes A and D" (Atipo-Ibara, 2015, Angounda et al., 2016). Despite the high burden of HBV infection in our country, there is a paucity of data on the genotype distribution in HIV infected patients. However, the HBV genotypes distribution in patients co-infected with HIV-1 in Brazzaville remains unknown. The aim of this study was to assess the prevalence and genetic diversity of HBV infection among HIV-infected patients in Brazzaville, Republic of the Congo.

2. MATERIALS AND METHODS

2.1 Study Design, Participants and Sample Collection

This cross-sectional study was conducted among HIV infected patients attending an outpatient treatment center for HIV/AIDS in Brazzaville, Republic of the Congo from June 2018 to February 2020. Their informed consent was obtained regarding the main objective of this study based on the monitoring of HIV and hepatitis B infected patients. Sociodemographic and epidemiological data were collected using a standardized form. Five milliliters of whole blood were collected in EDTA tubes and sera were frozen in small aliquots at -20℃ for subsequent

analyses. All samples were tested for HBsAg using the Monolisa HBsAg ULTRA kits (Bio-Rad, Marnes-La-Coquette-France) following the manufacturer's instructions.

2.1.1 HBV-DNA detection and sequencing

The QIAamp DNA Blood Mini kit was used to extract DNA from plasma according to manufacturer's instructions with a final elution volume of 30 µL (Qiagen, Hilden, Germany). HBV preS1 region was amplified by nested PCR with HBPr1 (nt 2850–2868, 5'GGGTCACCAT ATTCTTGGG-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'-CCACTGCATGGCCTGAGGA TG-3') for the second PCR as previously (Stuyver et al., 2000). 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. Type-specific PCR products were directly sequenced using the "Big Dye Terminator v3.1 Cycle Sequencing kit" (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

2.1.2 Genotype Determination by Phylogenetic Analysis

Hepatitis B genotypes/subgenotypes were determined by comparing with corresponding sequences of HBV belonging to genotype A–I obtained from GenBank database. Genetic distances were calculated using the Kimura two parameter algorithm and phylogenetic trees were constructed using MEGA version 6 software. HBV sequences were edited, aligned and amino acid substitutions in preS1 region were determined by comparing them against reference HBV genotypes amino acid sequences, using Bioedit version 7.0.4.1.

2.2 Statistical Analysis

Data analysis was done using scientific programme for social sciences (SPSS) version 21.0 and prevalence of HBV/HIV co-infections are expressed in percentages. The association between the presence of HBV/HIV and sociodemographics characteristics were determined using Pearson's chi-square test with the significant p value less than 0.05.

3. RESULTS

A total of 275 HIV-infected patients were included in this study. The prevalence of HBV-HIV coinfection was 6.9% (95%CI:4.21-10.58). The prevalence of HBV-HIV co-infection among male and female patients showed 7.7% and 6.5%, respectively. However, this was not significant (p=0.719) for both genders. The highest HBV-HIV co-infected age group was patients in the ages of 28-38 years (8.4%). This was, however, not statistically significant (p=0.350). Patients with primary (10.5%) and secondary (7.2%) education level had higher rates of HBV-HIV than those with university/graduate education (6.4%). Marital status revealed a higher HBV prevalence among HIV patients who were single (8.5%) compared to those who were married (3.5%). Level of education and marital status were also no significantly associated with the risk of HBV-HIV co-infection (Table 1). The highest rate of HBV-HIV coinfection was observed among respondents that are civil servant (7.8%), followed by unemployed (7.3%), laborer (7.0%) and student (4.2%). The result shows that there was no significant association between HBV-HIV co-infection and occupation of the respondents (P>0.05) (Table 1). Regarding their risks factors status, HBV was detected in HIV-infected participants who reported having histories of blood transfusion (7.8%), surgery (7.1%), multiple partner sexual (7.8%), scarification (7.4%) and ear piercing (3.3%). However, there was no significant association between HBV-HIV co-infection in relation to the risk's factors (P>0.05) (Table 2). HBV-DNA was detected and successfully sequenced in 15 of the 19 HBsAg positive samples. Phylogenetic analysis revealed two prevalent genotypes namely A and E. HBV genotype E was identified in 60% (n = 9) and HBV genotype A in 40% (n = 6) of the participants. Of the 6 HBV genotype A, 4 were categorized as subgenotype A3 and 2 as subgenotype A5 (Fig. 1).

Table 1. Demographics characteristics of HBV coinfection among HIV infected patients at Brazzaville

Characteristics	Tested (%)	HBV-HIV (%)	OR (95% CI)	*P-Value*
Gender				
Male	91(33.1)	7(7.7)	1.19(0.45-3.14)	0.719
Female	184(66.9)	12(6.5)	1	
Age group (years)				
18-27	81(29.5)	6(7.4)	1.65(0.39-6.9)	0.489
28-38	107(38.9)	9(8.4)	1.89(0.49-7.28)	0.350
39-48	65(23.6)	3(4.6)	0.98(0.097-9.98)	0.989
49-58	22(8.0)	1(4.5)	1	
Education level				
Primary	19(6.9)	2(10.5)	1.71(0.354-8.31)	0.502
Secondary	69(25.1)	5(7.2)	1.14(0.38-3.36)	0.813
University/Graduate	187(68)	12(6.4)	1	
Marital Status				
Single	189(68.7)	16(8.5)	2.56(0.72-9.03)	0.144
Married/cohabiting	86(31.3)	3(3.5)	1	
Occupation				
Civil Servant	90(32.7)	7(7.8)	1	
Trader	35(12.7)	2(5.7)	0.71(0.14-3.6)	0.689
laborer	71(25.8)	5(7.0)	0.89(0.27-2.95)	0.860
Student	24(8.7)	1(4.2)	0.51(0.06-4.41)	0.545
Unemployed	55(20.0)	4(7.3)	0.93(0.26-3.33)	0.911

%: percentage; OR: Odds ratio; CI: confidence Interval; 1: reference

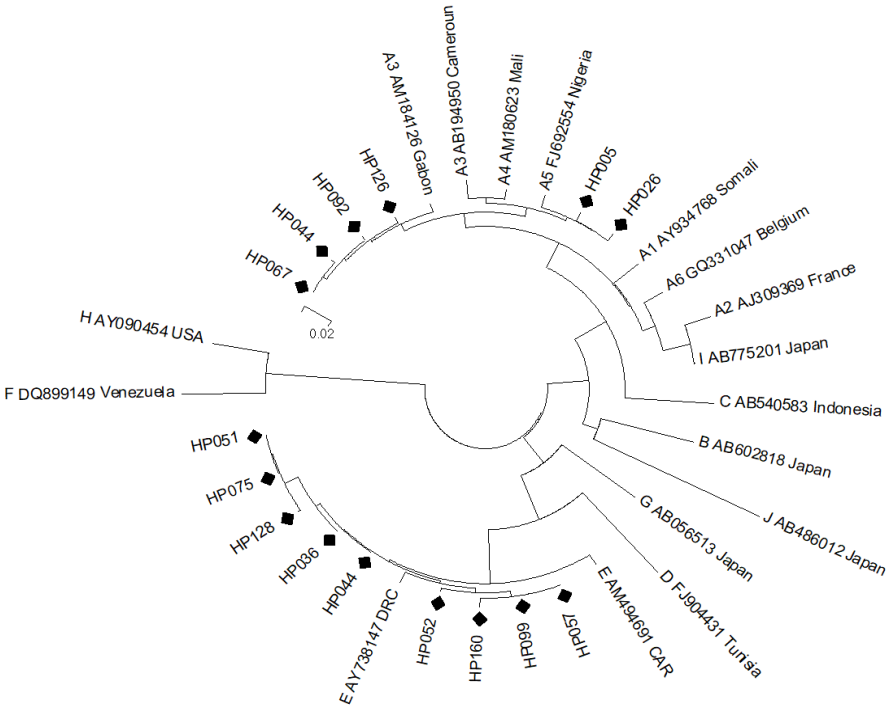

Fig. 1. Phylogenetic tree of partial pre-S1 sequences from 15 participants and GenBank references. Bootstrap support values obtained from 1000 replicates are indicated. Accession number and genotype/subgenotype of sequences obtained from GenBank are indicated [CAR, Central African Republic; DRC, Democratic Republic of Congo]. Sequences from this study are in bold font and their name starts with HP

Table 2. Distribution of risk factors for HBV infection among HIV infected patients at Brazzaville

Characteristics	Tested (%)	HBV-HIV (%)	OR (95% CI)	*P-Value*
Multiple partners sexual				
Yes	102(37.1)	8(7.8)	1.25(0.49-3.22)	0.639
No	173(62.9)	11(6.4)	1	
Surgery				
Yes	85(30.9)	6(7.1)	1.03(0.38-2.82)	0.947
No	190(69.1)	13(6.8)	1	
Ear piercing				
Yes	61(22.2)	2(3.3)	0.39(0.09-1.75)	0.220
No	214(77.8)	17(7.9)	1	
Blood transfusion				
Yes	51(18.5)	4(7.8)	1.18(0.37-3.73)	0.771
No	224(81.5)	15(6.7)	1	
Scarification				
Yes	121(44.0)	9(7.4)	1.15(0.45-2.94)	0.759
No	154(56.0)	10(6.5)	1	

4. DISCUSSION

Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) are major public health problems, particularly in developing countries. In our study, the overall prevalence of HBV-HIV coinfection was 6.9%. These results are higher compared to those previously obtained in Kenya with 4.93%, 2.5% in Brazil and 4.8% in Nigeria (Komu et al., 2017, Freitas et al., 2014, Ayodele et al., 2014). However, our findings were also in contrast with those previously conducted in Cameroun (25.5%), Gabon (6.2%), Mozambique (9.1%) and China (13.2%) (Magoro et al., 2016, Bivigou-Mboumba et al., 2018, Chambal et al., 2017, Huang et al., 2017). These differences of HBV infection in HIV infected patients can be explained by several factors, such as the category of participants, geographical location and the sensitivity of viral assay reagents.

The current study sought to establish an association between socio-demographic data, risk factors and HBV in HIV infected patients. The rate of HBV infection was higher in male (7.7%) than in female (6.5%). However, this difference was not significant. This result is in agreement with Kerubo et *al.*, in a study involving participants from two informal settlements in Nairobi, Kenya did not find any association between HIV/HBV co-infection with gender (Kerubo et al.,2015). “In contrast to the results of this study, male gender was significantly associated with HBV infection in HIV-positive participants in other studies conducted in Venezuela, Brazil and Nigeria” (Freitas et al., 2014, Jaspe et al., 2014, Balogun et al., 2012). Indeed, this association may be attributed to risk behaviours associated with HBV transmission, such as multiple sexual partners and more frequent intravenous drug use than women.

In our study, the 28-38 age group is the most affected by HBV-HIV co-infection. This has been reported in several studies and is probably due to the influence of age on the frequency of sexual intercourse, so that the probability of infection increases at the start of sexual activity, then decreases after the age of 50 (Freitas et al.,2014, Ott et al., 2012, Bado et al., 2013). “In Africa, HIV infections are mainly due to sexual transmission, while HBV infections mainly occur in early childhood due to horizontal transmissions” (Hwang and Cheung, 2011).

In this study, “there was no significant association with marital status, education, surgery, transfusion history or multiple partners sexual. On the other hand, the study carried out in Ethiopia, reported that people who injected drugs were five times more likely to be infected with HBV” (Zenebe et al., 2014). Other studies have documented those factors such as blood transfusion, tattooing/scarification, homosexuality to be associated with HBV infections among HIV positive patients (Mirambo et al., 2019, Fibriani et al., 2014).

“Phylogenetic analysis of the HBV genotypes in the current study revealed the presence of genotype E (60%) and A (40%). This observation is in agreement with other studies done in Cameroun (Bivigou-Mboumba et al.,2018),

Gabon (Magoro et al. 2016), Kenya (Komu et al. 2016), Mozambique (Chambal et al., 2017) Soudan (Yousif et al., 2014) that reported the predominance of circulating HBV genotypes E and A.

HBV genotype E is predominant in the sub-Saharan Africa, and has been discovered in Central African Republic, Senegal, Namibia, and in East Africa. It has widespread geographical distribution but a very low genetic diversity (Hübschen et al., 2014). All genotype E strains isolated in Europe and America have been derived from HBV carriers of African origin, regardless of their country of residence (Kramvis et al., 2007). “The HBV genotype A sequences belonged to A3 and A5 sub-genotypes. HBV genotypes A and the genotype E sequences clustered with sequences from countries in sub-Saharan Africa including Gabon, Cameroun and Central Africa Republic. This could be attributed to trade relationships between these African countries” (Makuwa et al., 2006, Kurbanov et al., 2004) Genotype determination of HBV in HIV infected patients is important in estimating disease progression and planning optimal antiviral treatment. Pathogenic differences between HBV genotypes explain the intensity of disease. Previous studies have shown that genotype A tends to become chronic, while viral mutations are frequently in genotype C. Acute infection with genotypes A and D leads to higher rates of chronicity than genotypes B and C (Lin and Kao, 2016, Kao and Chen, 2006). Genotype E has been reported to be associated with progression to chronic HBV carriers (Cuenca-Gómez, 2018).

5. CONCLUSION

This study showed that HBV coinfection was prevalent in HIV infected patients from Brazzaville. HBV genotype E being the most common, followed by genotype A with subgenotypes A3 and A5. Overall, these data underline the need for routine HBV screening in HIV-infected patients and highlight the importance of HBV genetic diversity in treatment strategies. Further molecular characterization of full-length HBV nucleotide sequences will enable assessment of their genetic variability and mutation patterns.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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