

HBV Seroprevalence and Genetic Diversity among HIV Patients in the Department of Lekoumou in Congo Brazzaville

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ABSTRACT

Introduction: HBV-HIV co-infection is a major public health problem. The objective of this study was to contribute to the improvement of management of HBV-HIV co-infected patients in Congo.

Methods: This was a cross-sectional descriptive study conducted from January 1st to September 30th, 2016. We studied outpatients seen in hospitals and Integrated Health Center of Department of Lékoumou. Screening for HBsAg was done by rapid test (Heath Mate HBsAg plus test) and that of HIV 1 and 2 was done by immunoassay (ImmunocoCOMB II HIV). The detection of HBV DNA was made by the QIAGEN QIA Kit and the identification of genotypes by PCR product sequencing at the National Public Health Laboratory of Brazzaville.

Results: During nine months of study period, 204 patients were enrolled to participate in this study. Male patients were 37.3% and female were 62.7%, with a sex ratio of 0.5. The mean age was 40.9 ± 13.4 . The frequency of HBV-HIV co-infection was 2.4% ($n = 5$). Single infections accounted for 6.3% ($n = 13$) for HBV and 12.3% ($n = 23$) for HIV. Carriers of HBV DNA accounted for 7.8% ($n = 16/204$). Condom non-use was significantly associated with co-infection and mono-infection ($P < 0.05$). HBV genotype E was more common in the study participants than genotype A.

Conclusion: HIV-HBV co-infection exists. Despite the small sample size, the prevalence of infection with hepatitis B virus remains high. Two HBV genotypes, A and E, have been identified in co-infected and mono-infected patients.

Keywords

Hepatitis B virus, HBV-HIV co-infection, HBV genotypes.

Introduction

Infection with hepatitis B virus (HBV) is a public health problem of concern worldwide. The World Health Organization (WHO) estimates that 2 billion people are infected with HBV, about 350 to 400 million are chronically carriers, and nearly one million die each year [1].

HBV is a deoxyribonucleic acid (DNA) virus belonging to the family of hepadnaviridae, responsible for chronic infection of the liver [2]. The prevalence of viral hepatitis B mono-infection in Congo varies between 5 and 10%, placing Congo in an area of high endemicity [3,4].

The Human Immunodeficiency Virus (HIV) is a ribonucleic acid (RNA) virus of the family of retroviridae. It is responsible for an acquired immunodeficiency facilitating the occurrence of

infections. Its seroprevalence in Congo was 3.2% between 2009 and 2013 [5]. Molu et al. in 2018 reported an 8.9% prevalence of HBV infection in HIV-infected people in Cameroon [6].

In Congo, the exact prevalence of hepatitis B in the HIV population has not been accurately established. In 2013, Atipo Ibara et al. reported a 20.1% prevalence of HBV-HIV co-infection in the Gastroenterology and Internal Medicine department of the Brazzaville University Hospital Center [7]. To our knowledge there is no conducted study on the molecular epidemiology of HBV-HIV coinfection in Congo. The aim of this study was to contribute to the improvement of management of patients co-infected with HBV-HIV in Congo.

Methods

This was a cross-sectional descriptive study, conducted over a period of nine months from January 1st to September 30th, 2016. Epidemiological data were collected in the basic hospitals and integrated health center of the department of Lékoumou. Serological analysis was done at the Loandjili General Hospital in the department of Pointe Noire and molecular analysis at the National Public Health Laboratory in the Brazzaville department.

The study population was composed by patients seen in outpatient clinics in hospitals and integrated health center of the department of Lékoumou. We selected participants with unknown HBV-HIV serologic status, older than 18 years old, who agreed to voluntarily participate in the study after reading and freely signing the consent form. We excluded those who refused to consent for the study and admitted inpatients. By simple random method, 204 participants were selected and consent to participate in this study.

We collected the epidemiological data on a survey form developed solely for the study. The blood sample was taken on a 5 ml EDTA tube with separator gel. The tube was immediately centrifuged for 5 minutes at 3000 rpm. This allowed to obtain two compartments separated by the gel: plasma and the red cell. About 1000 microlitres (μL) of plasma were collected and placed in the cryovials for serological and molecular study.

Samples from the department of Lékoumou were transported to Pointe-Noire for serological analysis by vehicle in a cooler containing cold accumulators. The transport lasted 4 hours. Arrived at Pointe Noire, the samples were transported to Brazzaville by an air plane in dry ice (-20°) placed in the hold. The transportation lasted 45 minutes. The samples were kept at the national laboratory at -80°C until the day of the molecular analysis.

The epidemiological variables were: gender, age, risk factors for transmission (tattoos, blood transfusion, piercing, scarification, intravenous drug use, vertical transmission, surgical history, multiple sexual partners). The biological variables were: HBsAg, HIV-1 and HIV-2 Ab, DNA and HBV genotype strains.

Screening for HBsAg was done by rapid screening test (Heath Mate HBsAg plus test). This was a test based on immunochromatography to determine hepatitis B surface antigen (HBsAg) in serum. The

detection of anti-HIV 1 and 2 antibodies was made by immunoassay (ImmunocoCOMB II HIV).

Molecular analysis consisted of the detection of HBV DNA and the identification of genotypes. The viral extraction was carried out from 200 μl of plasma using the QIAGEN QIA amp DNA Blood Kit. The elution volume was 60 μl . We then proceeded to an assessment of the quality of the DNA by performing β -globin PCR. The preparation of the Mix was carried out based on: 5 μl of PCR buffer and water. In 20 μl of Mix, we added 5 μl of DNA to be amplified. The final volume of the reaction mixture was 25 μl . The amplification of this gene was done using the pair of PCO4 / GH20 primer that makes it possible to amplify a 268 bp fragment of the β -globin gene.

Detection of HBV viral DNA was performed from nested PCR. It focused on the HBPol region of the PreS1 / PreS2 / HBsAg domains using the HBPr1 / HBPr135 and HBPr2 / HBPr3 primer pairs. The principle of a nested PCR is a double amplification by two successive PCRs whose 2nd PCR amplifies an internal sequence at the first PCR and the product of this serves as a template for the 2nd PCR.

Genotyping was performed after sequencing of the Nested PCR product. The sequencing reaction was carried out according to the technology of Big Dye Terminator (BDT) ABI PRISM Big Dye Terminator Kit in a HITACHI type sequencer. The reaction was carried out in the TC3000 thermocyclers, thus in 18 μl of Mix, we added 2 μl of purified DNA. The final volume of the reaction mixture was 20 μl . Once the electropherogram was generated, bioinformatic processing of the data was performed to identify the genotypes present in each treated sample. To do this, the sequences obtained were aligned corrected and compared to the set of genomic sequences of the HUBs available in the Genbank folder bank from the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST) algorithm (NCBI) www.ncbi.nlm.nih.gov/BLAST.

Data entry and analysis were done using microsoft Excel version 2007 and Epi infoTM7 software respectively. The calculation of the p values was performed using Pearson's chi-square test (χ^2) or Fisher's exact test. The probability less than 0.05 ($p < 0.05$) was considered to be the threshold of statistical significance between two variables.

Results

During the nine months of study period, 204 patients were selected and participated in this study. Male gender accounted for 37.3% whereas female gender accounted for 62.7%, with a sex ratio of 0.5. The average age was 40.9 ± 13.4 years ranging from 18 to 76 years old.

In our study, 85.3% ($n = 174$) of study participants had risky sexual behavior (no condom use), 45.1% ($n = 92$) had scarification, 14.7% ($n = 30$) had a history of surgery, 13.7% ($n = 28$) had a multiple sexual partners and 7.4% ($n = 15$) had tattoo. No cases of

Intravenous drug use had been identified.

For all 204 subjects, the prevalence of HBV-HIV coinfection was 2.4% (n = 5). Single infections accounted for 6.3% (n = 13) for HBV and 12.3% (n = 23) for HIV. Our study did not show any statistically significant difference between co-infection with age (p = 0.98) and sex (p = 0.5), respectively.

In this study, 7.8% (n = 16/204) of study participants had HBV DNA. The table below is showing the molecular prevalence of HBV according to the serological groups.

ADN	Co-infection		mono-infection				no-infection	
	VHB+/VIH+ N %\		VHB+/VIH- N %		VHB-/VIH+ N %		VHB-/VIH- N %	
Positive	4	80	11	84,6	1	4,3	0	0
Négative	1	20	2	15,4	22	95,7	163	100
Total	5	100	13	100	23	100	163	100

Table 1: Molecular Frequency vs. Serological Subgroups.

Non-condom use was significantly related to co-infection and mono-infection (Table 2).

Risk factors		Co-infection		Mono-infection				No-infection		P
		VHB+/VIH+		VHB+/VIH-		VHB-/VIH+		VHB-/VIH-		
		n	%	n	%	n	%	n	%	
Blood transfusion	Yes	0	0	1	7,7	1	4,3	16	9,8	0,92
	No	5	100	12	92,3	22	95,7	142	87,2	
surgical	Yes	1	20	1	7,7	5	21,7	23	14,1	0,76
	No	4	80	12	92,3	18	78,3	140	85,9	
No codom use	Yes	5	100	12	92,3	23	100	135	82,8	0,76
	No	0	0	1	7,7	0	0	28	17,2	
Multiple sexual partners	Yes	2	40	2	15,4	4	17,4	20	12,3	0,26
	No	3	60	11	84,6	19	82,6	143	87,7	
scarification	Yes	4	80	5	38,5	14	60,9	69	42,3	0,23
	No	1	20	8	61,5	9	39,1	94	57,7	
tattoos	Yes	0	0	0	0	1	20	14	8,6	0,89
	no	5	100	13	100	22	80	149	91,4	

Table 2: Molecular Prevalence Based on Risk Factors.

Sixteen patients with HBV had detectable viral DNA. We found that 40% of co-infection group (n=5) had genotype A and 60% had the genotype E. Among 11 mono-infected HBV, the genotype A accounted for 36.4% and genotype E accounted for 63.4%.

Discussion

In our study, the mean age was 40.9 ± 13.4 years old. This average age could be explained by the young population of our country. Our population is mostly represented by women. The same observations were made by Sawadogo et al. in Burkina Faso, who found a female predominance in their HIV study populations [8].

It should be noted that Congolese demography has been more represented by women in recent decades. In England, Frater et al. reported a male predominance [9]. This difference can be explained by the high frequency of intravenous drug use and homosexuality, which are confined to male subjects in Europe. These two practices seem to be rare in Congo.

The prevalence of HBV-HIV co-infection was 2.4% in this study. In 2014 in Brazil, Freitas et al. reported a similar prevalence. The co-infection was 2.5% in their study population [10]. Our prevalence is low compared to Zoufaly et al. and Molu et al. who reported the prevalences of 12.6% in 2012% and 8.99% in 2018 respectively [6,11]. One of the most likely causes of the high prevalence of HBV-HIV co-infection is that both viruses have similar transmission pathways [12].

Atipo Ibara et al. in 2012, in a study of the epidemiological aspects of HIV / HBV and / or HCV coinfection in HIV subjects, found a prevalence of 20.1% [7]. This could be explained by the fact that they studied patients hospitalized for retroviral disease. On the other hand, the low prevalence in our study could be explained not only by the small sample size but also the fact that in Congo there is high prevalence of HIV in Brazzaville and Pointe-Noire.

The mono-infection represented respectively 6.3% for HVB and 11.2% for HIV. The viral DNA of hepatitis B was found in 7.8% of subjects. According to the serological groups, the molecular prevalence of hepatitis B in the HBV + / HIV + serologic group was 80% (4/5). Our results are superior to those of Larsen et al. [13] who in 2005 reported a frequency of 4, 7%.

The presence of viral DNA was identified in 11 subjects in mono-infected HBV patients. On the other hand, in the serotype group of mono-infected patients with HIV and negative for HBsAg, hepatitis B DNA was detected in one patient representing 4.3% of HIV mono-infected participants. This result proves the existence of certain so-called occult viral B hepatitis with negative HBsAg but detectible DNA. It has also been shown that HBsAg levels are not always associated with serum HBV DNA levels since HBsAg synthesis may occur independently of HBV replication [14]. It is necessary in these conditions to carry out a molecular search for viral DNA, especially in blood donors.

Our study identified genotype A and genotype E in the co-infected group and in the mono-infected group. Genotype E was predominant in both groups. This could be explained by the fact that genotyping was done to a very limited number of subjects, which could not give us an expanded profile of genotypes in our study population; increasing the size of the sample could better help to appreciate this profile. However, the profile found in our study is the same as that found in previous studies. Indeed, Atipo Ibara et al. among blood donors in 2015 reported a predominance of genotype E [15]. Literature reports that the two genotypes are the most predominant in Central Africa. This observation is in agreement with previous studies that have shown the endemicity of this genotype A and E which extends from Senegal to Namibia

in the south and to The Republic of Central Africa in the east [16, 17].

Conclusion

HBV-HIV coinfection exists. The absence of surface antigen is not synonymous with the absence of HBV DNA. Molecular biology remains the key element of diagnosis and management. Despite our small sample size, the prevalence of infection with hepatitis B virus remains high. In our study, the non-use of condoms was the only risk factor for co-infection. Two genotypes A and E were identified among co-infected and mono-infected patients with a predominance of genotype E in both groups.

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