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Genotypes of The Hepatitis B Virus in Congo-Brazzaville

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ABSTRACT

Introduction: *The hepatitis B virus (HBV) is characterized by significant genotypic heterogeneity. Circulating genotypes in Congo are not all known. The purpose of this work is to establish a national genotypic mapping of HBV.*

Patients and Methods: *Descriptive, cross-sectional study carried out in the blood transfusion centers and integrated health centers of the Congo departments, from 1 January 2010 to 31 December 2016. Asymptomatic, blood donor or consulting patients formed the study population. The search for HBsAg was carried out by rapid tests, supplemented by a discriminatory screening test by the ELISA technique ; then detection of the HBV viral DNA by nested PCR on the HBPol region of the PreS1 / PreS2 / HBsAg domains of the S gene, using the HBPr1 / HBPr135 and HBPr2 / HBPr3 primer pairs and determination of the genotypes by sequencing from PCR product.*

Results: *A total of 3017 patients with mean age of 39 +/- 16 years were included, including 1576 men and 1440 women. Of these, 379 (12.5%) were positive for HBsAg. Of these 379 samples submitted to the molecular study, 321 (84%) were positive for HBV viral DNA. The genotypes found were genotype E (n = 207, 54.6%), A (n = 88, 23.2%), D (n = 4, 1%), B (n = 1, 0.3). %), B / C (n = 1, 0.3%) and C (n = 1, 0.3%). In 19 cases (5%) the genotype could not be determined. The identified subgenotypes were A3 and A6 for genotype A and D7 for genotype D. The distribution of genotypes was almost homogeneous across all departments. Genotype D was only identified in one department, as were genotypes B and C in only one other department.*

Conclusion: *Genotypes E and A are the most common in our country. The presence of genotypes D, B and C is probably related to population migrations. Subgenotypes A3, A6 and D7 were the only ones identified in our work.*

Keywords

Hepatitis B virus, Genotype, Congo.

Introduction

The hepatitis B virus (HBV) chronically affects about 400 million people worldwide [1]. It is characterized by a large genotypic heterogeneity. The genotypes identified in Africa in general are known [2]. Congo, a country in sub-Saharan Africa, is an area of high endemicity [2-5]. Although the most common genotypes in Africa are known, in Congo, circulating genotypes are still poorly understood. It is in this context that we undertook this work in order to establish a national genotypic mapping of HBV.

Patients and Methods

This is the synthesis of cross-sectional descriptive surveys that we conducted in the various departments of our country, from January 1, 2010 to December 31, 2016. It took place in the different departments of Congo: Lékoumou, Likouala, Niari, Sangha, Bouenza, Cuvette, Plateaux, Brazzaville and Pointe-Noire. Patients were counted in integrated health centers and blood centers. The choice of survey structures was random. Patients aged 18 years and older, asymptomatic, who came for a consultation

or for a blood donation and who consented to participate in the study were included. Patients with unusable sera were excluded from the study. The variables studied were socio-demographic and biological. Paraclinical examinations were carried out either at the National Public Health Laboratory of Brazzaville or in foreign laboratories, particularly in Cameroon and Morocco, when local conditions did not allow their implementation. In all patients 5 ml of blood were collected in an EDTA tube.

The plasma obtained after centrifugation was distributed in two cryotubes, stored at -20°C., one of which was used to search for HBsAg and the other for molecular analysis. The search for HBsAg was made by a rapid test (Health Mate HBsAg plus test) and then a discriminatory screening test by the ELISA technique. The search for HBsAg was made by a rapid test (Health Mate HBsAg plus test) and then a discriminatory screening test by the ELISA technique. In HBsAg-positive sera, a discriminatory screening test was performed for the detection of HBsAg using the fourth-generation ELISA using the Human HBs AG reagent. DNA extraction was performed using the «QIAamp DNA Blood Kit mini kit handbook (QIAGEN)». The detection of HBV viral DNA by nested PCR focused on the HBPol region of the PreS1 / PreS2 / HBsAg domains of the S gene using HBPr1 / HBPr135 and HBPr2 / HBPr3 primer pairs.

Results

In total, 3017 patients were included, 1576 men for 1440 women, a sex ratio of 0.9. The average age was 39 ± 16 years, with extremes of 18 and 87 years. Mean age was 39 ± 16 years, with extremes of 18 and 87 years. Of these, 379 tested positive for HBsAg, a frequency of 12.5%. The majority of infected patients were between 20 and 39 years old. The average age of the infected population was 38.9 ± 16.4 years with extremes of 18 and 70 years. There was a male predominance of the infected population with a sex ratio of 1.6. Of the 379 HBsAg positive specimens submitted to the molecular study, 321 tested positive for HBV DNA after nested PCR, a molecular prevalence of 84%. The genotype could be identified in 302 patients positive for HBsAg and HBV viral DNA, ie 79.7% of the infected population; in 19 cases (5%) the genotype could not be determined. Five genotypes were identified in our population, divided into the following proportions : genotype E (n = 207, 54 ,6%), A (n = 88, 23.2%), D (n = 4, 1%), B (n = 3, 0.3%), C (n = 2, 0.6%).

AgHBs	PCR1 avec HBPr1/HBPr135		PCR2 avec HBPr2/HBPr3	
	Effective	Frequency %	Effective	Frequency %
Negative	277	86.3	58	20.9
Positive	102	31.8	219	79
2638 379	Positive by PCR 1 102		Positif by PCR 2 219	
Total 3017	Total PCR1 et PCR2 321			

Note that some patients had two genotypes: A/E (n = 8, 2.5%) and B/C (n = 1, 0.3%). Depending on the departments, the distribution of genotypes was almost identical, genotype E was predominant in all departments, followed by genotype A in all departments. The genotype D was only found in one department, and genotypes B and C were only found in one department. One patient in this department, had both genotypes, B and C. Three subgenotypes were identified during this work: genotype A, subgenotypes A3 and A6, and genotype, subgenotypes D7. The following figure shows the characteristics of the population and the distribution of genotypes by department.

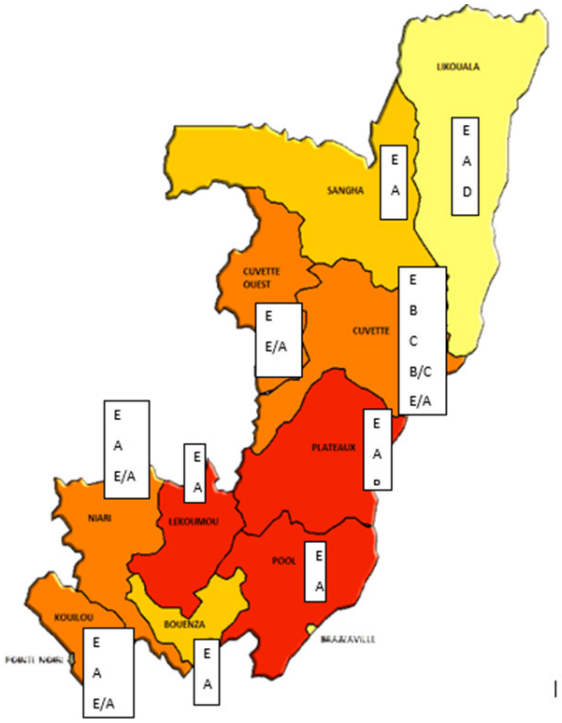

Figure 1: Genotypic mapping of HBV in Congo.

Depart-ment	Genotypes						
	A	B	C	D	E	A/E	B/C
Brazza-ville	32 (28%)	-	-	-	56 (72%)		
Pointe-Noire	17 (19.8%)	-	-	-	59 (68.6%)	4 (4.6%)	-
Sangha	16 (53.3%)	-	-	-	14 (46.7%)	-	-
Likouala	11 (36.7%)	-	-	4 (13,3%)	15 (50%)	-	-
Bouenza	6 (27.3%)	-	-	-	16 (72.7%)	-	-
Plateaux	7 (35%)	1 (5%)	-	-	12 (60%)	-	-
Niari	6 (50%)	-	-	-	6 (50%)	1 (8,3%)	-
Lékoumou	3 (30%)	-	-	-	7 (70%)	-	-
Cuvette	-	1 (10%)	1 (10%)	-	8 (80%)	2 (20%)	1 (10%)
Cuvette-West	-			-	8 (80%)	2 (20%)	-
Total	88	2	1	4	207	9	1

Table 2: Frequency of different genotypes by department.

Discussion

The present study has the merit of covering all the departments of the country, and thus giving a better estimate of the prevalence of HBV in the Congo. We found an overall prevalence of 12.5% of the carriage of HBsAg. The available data on the prevalence of HBV classify our country as an area of high endemicity, with a prevalence of between 12 and 15% [6-9]. All of these existing data concern fragmentary studies, concerning specific populations. The molecular prevalence was 84% of all HBsAg carriers. Such high rates of molecular prevalence are also described by Araujo, who found a rate of 84% with positive HBsAg samples from Brazilian patients and even higher prevalences were described by Allain who found 98% of molecular prevalence [10,11].

However, in sub-Saharan African countries, studies also describe lower molecular prevalence rates, such as the work of Fatima Valente, who has a molecular prevalence of 53% in Luanda [12]. These differences may reflect a real geographic difference or simply be related to different methods used in studies or low levels of viral DNA. Young adults aged 20 to 39 were the most represented in our population. The fact that the contamination occurs mainly in the perinatal period and in infancy could explain this high incidence of HBV in the active class due to the fact that in this context the risk of progression to chronicity is very high. Several African studies also describe a high representativeness of this young class [13,14]. However, this predominance of the active class may be biased by the recruitment method, which involved consecutive, voluntary sampling in asymptomatic patients.

Genotyping performed on the sera of 321 HBV DNA positive patients allowed us to identify five genotypes, mainly represented by genotypes E and A. Genotype E was the predominant genotype in the different departments of our country with an almost homogeneous distribution.

In The majority of studies seem to indicate its predominance in West African countries which is described as its main focus [1517]. Deed, genotype E is described as predominant in Africa according to several studies [15,16].

However, other studies such as ours show its predominance in Central African countries, particularly in Angola, Gabon and Central Africa where genotype E accounted for more than 80% of the identified genotypes [2,12,18]. This genotype E, the main genotype in sub-Saharan Africa, is exclusively found in this geographical region, or exceptionally outside this continent. Indeed, when it was encountered in Western countries, it was the descendants of black African subjects living in the West. But among the black subjects of America this genotype is not found. This distinction suggests that the genotype E is of recent appearance, and that it would result from genetic mutations occurring in Africa [16,19-21].

Genotype A in our work is the second most common genotype, found in all departments of our country. F. Valente et al in Angola describe results similar to ours in Angola, genotype A accounting for 10% of all identified genotypes [12]. Genotype A is a ubiquitous genotype, but predominant in northwestern Europe, North America, and southern, eastern and central Africa [2,20,22,23]. A Burundian study of 51 patients from the five regions of this country, objectified genotype A as the only identified genotype; as well as a study of genomes of subjects from different African countries, had shown a predominance of genotype A in Cameroon [16,24]. In two departments of our country, genotype D was isolated with a relative frequency of 1% (n = 4). En Angola, le génotype D était retrouvé chez un seul sujet originaire du Brésil [12].

Genotype D is rare in sub-Saharan Africa, most African studies do not isolate this genotype. Indeed, the genotype D is frequent around the Mediterranean basin and in the Middle East, the dominant genotype in North Africa [2,23,25]. The demonstration of this genotype in only two departments of our country could be linked to an importation of the genotype favored by the migrations of the populations. It is the same for genotypes B and C that were found in two other departments of the country. Geographical distributions of genotypes worldwide describe genotypes B and C as restricted to Asia (Indonesia, China, Japan, Korea, Vietnam) [2,25,26].

In our work 3% hybrid genotypes A / E and B / C were found. It is thanks to the movements of populations that we describe the mixing of genotypes. Hybrid genotypes are usually found in cosmopolitan cities. According to a French multicentre study, hybrid genotypes were found in about 10% of cases; the main genotypes found were genotypes A and D (24%), A and G (12%) and then C and D (10%) [27]. The main recombinations described to date involve genotypes A and D, B and C as well as C and D [28,29]. The sub-genotypes identified in our departments were genotypes A1, A3, A6 and D7. Genotypes A1 and A3 are classically described in published studies [2,19,23,30,31].

But it appears in our work two new genotypes A6 and D7 that can result from mixing genotypes. The impact of genotypes and subgenotypes on the natural course of the disease and the sensitivity to Lamivudine or Interferon treatments is controversial. Indeed, several authors have reported that chronic evolutionary carrier profiles of HBV as well as viral mutations and anti-HBe seroconversions largely depend on genotypes [24]. According to some Asian studies, genotype C seems to be preferentially associated with a progression towards chronicity, the occurrence of HBe seroconversion that is less frequent and greater histological damage; genotype B is more readily associated with the development of hepatocellular carcinoma at a younger age [25,26].

Patients with Genotype C after anti-HBe seroconversion had a high incidence of reactivation of HBeAg [24]. Whereas in Europe, chronic infection is more common in patients infected with genotype A than in those infected with genotype D, but the prognosis seems better for those infected with genotype A than with genotype D or F [32]. All these data are not yet confirmed because the studies highlighting them are of low amplitude and

there are results contradicting these data in other studies. It is therefore necessary that large-scale studies be conducted to confirm the results of these different studies, as the emergence of mutated strains can have an impact on the diagnosis and compromise certain public health measures such as screening for infection. In the blood donor population or the full effectiveness of large-scale vaccination campaigns in highly endemic countries. But until now, these studies have not concerned genotype E, present exclusively in Africa. Studies specific to Africa must therefore be carried out in order to determine with certainty the impact of genotypes and under genotypes on the therapeutic management.

Conclusion

In Congo circulating genotypes almost homogeneously in all departments are genotype E followed by genotype A. Genotypes D, B and C, probable import strains, are sporadically found in the country. The results of this study will guide patient management programs by better selecting patients for treatment and the most appropriate treatments.

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