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Molecular Characterization and Resistance Profile of the Hepatitis B Virus to Polymerase Inhibitors in Infected Treatment-Naïve Patients in Abidjan

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Abstract: Mutant selection is due to the high rate of viral replication and lack of proofreading activity in the hepatitis B virus (HBV) polymerase thus leading to the generation of mutations in HBV. However naturally occurring HBV strains carrying primary drug resistance mutations are very rare in the absence of prior treatment. Monitoring changes in primary and secondary resistance mutations in patients who haven't been treated is crucial in order to optimize and promote the best treatment, to obtain a sustained virological response (SVR) and therefore, reduce the progression to cirrhosis and later to hepatocellular carcinoma (HCC). The main purpose of this research was to evaluate the resistance of HBV to antivirals and show the genetic variability of HBV in a population of blood donors, carrying HBs antigen, naïve to anti-HBV treatment in Abidjan. A descriptive and analytical cross-sectional method was used to establish the molecular profile and to identify the polymorphism of HBV in treatment-naïve infected study participants. Adults blood donors, of any sex, with a positive result for HBsAg, naïve to any antiviral treatment but with an HBV viral load superior to 1000 IU/ml were included. The ABI 3130 Avant sequencer (Applied Biosystems, Courtaboeuf, France) was used to sequence the polymerase (pol) gene to determine HBV resistance genotypes. Fifty-three (N=53) blood donors infected with HBV (HBs Ag positive) were screened. All patients were naïve to any antiviral treatment. Of all these patients, 30 (56.6%) blood donors, carrying HBsAg with a viral load superior to 1000 IU/mL were included in the study. The median age was 34 years old (21-52). The median viral load was 6561 IU/mL (103 – 1.65 x 109). Two mutations of a single base, notably A181T and A181S were highlighted in this study. The A181T mutation was associated with resistance to adefovir, lamivudine and telbivudine. As for the A181S mutation, it was associated with resistance to adefovir only. Analysis of phylogenetic trees obtained by sequencing confirmed the circulation of 2 genotypes: E (22; 92%) and A (2; 8%). The circulation of genotypes A and E of HBV in Côte d'Ivoire has been confirmed by this study, with an estimated genotypic mutation prevalence of 8%. The resistance of HBV to some antiretroviral drugs in the class of HBV polymerase inhibitors, such as lamivudine (3TC), telbivudine (LdT) adefovir (ADV) may be attributed to these mutations.

Keywords: HBsAg, Hepatitis B, Primary Resistance

1. Introduction

Viral hepatitis B is a global public health problem [1]. Hepatitis B Virus (HBV) affects more than 2 billion people worldwide with more than 400 million chronic carriers, including 65 million in Africa [2-4]. In Côte d'Ivoire, the prevalence of hepatitis B is about 12% [5]. The risk of developing serious liver diseases is significantly increased in chronic HBV infection which can include liver cirrhosis and hepatocellular carcinoma (HCC), a common form of human cancer [6]. The estimated risk of HCC in chronic HBV carriers is one hundred times that of non-infected people. For therapeutic management, guidelines recommend the administration of virostatic nucleos(t)ide analogues (NA) for a long period [6, 7]. This could be a selection factor for resistance mutations of HBV to these molecules. These NAs block reverse transcriptase (RT) and HBV polymerase activities. However, studies have shown that mutations in the RT region of HBV polymerase are strongly associated with NA resistance during antiviral therapy. This selection of mutations is due to the high rate of viral replication and the lack of proofreading activity of the HBV polymerase thus leading to the generation of mutations on the virus genome [8]. Several studies have been carried out on molecular characterization and antiviral resistance profile of HBV in treatment-naïve patients in Africa. Based on these studies, no resistance to Tenofovir Disoproxil Fumarate (TDF) has been demonstrated [8, 9] However, data on HBV molecular and drug resistance in Côte d'Ivoire are scarce to NA in treatment-naïve patients in particular and in infected population in general.

Therefore, to help understand genotypic HBV resistance in treatment-naïve patients, it is important to monitor changes in NA resistance mutations in these patients in order to optimize and promote for better therapeutic management, achieve sustained virologic response (SVR), and consequently reduce cirrhosis progression and progression to HCC. This study aimed to identify genetic polymorphisms and assess the prevalence of genotypic mutations resistant to NA in Abidjan treatment-naïve patients (Côte d'Ivoire).

2. Material and Methods

2.1. Type and Period of Study

During this descriptive and analytical cross-sectional study patients were enrolled in a prospective cohort followed at the National Blood Transfusion Center (CNTS) between March and April 2021. They study population consisted in adult blood donors, with a positive HBsAg serological test, naïve to any antiviral treatment and who have given their consent.

2.2. Ethical Considerations

The study was approved by the National Ethics Committee of Life Sciences and Health (N/Réf: 196-22/MSHPCMU/CNESVS-km) of Cote d'Ivoire. Moreover, to ensure confidentiality during our study, only the inclusion number was reported. No names or information identifying any patient were mentioned. Biological data were collected as part of routine care activities. Patients directly benefit from the results.

2.3. Biological Analysis

Blood was obtained by venipuncture and collected on tubes without additive and tubes containing ethylene diamine tetra acetic acid (EDTA) for this study. No additive tubes were used for biochemical tests such as creatinine, urea, total protein levels, alanine aminotransferase activity (ALT) and serological tests such as HBs Ag. For virological tests, the plasma was obtained after centrifugation of EDTA tubes at 3500 rpm for 5 min and stored at -80°C until the genotypic tests were carried out.

2.4. Determination of HBV Genotypes and Resistance Genotypes

Extraction of viral DNA following lysis of viral particles and purification on filter columns (QIAamp Viral RNA Mini Kit, Qiagen, Germany) according to the manufacturer's recommendations. The amplification of the HBV polymerase (*pol*) was carried out after a "nested PCR" using the specific primer pairs 5'POL m1/3'POL m2 and 5'POL m3/3'POL m4 previously described [10]. The 808 bp fragments obtained were purified and sequenced on an automatic sequencer, the ABI 3130 Avant, (Applied Biosystems, Courtaboeuf, France). The obtained sequences were aligned using SeqScape 3 software (Applied Biosystems, Courtaboeuf, France) to generate consensus sequences. HBV genotypes were determined by phylogenetic analysis after alignment of consensus sequence with reference sequences corresponding to A-J genotypes thanks to Bio Edit and Mega 7 softwares [11]. Resistance genotypes were determined using the online software (http://www.hiv-grade.de/hbv_grade) based on the European Association for The Study of the Liver (EASL) algorithm.

2.5. Statistical Analysis

Statistical analyzes were performed using SPSS 17.0.1 software. This software allowed us to accomplish the following tasks: (i) Univariate analysis (calculation of the prevalence of different genotypes and subtypes, mean age and standard deviation, etc.); (ii) bivariate analysis.

3. Results

3.1. Selection Process for the 30 Patients in the Study

Fifty-three blood donors carrying HBsAg were screened.

Among them, 30 blood donors (56.6%) with a viral load superior to 1000 IU/mL were included (Figure 1).

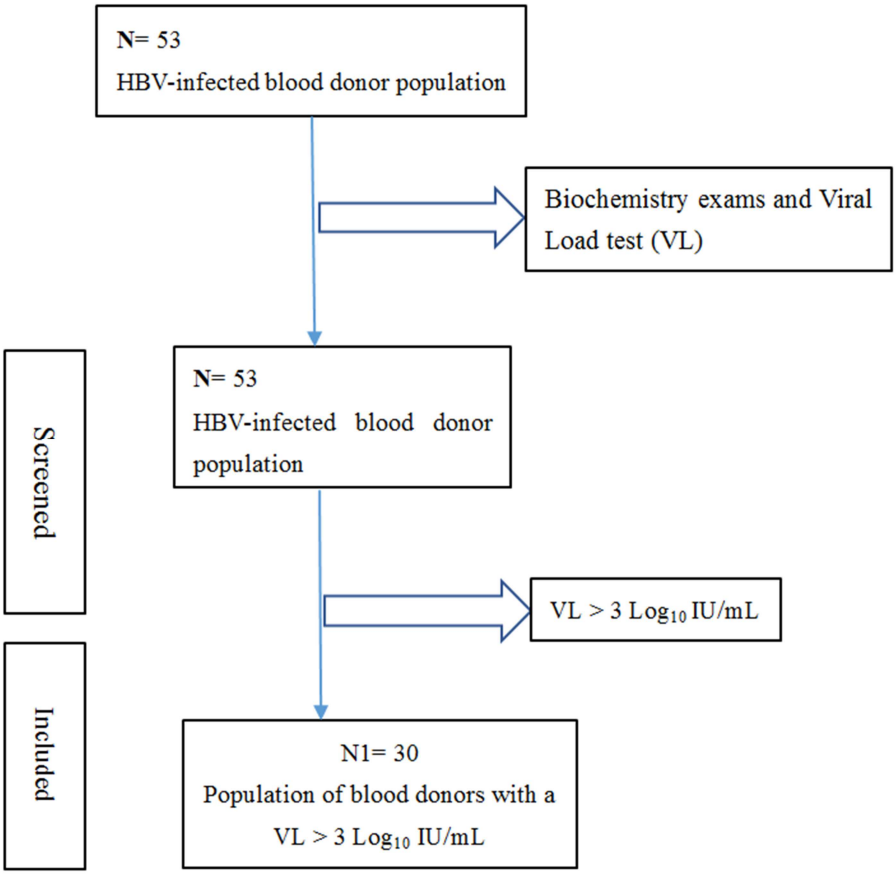

Figure 1. *Selection process of 30 patients in the study.*

3.2. Characteristics of the 30 Patients with Positive Viremia in the Study

The median age of patients with detectable viremia was 34 years [21-52]. The median viral load was 6.56×10^3 IU/mL [1.10^3 – 1.65×10^9] with a median quantitative titration of $1.06.10^4$ IU/mL [$15.42–2.21.10^5$]. All patients were naïve to any antiviral treatment (Table 1).

Table 1. *Characteristics of the 30 patients with positive viremia in the study.*

Characteristics	values	Percentage
Sex		
Male (N, %)	23	77
Female (N, %)	7	23
Age (year) (median, range)	34	(21-52)
VL-HBV (IU/ mL)	$6.56.10^3$	($103-1.65.10^9$)
ALT (UI/mL)	12.5	(10-73)
Quantitative HbsAg (median, range)	$1.06.10^4$	($15.42 – 2.21.10^5$)

3.3. Distribution of Circulating HBV Subtypes

24 samples could be amplified by PCR. The fragments of the *pol* gene attained by sequencing were aligned with reference sequences using the Bio Edit software (Figure 2). Phylogenetic trees were produced using the Neighbor joining method on Mega 7 software with HBV reference sequences. The analysis of these trees corroborated the circulation of two genotypes which were the genotype E (92%; (22/24)) and the genotype A (8%; (2/24)).

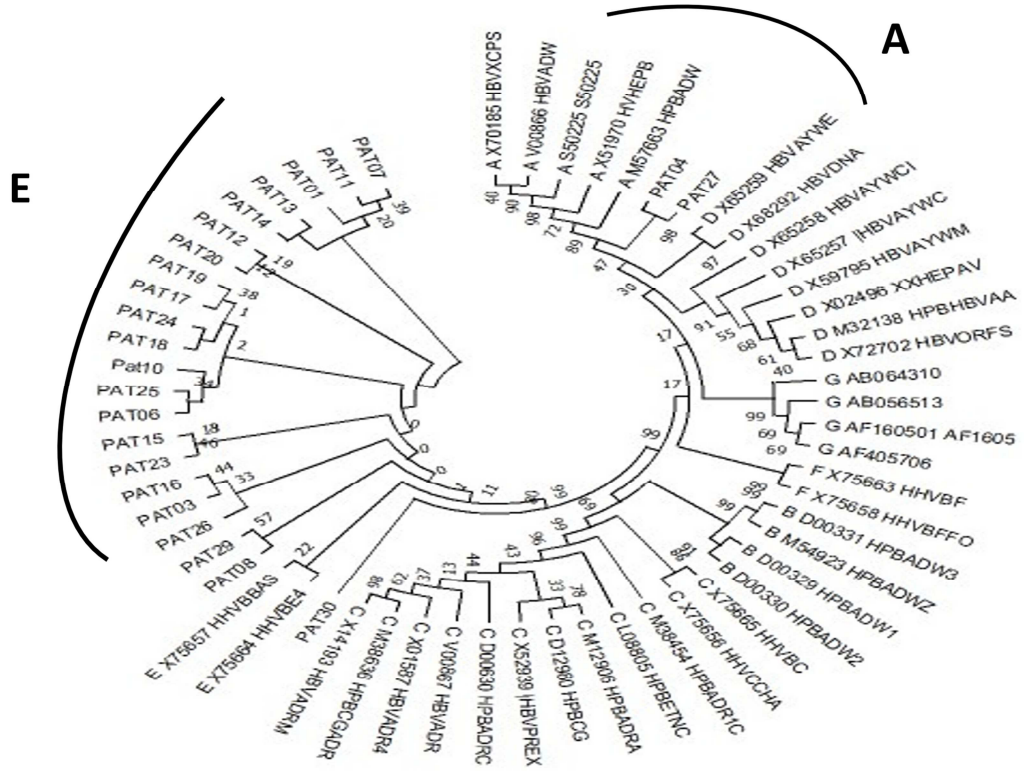

Figure 2. *Phylogenetic tree of circulating HBV subtypes of the polymerase (pol) gene in 24 patients.*

Consensus sequences were aligned and compared with reference sequences.

3.4. Molecular Profile of HBV Strains Sequenced in Treatment-Naïve Patients

Of the 24 strains which could be sequenced, analysis of resistance profiles main drugs was carried out. A total of 22 (92%) patients had strains susceptible to nucleos(t)idic analogues (NA) (Table 2). Only two patients (8%) had bore a virus exhibiting resistance to NAs (Table 3) No strain showed resistance to tenofovir.

Table 2. *Molecular profile of HBV strains sequenced in treatment-naïve patients.*

ID	Genotypes	Resistance	Mutation
Pat 1	E	ADV; 3TC; LdT	A181T
Pat 2	A	S	
Pat 3	E	S	
Pat 4	E	S	
Pat 5	E	S	
Pat 6	E	ADV	A181S
Pat 7	E	S	
Pat 8	E	S	
Pat 9	E	S	
Pat 10	E	S	
Pat 11	E	S	
Pat 12	E	S	
Pat 13	E	S	
Pat 14	E	S	
Pat 15	E	S	
Pat 16	E	S	
Pat 17	E	S	
Pat 18	E	S	
Pat 19	E	S	
Pat 20	E	S	
Pat 21	E	S	
Pat 22	A	S	
Pat 23	E	S	
Pat 24	E	S	

Legend: Pat: patient code; ADV: adefovir; 3TC: lamivudine; LdT: telbivudine; S: Sensitive; Resistance; A: Alanine; T: Tyrosine; S: Serine

Table 3. *Mutation profile of Pol sequences in strains from the 2 treatment-naïve patients with drug resistance among the 24 patients with positive nested PCR.*

Combination of Mutations	(N = 2)	Frequency (%)	Associated molecule
A181T	1	4	3TC; LdT; ADV
A181S	1	4	ADV
No major mutations	22	92	-
Quantitative HbsAg (median, range)	$1.06.10^4$	$(15.42 – 2.21.10^5)$	

Legend: N: Number of people having a positive nested PCR in the study; A: Alanine; T: Tyrosine; S: Serine.

4. Discussion

Phylogenetic analysis of the pol sequence of the twenty-four isolates obtained in this study revealed the circulation of genotypes A and E of HBV in Ivory Coast with respective prevalences of 8% and 92%. The circulation of genotypes A and E is confirmed by these results with a high prevalence of genotype E in Côte d'Ivoire as published in a retrospective study to identify the distribution of HBV genotypes in 4 countries of Sub-Saharan Africa, in particular Côte d'Ivoire, Ghana, Cameroon and Uganda and in a second study in 33 chronic carriers of the hepatitis B virus in Côte d'Ivoire [12, 13]. However, other authors have published the circulation of other genotypes in this country. Indeed, in addition to genotypes A and E, genotype D has been demonstrated in an HBV/HIV coinfected population in Abidjan [14]. The circulation of E genotypes remains predominant with more than 92% of cases in our study.

HBV molecular profile in treatment-naïve individuals made it possible to describe resistance genotypes in this study. HBV resistance mutations prevalence in antiviral-naïve patients enrolled in this study was 8% (2/24). The comparison of our sequences with the reference sequences shows the presence of A181T mutations at 50% (1/2) and A181S at 50%; (1/2). These mutationsA181T and A181S are resistance mutations to ADV, 3TC and LdT [15].

Results of this study confirm the circulation of viruses carrying primary resistance mutations in the DNA polymerase of hepatitis B virus in naive patients in our setting. This could occur either as a result of transmission events or by virological factors intrinsic to the virus. Indeed, these pre-existing mutations have been described in the HBV reverse transcriptase gene in naive patients in China and the same resistance patterns were observed [8].

A primary resistance process associated with a greater number of resistance mutations has also been described. It involves the accumulation of mutations S202I, A194T, M204I, A181S, L180M and M204V [16]. This situation can be observed in contexts with a longer therapeutic history. Several genetic resistance mutation selection pathways have been described. In a Chinese study, the authors described no A181T neither A181S mutations, but rather five primary drug resistance mutations (rtT184G, rtS202I, rtM204I/V, rtN236T and rtM250V), one secondary resistance mutation (rtL180M) and one codon mutation that could be basic polymorphisms without any clinical significance (rtV207I) [17]. The accumulation of resistance mutations may be associated with the severity of liver disease in patients with chronic hepatitis B who are not receiving treatment. [18].

5. Conclusion

This study provided important data on the molecular profile of HBV in treatment-naive infected patients in Abidjan. This study is a key reference for the health authorities, as it enabled them to determine the circulating HBV genotypes in this population, as well as the antiviral resistance profile. Two resistance mutation profiles were observed in the HBV pol gene in two patients. These mutations may contribute to HBV resistance to antiretroviral drugs in the HBV polymerase inhibitor family, including lamivudine, telbivudine and adefovir. Sequencing for HBV genotyping is accessible and can be used for epidemiological surveillance to effectively monitor and optimize patient follow-up in Côte d'Ivoire.

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