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RESISTANCE OF HBV TO NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS AND ITS IMPACT ON SURFACE ANTIGEN IN HBV-HIV CO-INFECTED PATIENTS IN ABIDJAN

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ABSTRACT: *Mutations in the viral hepatitis B virus (HBV) on genes pol and S, can confer the variants a clear survival advantage. The objective of this work was to describe HBV resistance mutations to nucleoside reverse transcriptase inhibitors (NRTIs) and their impact on surface antigen HBs antigen (HBs Ag) in HBV -HIV coinfected patients undergoing antiviral treatment in Abidjan (Ivory Coast). The genotypic resistance interpretation was performed using the HBV Tool algorithm available on the online software (http://www.hivgrade.de/hbv_grade/). In this study, the prevalence of HBV resistance to NRTIs was 17% (5/30). The prevalence of HBsAg escape mutations in patients co-infected with HBV and HIV was 50% (15/30). The prevalence of resistant mutations associated with potential vaccine escape mutations (ADAPVEMs) in HBV-HIV co-infected patients was 13% (4/30). This study has provided important data on the mechanism of HBV escape from NRTIs, immune systems and HBV vaccination in Ivory Coast.*

KEY WORDS: HBV-HIV co-infection, HBV resistance, HBs antigen escape mutations.

INTRODUCTION

Viral hepatitis B is a public health problem affecting approximately 325 million people worldwide (WHO, 2018). According to the World Health Organization (WHO), the global prevalence of HBV infection in the population of patients living with HIV (PLHIV) is estimated at 7.4% (WHO, 2018). Ivory Coast is one of the most affected West African countries with an estimated prevalence of HBV-HIV co-infection of 13.4% (Attia et al., 2012).

In Ivory Coast, the therapeutic strategies are based on the WHO's strategy, which recommends the introduction of molecules that are active on both HBV and HIV in HBV-HIV co-infected patients (WHO, 2018). The active molecules on both viruses recommended by the national AIDS Control Program (PNLS) are the nucleotide and nucleoside reverse transcriptase

inhibitors (NRTIs) which are Tenofovir, Disoproxyl, Fumarate (TDF) and lamivudine (3TC) respectively (PNLS, 2015).
Monitoring response to HBV treatment in patients co-infected with HIV should include virological tests such as HBV plasma viral DNA quantification and TGR. Indeed, these tests are important for the early detection of therapeutic failures and to guide physicians in the choice of HBV-active drugs. Unfortunately, the costs and the limited number of molecular biology facilities makes the test inaccessible to the majority of patients.

Therefore, HBV treatment in patients co-infected with HIV is usually initiated or modified without taking into account the HBV plasma viral load and the resistance to antivirals. Consequently, the efficacy of NRTIs may be compromised by the transmission of resistant viruses or the acquisition of resistance during treatment (Caligiuri et al., 2016). In addition, these NRTI-resistant viruses may develop escape mechanisms (EM) and/or potential vaccine-escape mutations (VEM) in *HBsAg* due to the overlap of the polymerase (*Pol*) and surface (*S*) genes (Sayan et al. 2012; Sun et al., 2016; Ozguler and Sayan 2016; Zhao and al., 2016; Ozguler and Sayan 2018). Such strains are called antiviral drug-associated potential vaccine-escape mutants (ADAPVEMs) (Sayan and al. 2012; Sayan & Bugdaci, 2013; Pal and al., 2015).
Similarly, *HBsAg* immune escape mutations (IEM) can make serological tests unreliable, often leading to so-called occult hepatitis (AFSSAPS, 2009; Zhao et al., 2016). In Ivory Coast, the prevalence of occult hepatitis B is estimated at 21.3% in HIV-infected patients (Attia and al., 2012). HBV strain evolution surveillance is therefore necessary to improve the management of HBV infection.The objective of this work was to estimate the prevalence of HBV virological failure, describe HBV resistance mutations to NRTIs and their impact on *HBsAg* in HBV and HIV-infected patients undergoing antiviral treatment in Abidjan (Ivory Coast).

MATERIALS AND METHODS

Study population

This was a retrospective monocentric descriptive and analytical study conducted between January 2016 and July 2018. HBV-HIV co-infected patients were enrolled in a prospective national cohort of 3,000 HIV-infected patients treated at the Centre Intégré de Recherche Bioclinique d'Abidjan (CIRBA). Patients (adult) on antivirals (AV) with a detectable HBV DNA level (> 20 IU/mL) were included. The study was approved by the National Ethics Committee of Life and Health Science's (CNESVS; N/Ref: 137-18/MSHP/CNESVS-km).

Determination of NRTI resistance and *HBsAg* escape mutations

Determination of NRTI resistance mutations and *HBsAg* escape mutations was performed after sequencing the *pol* and *S* genes. The *pol* and *S* genes were amplified after extraction of the viral DNA with the QIAamp viral DNA mini kit (50) (Qiagen, GmbH). Two PCRs were performed with the HotStarTaq® DNA Polymerase (Qiagen, GmbH). The outer primers used for the first PCR was: *HBV pol1* (5′-CCCTGCTCGTGTTACAGGCGGG-3'; nucleotide position 186–206) and *HBV pol2* (5′-GTTGCGTCAGCAAACACACTTGGGCA-3'; nucleotide position 1196-1174).

The nested PCR primers were *HBV pol3* (5'-GACTCGGTGGTGGGGACTTCTCTCTCA-3'; nucleotide position 251–272) and *HBV pol4* (5'-

GGCATTAAAAGCAGGATAACCACATTG-3'; nucleotide position 1058-1033). The thermal amplification program for both PCRs was previously described by N'din et al. (N'din and al., 2018). The final size of the obtained amplicons was approximately 1010 bp. The amplicons were purified and sequenced on the automatic Genetic Analyzer 3130 sequencer (Applied Biosystems, Courtaboeuf, France) using the Big Dye Terminator Kit v3.1 cycle sequencing Kit (Applied Biosystems, Courtaboeuf, France). The determination, interpretation of NRTI resistance and *HBsAg* escape mutations of the HBV were performed using the online Genotypic Resistance-Algorithm Deutschland HBV Tool algorithm available on the online software *http//www.hiv-grade.de/HBV_grade/* based on the Stanford hivDB algorithm. Analysis of nucleotide sequences on Mega 3 software also allowed the identification of circulating viral subtypes in Ivory Coast. The phylogenetic relationships of the newly derived viruses were estimated from sequences comparison with the reference sequences. Nucleotide sequences were aligned using Bioedit v7 software (Hall, 1999) and trees were edited with Mega 10 software (Kumar and al., 2018).

Statistical analyses

SPSS Statistics v.13.0.0 was used for univariate and multivariate statistical analyses.

RESULTS

Patient Characteristics

The study involved three hundred (n = 300) plasma samples from HBV and HIV co-infected ART treated patients. A total of 15% (n = 44/300) of plasma samples with viral DNA levels greater than 20 IU/mL were submitted to genotyping assay. HBV DNA sequencing was successfully performed in 68% (n = 30/44) of patients with viral DNA levels greater than 100 IU/mL mostly. Of these patients, 87% (n = 26/30) were on TDF + 3TC as an NRTI as part of triple therapy. The sex ratio (M/F) was 1.6 in favor of males and the median age was 51 years (34–68). The percentage of patients with HBV DNA below 2000 IU/mL was 69% (n = 18/26). The percentage of patients with HBV DNA above 20,000 IU/mL was 19% (n = 5/26). The median HBV DNA level was $1.38x10^7$ IU/mL and the median transaminase level (ALAT) was 43 IU/ml. On the other hand, 13% (n = 4/30) of patients were on 3TC only as NRTI against HBV in the triple therapy. The sex ratio was 100% in favor of males and the median age was 54 years (50–58). In this group, two (n = 2; 50%) patients had an HBV DNA level below 2000 IU/mL and two (n = 2; 50%) patients had an HBV DNA level more than 20000 IU/mL. The median HBV DNA level was 55.10^7 IU/mL and the median transaminase was 57 IU/mL (Table 1). The median duration of antiviral therapy for the included patients was 5 years (1–10 years).

Table 1. Social, biological, and clinical characteristics of the 30 HBV-HIV coinfected patients whose HBV sequences were obtained at CIRBA in 2016 and 2018 (N=30/44, 68%)

Characteristics	Patients with TDF + 3TC	Patients with 3TC
Nombre (N ; %)	(26 ; 87)	(4 ; 13)
Sex ratio (H/F)	16/10	4/0
Median age (year ; interval)	51 (34 – 68)	54 (50 – 58)
Median treatment duration	5 (1–10)	5 (1–10)
median HBV viral load (UI/mL)	1,38x 10^7	55x10^7
ALAT Transaminases (IU/mL)	43	57
HBV DNA < 2000 IU/mL (N ; %)	(18 ; 69)	(2 ; 50)
HBV DNA > 20000 UI/mL (N ; %)	(5 ; 19)	(2 ; 50)
2000 < HBV DNA < 20000 UI/mL (N ; %)	(3 ; 12)	(0 ; 00)

N : number ; M : male; F : Female ; 3TC : lamivudine ; TDF : tenofovir disoproxil fumarate ; HBV : hepatitis B virus ; ALAT : Alanine Amino Transferase

HBV genotypes

Phylogenetic analysis of the pol gene was performed on the 30 successfully sequenced viruses. These results showed a majority of genotype E (n=22, 73%) followed by genotype A (n=5, 17%) and genotype D (n=3, 10%) **(Figure 1)**.

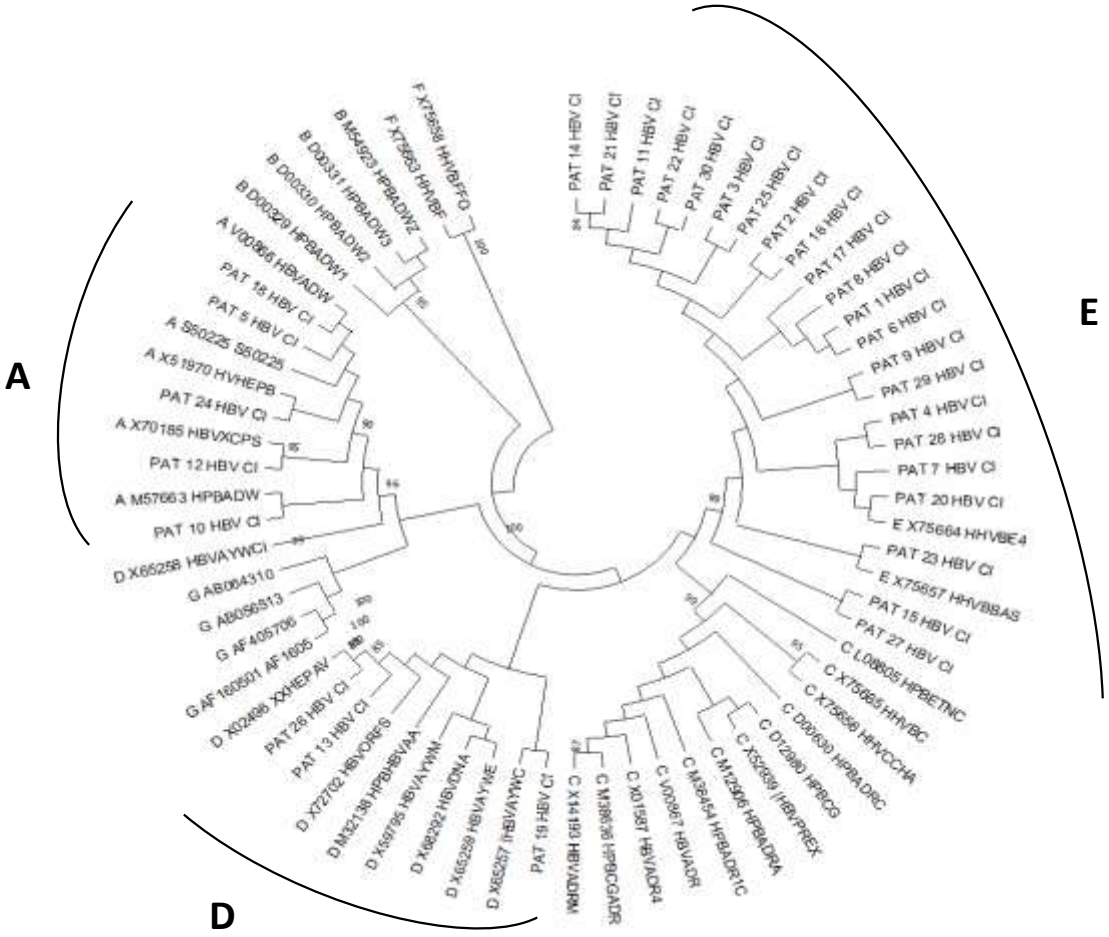

Figure 1: **Phylogenetic trees of HBV viruses subtypes isolated from 30 HIV-HBV co infected subjects. Aligned nucleotides sequences from the *pol* gene were compared with reference sequences using BioEdit v7 and Mega 7 software.**

Frequency of HBV resistance mutations to NRTIs

The primary resistance mutations rtM204V and rtM204I observed on TI, inducing HBV resistance to lamivudine (3TC) and telbivudine (LdT) were respectively found in 13,33% (n = 4/30) and 3% (n = 1/30) of the generated nucleotide sequence(s). The secondary resistance mutation ns rtL180M, rtV173L and rtS202C were respectively found in 17% (n = 5/30), 10% (n = 3,33/30) and 3% (1/30) nucleotide sequence(s). However, no TDF resistance mutations were detected in this study. The primary and secondary HBV resistance mutations are shown in **Figure 2**. The prevalence of HBV resistance to 3TC and LdT in patients co-infected with

HBV and HIV was estimated at 17% (5/30). However, no resistance to TDF was observed (**Figure 3).**

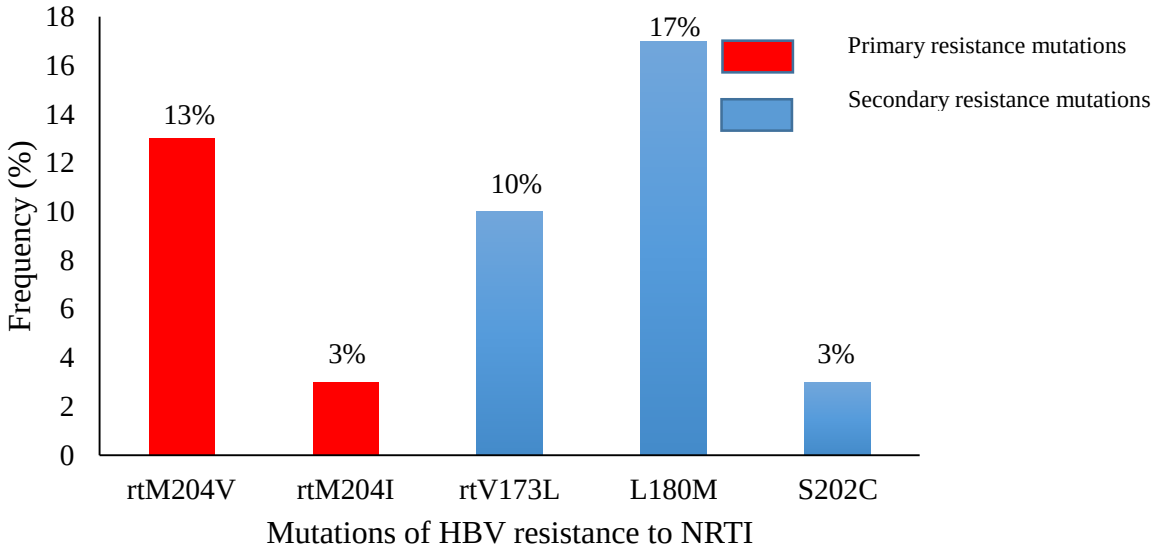

Figure 2. Frequency of detected NRTI resistance mutations (according to the HBV Tool algorithm available on the online software HIV-GRADE derived from the Stanford HIVdb software) in HBV-HIV co-infected individuals at CIRBA in 2016 and 2018.

NRTI : Nucleoside(t)idic Reverse Transcriptase Inhibitors; M: Methionine; V: Valine; I : Isoleucine; L : Leucine; S : Serine; C : Cysteine

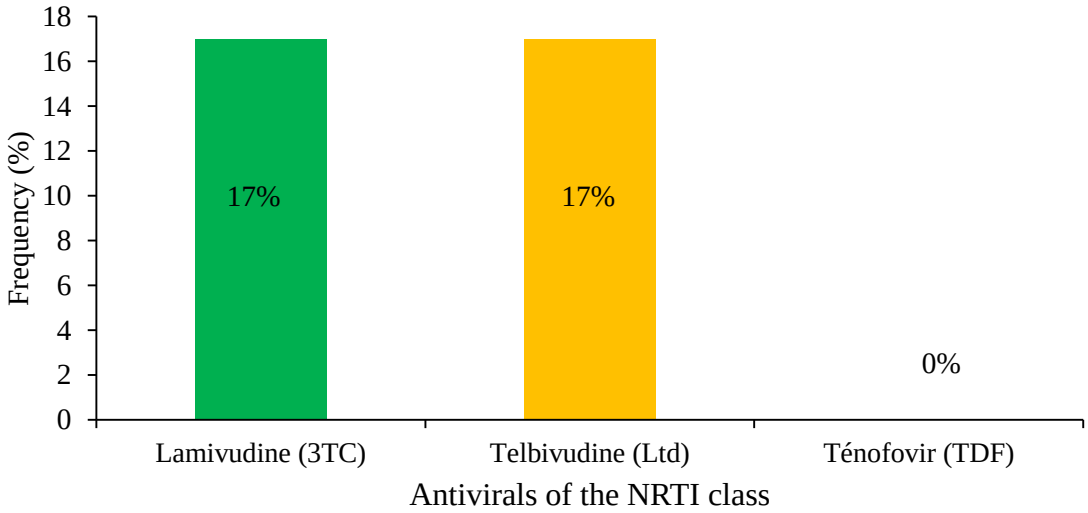

Figure 3. Prevalence of NRTI resistance in HBV-HIV co-infected patients at CIRBA in 2016 and 2018.

3TC: Lamivudine; LdT: Telbivudine; TDF: Tenofovir; NRTI: Nucleoside(t)idic Reverse Transcriptase Inhibitors.

Frequency of combinations of HBV resistance mutations to NRTIs

The analysis of NRTI resistant viruses, identified the triple combination of HBV resistance mutation to 3TC: rtV173L + rtL180M + rtM204V with a frequency of 60% (3/5). The double combinations of resistance mutation to 3TC and Ltd: rtL180M + rtM204I and rtL180M + rtM204V were each observed at a frequency of 20% (1/5). The combinations of HBV NRTIs resistance mutations are shown in **Figure 4.**

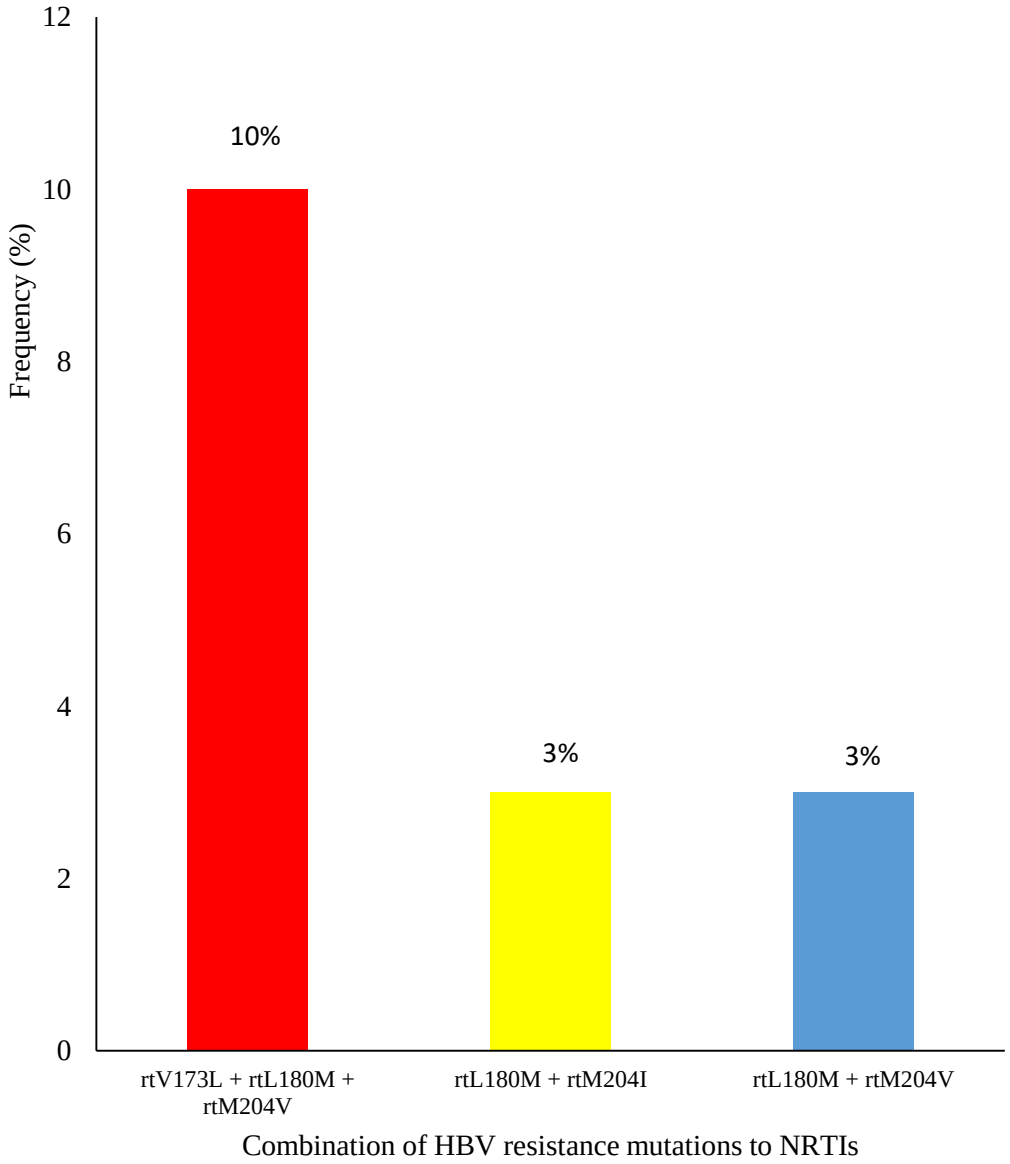

Figure 4. Frequency of 3TC HBV resistance mutation combinations detected on RT in HBV-HIV co-infected patients at CIRBA in 2017 and 2018.

3TC : Lamivudine ; Ltd : telbivudine ; M : Méthionine ; V : Valine ; I : Isoleucine ; L : Leucine ; S : Sérine ; C : Cystéine.

Frequency of *HBsAg* escape mutations in HBV

Eight (n = 8) immune escape mutations (IEM) of *HBsAg* were observed on the HBV *HBsAg S* gene in this study. The mutations sL109I 3% (1/30), G130N 3% (1/30), sM133L 10% (3/30), sG145K 3% (1/30), sT126N 3% (1/30), C121Y 3% (1/30), sK141E 3% (1/30), P127T 3% (1/30) Figure 4. The frequency of *HBsAg* IEMs observed in patients co-infected with HBV and HIV was 33% (10/30). Four (n = 4) NRTI-induced *HBsAg* vaccine-escape mutations (VEM) were also observed: sE164G 3% (1/30), sI195M 10% (3/30), sW196L 3% (1/30), sE164D 3% (1/30). The frequency of *HBsAg* IEMs observed in patients co-infected with HBV and HIV was 20% (6/30) (**Figure 5**).

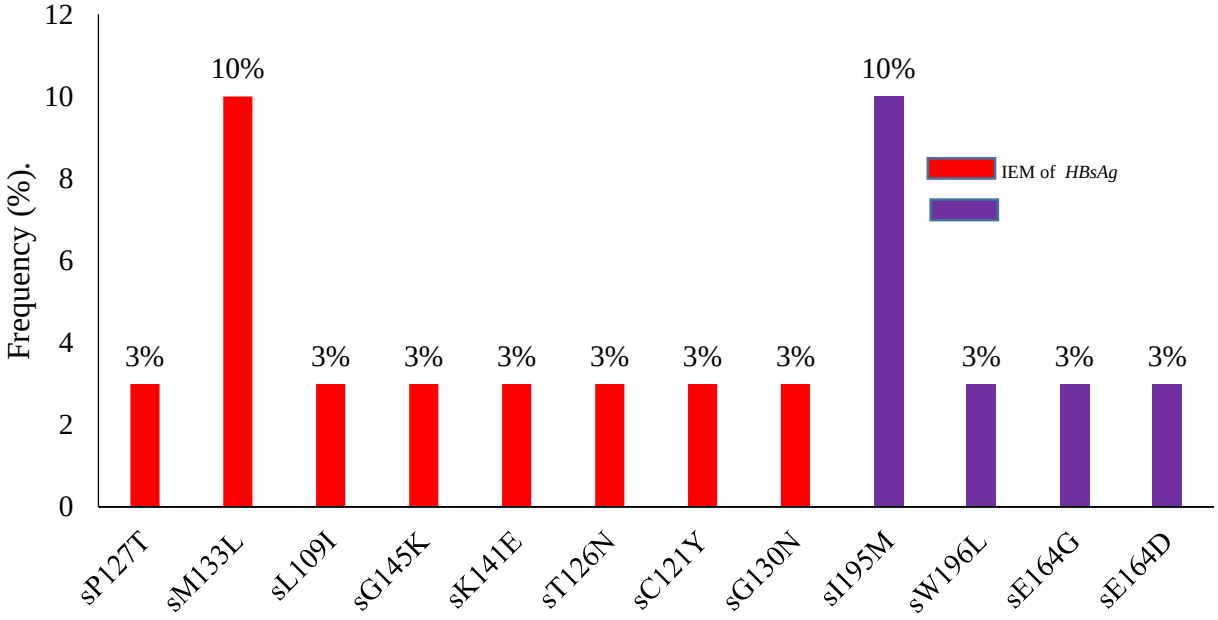

Figure 5. Frequency of escape mutations on *HBsAg* detected in HBV-HIV co-infected patients

VEM : Vaccine-escape Mutations S : Surface gene; M: Methionine; I: Isoleucine; L: Leucine; G: Glycine; N: Asparagine; K: Lysine; T: Threonine; C: Cysteine; Y: Tyrosine; E: Glutamic acid; P: Proline; W: Tryptophan; D: Aspartic acid.

Frequency of escape mutation combinations in *HBsAg* of HBV

The identified combinations of escape mutation in are shown in **Figure 6.** The double escape mutation combination sL109I+sM133L was observed with a frequency of 3% (1/30). The double VEM sI195M+sE164D was identified with a frequency of 3% (1/30). The triple escape mutation combinations sT126N+sE194G+sI195M was identified with a frequency of 3% (1/30). The sC121Y and sG145K mutations associated respectively with the sW196L and sI195M vaccine-escape mutations were each found with a frequency of 3% (1/30). A total of 5 combinations of HBV *HBsAg* escape mutations were identified in 5 different patients in the present study.

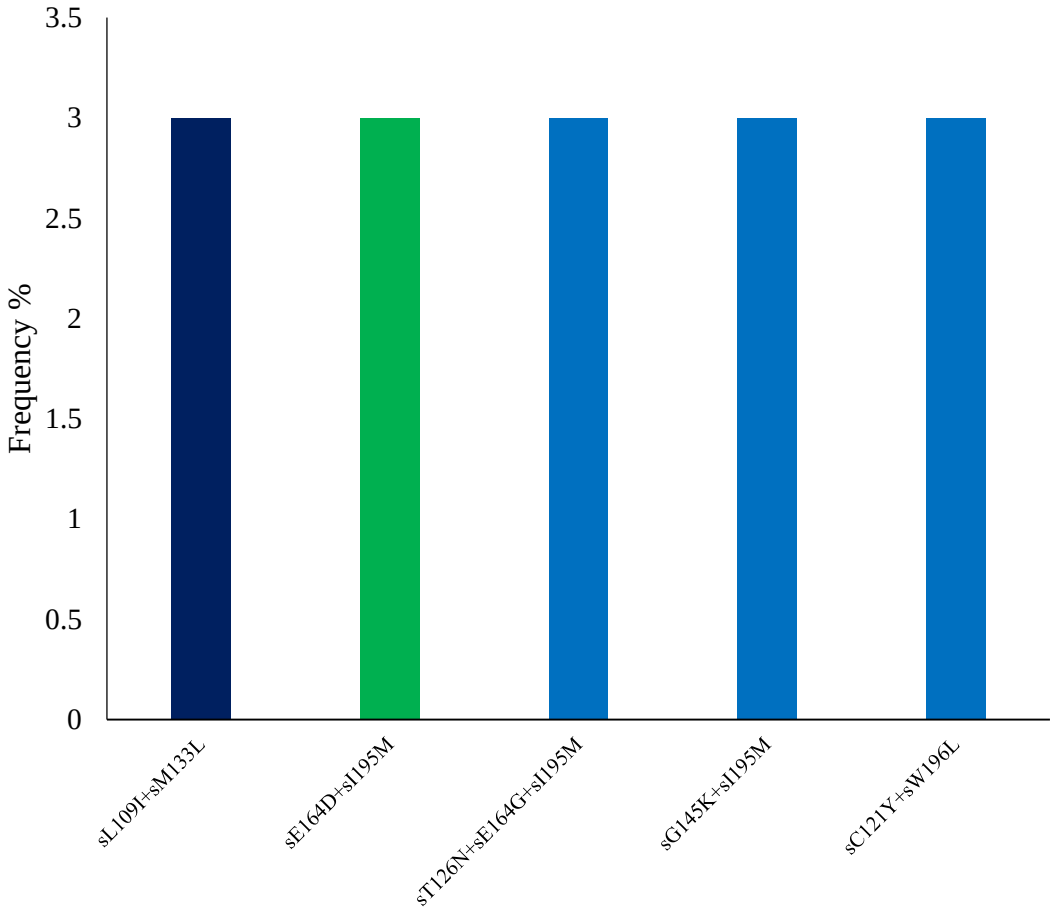

Figure 6. Frequency of *HBsAg* escape mutation combinations detected in HBV-HIV co-infected patients.

HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; T: Threonine; N: Asparagine; M: Methionine; K: Lysine; L: Leucine; A: Alanine; E: Glutamic acid; Y: Tyrosine; S: Serine; G:; I: Isoleucine; D: Aspartic acid; W: Tryptophan.

Frequency of HBV antiviral resistance mutations associated with potential vaccine escape mutations (ADAPVEM)

HBV viral strains with antiviral resistance mutations associated with potential vaccinal-escape mutation or ADAPVEMs were detected in this study. We identified three ADAPVEMs types, all of genotype E, in the 5 patients with NRTI-resistant HBV strains. ADAPVEM 1 with a triple NRTI resistance mutation rtV173L+rtL180M+rtM204V, associated with a double NRTI-induced VEM sE164G + sI195M was observed in a frequency of 40% (n=2/5). ADAPVEM 2 with a dual at NRTI resistance mutation rtL180M+M204V combined with a sI195M VEM was found in 1 patient. i.e., a frequency of 20% (1/5). ADAPVEM 3 with a dual NRTI resistance mutation rtL180M+M204V associated with a VEM of *HBsAg* sW196L, was found in 1 patient (20%) with NRTI-resistant HBV strains (Table 2).
Most of the ADAPVEMs strains were identified in 43% (3/7) of patients with HBV DNA > 20,000 IU/mL and in 5% (1/20) of patients with HBV DNA < 2,000 IU/mL. This represented an estimated prevalence of ADAPVEMs strains of 13% (4/30) in patients co-infected with HBV and HIV in the present study.

Biological characteristics of patients who have developed NRTI resistance and/or *HBsAg* escape mutations.

The biological characteristics and the different HBV resistance mutations to NRTIs and escape mutations of *HBsAg* are shown in Table 3. Overall, 33% (10/30) of the patients included in the study carried HBV strains that developed *HBsAg* escape mutations with or without NRTI resistance mutations and 3% (1/30) of patients with resistant HBV only. The HBV strains detected were genotype *E, A* and *D*. Genotype *E* strains (n = 9/11) was predominant.

HBV resistance mutations to 3TC and Ltd were detected in five patients. Antiviral regimens were 3TC (2/5). Both individual had *HBV* DNA viral load > 20,000 IU/mL. And, three with 3TC+TDF. One participant had a *HBV* DNA viral load < 2,000 IU/mL and the two remaining *HBV* DNA viral load > 20,000 IU/mL). *HBsAg* escape mutations were detected in all these patients except one patient (PAT_01). HBV resistance mutations associated with potential vaccine escape mutations or ADAPVEMs were found in patients for patients (PAT_02, PAT_07, PAT_08, and PAT_06).

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Table 2. Distributions of combinations of HBV resistance mutations to NRTIs associated with potential vaccine-escape mutations (ADAPVEMs) detected in the *pol* and *S* gene.

NRTI	*RT* Resistance mutations	*HBsAg* Vaccine-escape mutations	ADAPVEMs (%)
3TC, Ltd	rtV173L+rtL180M+rtM204V	sE164G/D+sI195M	2 (40%)
3TC, Ltd	rtL180M+rtM204I	sW196L	1 (20%)
3TC, Ltd	rtL180M+M204V	sG145K+ sI195M	1 (20%)

Legend. *Pol* : polymérase of HBV ; *S* : surface ; *RT*: trancriptase inverse; NRTI : Nucleoside(t)idic Reverse Transcriptase Inhibitors ; 3TC : Lamivudine ; LdT : Telbivudine ; M : Méthionine ; V : Valine ; I : Isoleucine ; L : Leucine ; G : Glycine ; K : Lysine ; E : Acide glutamique ; W : tryptophane ; D : Acide aspartique.

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Patient ID	HBV Genotypes	NRTI	viral load (UI/mL)	RM in the *RT*	EM in the *HBsAg*	VEM in the *HBsAg*
PAT_01	E	3TC	109.10^6	M204V+L180M+V173L	-	-
PAT_03	E	TDF+3TC	1740	M204V+L180M	C121Y, W196L	W196L
PAT_04	E	TDF+3TC	5520	-	K141E	-
PAT_06	E	TDF+3TC	141.10^5	M204V+L180M+V173L	T126N, E164G, I195M	E164G+I195M
PAT_07	E	TDF+3TC	276.10^5	M204V+L180M	G145K, I195M	G145K+I195M
PAT_08	E	3TC	852.10^5	M204V+L180M+V173L	E164D, I195M	E164D+I195M
PAT_13	D	TDF+3TC	24	-	P127T	P127T
PAT_17	E	TDF+3TC	5230	-	M133L	-
PAT_18	A	TDF+3TC	105	-	M133L	-
PAT_20	E	TDF+3TC	61	-	G130N	-
PAT_21	E	TDF+3TC	2910	-	L109I, M133L	-

Table 3. Biological characteristics of patients who have developed NRTI resistance and/or *HBsAg* escape mutations

RM: resistance mutations; *RT*: reverse transcriptase; EM: escape mutation; *AgHBs* : HBV surface antigen; VEM: vaccine-escape mutation;

NRTI: Nucleoside(t)idic reverse transcriptase inhibitors; AV: antivirals; 3TC: lamivudine; TDF: tenofovir; CV HBV: Hepatitis B ; M: Methionine; I: Isoleucine; L: Leucine; G: Glycine; N: Asparagine; K: Lysine; T: Threonine; C: Cysteine; Y: Tyrosine; E: Glutamic acid; P: Proline; W: Tryptophan; D: Aspartic acid; V: Valine.

DISCUSSION

In Africa, most studies in HBV resistance in patients co-infected with HBV and HIV in therapy failure are limited only to the detection of antiviral resistance mutations that may appear on reverse transcriptase (*RT*). However, the selection of resistance mutations on *RT* could also have an impact on HBV *HBsAg*, the preferred target of preventive or therapeutic immunization strategies for HBV infection, due to the overlap of the *pol* and *surface* (*S*) genes (Zhao and al., 2016; Wang and al., 2017). To our knowledge, this is one of the first studies on resistance mutations in the regions of the HBV *pol* and *S* genes in HBV-HIV co-infected patients undergoing antiviral treatment in Ivory Coast.

In the present study, analysis of the HBV sequences of co-infected HBV-HIV patients, allowed the detection of the primary resistance mutations rtM204V and rtM204I on the HBV *RT* at respectively frequencies of 13% and 3%. These mutations are capable of inducing a HBV resistance to 3TC and may be responsible for cross-resistance to telbivudine according to the Stanford hivDB algorithm (*http//www.hiv-grade.de/HBV_grade/).* Some authors have shown that these mutations can occur not only in patients treated with NRTIs, but also in patients receiving any treatment (Bottecchia and al., 2016; Zhao and al., 2016; Asan and al., 2018).
In our study, the prevalence of HBV resistance to 3TC in HBV-HIV co-infected patients was 17% (n = 5/30). This prevalence remained relatively high although some authors reported higher prevalence in the West African sub-region. Archampong et al. in a population of HBV-HIV co-infected patients in Ghana, reported a prevalence of 77.8% of 3TC resistance (Archampong and al., 2017). However, studies reported lower 3TC resistance. it was higher than that of Saran and collaborator (9.1%) (Saran and al., 2017). The duration of treatment has a negative impact on 3TC resistance mutation selection. The high prevalence of HBV resistance to 3TC in our study is thought to be due to this factor.

The secondary HBV resistance mutations rtV173L and rtL180M inducing resistance to 3TC and Ltd were observed with frequencies of 10% and 17% respectively. These mutations were observed by Arikan et al with a frequency of 36.7% in chronic HBV carriers (Arikan and al., 2019). Secondary resistance mutations compensate for replication defects caused by primary NRTI resistance mutations and may reduce drug sensitivity by restoring HBV viral replication capacity (Yamani and al., 2017). However, previous studies have shown that the selection of secondary resistance mutations can lead to an increase in HBV drug resistance when they are combined with primary resistance mutations (Lim Y. S., 2017).

Combinations to 3TC resistance mutations such as the triple mutation of resistance rtV173L+rtL180M+rtM204V and duals mutation of resistance rtL180M + rtM204I and rtL180M + rtM204V have been observed in the genome of resistant HBV strains. The triple combination of resistance mutation was the most observed with an estimated prevalence of 60% (3/5). The high prevalence of the 3TC HBV resistance triple mutation observed in patients in treatment failure could be explained by the long duration of treatment and the selection of potential NRTI-induced vaccine-escape mutations on HBV *HBsAg* according to Pal and colleagues (Pal and al., 2015). Similarly, high prevalence of the 3TC triple mutation resistance was noticed in HIV and HBV co-infected patients from others low-income and middle-income countries in central Africa and Asia (Kouanfack and al., 2012; Pal and al., 2015). These studies

pointed out 3TC triple mutation resistance prevalence of 14% and 32.26% in Cameroon and India respectively.
However, our study showed that no HBV resistance was associated with TDF. Indeed, TDF retains effective antiviral activity and a very high genetic barrier. This result corroborated previous findings in the treatment of chronic HBV patients with TDF in Ivory Coast (Anzouan and al., 2016). Thus, this molecule remains a molecule of choice for the therapeutic management of HBV-HIV co-infected patients in Ivory Coast and elsewhere. The resistance of HBV to 3TC observed in some patients on TDF+3TC in the present study could therefore be explained by poor observance to dual active therapy on HBV and HIV.

Analysis of the nucleos(t)id sequences of the HBV *S* gene, has allowed the detection of *HBsAg* escape mutations in some patients. Indeed, the sP127T and sG145K mutations observed respectively in patients PAT_13 and PAT_07 may result in *HBsAg* escape to immune responses and HBV vaccines according to a study on the evaluation of mutations due to overlapping *pol/S* gene in chronic HBV carriers in north of Cyprus (Arikan and al., 2019).

Others mutations sM133L and sG130N, in PAT_18 and PAT_20 patients, that could lead to immune escape of *HBsAg* antibodies and the insufficient detection of *HBsAg* in serum were also observed in our study (Cooreman et al., 2001; Muhlbacher and al., 2008; Avellon and al., 2006; Baclig and al., 2014; Duda and al., 2015). Moreover, the C121Y mutation detected in PAT_3 patients may be the cause of *HBsAg* escape of hepatitis B diagnostic tests.

The frequency of immune escape mutations (IEMs) of *HBsAg* observed in patients co-infected with HBV and HIV was 33% (10/30). The frequency of IEMs in this study is higher than that of Collagrossi and al., who detected at least one immune system-associated escape mutation in 22.1% of treated chronic HBV patients. This high frequency of *HBsAg* IEMs may therefore pose a problem in terms of increased risk of HBV reactivation in HBV and HIV-infected patients in Ivory Coast. This can be a risk factor in the biological management of hepatitis B, as a false diagnosis of *HBsAg* in serum can lead to misdiagnosis of occult hepatitis (Asan and al., 2018).

Others vaccine escape mutation (VEM) in HBV viral polymerase may also lead to mutations in *HBsAg* due to overlapping *pol/S* genes (Zaaijer and al., 2007; Simon and al., 2013). These mutations are induced by NRTIs and may escape HBV vaccines (Sayan and al., 2011; Lacombe and al., 2013). Among them, mutations sE164G, sE164D, sI195M, sW196L were detected on *HBsAg* in some patients with NRTI-resistant HBV strains in the present study. The frequency of HBV *HBsAg* VEM observed in patients co-infected with HBV and HIV was 20% (6/30).
In addition, due to the overlap between genes encoding reverse transcriptase (*RT*) and *HBsAg*, the selection of resistances mutations by NRTIs may lead to spontaneous appearance of mutations in the main hydrophilic region of *HBsAg.* This situation might result in a reduction in the binding affinity of *HBsAg* for neutralizing antibodies and an escape vaccinal of *HBsAg* (Torresi and al., 2002; Colagrossi and al., 2018).

The combinations of escape mutations sG145K+ sI195M and sC121Y+sW196L found respectively in two patients (PAT_03 and PAT_07), could result in both an escape of *HBsAg* as diagnostic test of hepatitis B and escape at anti-VHB vaccine. These results demonstrate that

surveillance for HBV strains capable of escape preventive immunization strategies should be a priority in all patients receiving or not receiving antiviral treatment in Ivory Coast.
Furthermore, NRTI-resistant HBV mutations associated with potential vaccine escape mutations (ADAPVEM) were observed in NRTI-resistant HBVs in the study. Three types of ADAPVEMs were detected in our study, of which the ADAPVEM-1 composed of the HBV triple resistance mutation rtV173L+rtL180M+rtM204V associated with a NRTI-induced vaccine escape double mutation sE164G+sI195M was the most observed. It was found in 43% (3/7) of patients co-infected with HBV and HIV and with HBV DNA > 20,000 IU/mL. In contrast, Sayan et al. detected seven types of ADAPVEMs in patients with chronic HBV. According to these authors, ADAPVEMs have been observed more in immune-tolerant patients (Sayan and al., 2011). These results show that the appearance of ADAPVEMs strains in HBV infected patients could be related to the high viral replication of HBV resistant to NRTIs. Indeed, HBV replication taking place at high rates (estimated at more than 10^{12} virions produced per day) with the accumulation of viral polymerase errors (estimated at 1 error per day and per site every 10^4 bases incorporated) may be the cause of the appearance of several distinct but genetically very close variants. (Yano and al., 2015).

The prevalence of ADAPVEMs strains in HBV-HIV co-infected patients in Côte d'Ivoire was therefore 13% in the present study. The circulation of such strains was described in other region and at different frequencies. The prevalence of ADAPVEMs was estimated at 23% and 10% in cohorts of HBV infected patients in South Africa and Turkey respectively (Lacombe and al., 2013; Sayan and al., 2011). According to these findings, the emergence of ADAPVEMs strains could present a risk to local populations. Above all, in Côte d'Ivoire where there is a real lack of knowledge of viral hepatitis by the population (Enel and al., 2015). Indeed, the ADAPVEMs strains being transmissible (Frederico and al., 2017), may pose a public health problem because of their pathogenic potential and their possibility of transmission to people vaccinated against hepatitis B. This could also pose a risk to hepatitis B vaccination programs. The results of the study showed that NRTIs such as 3TC, commonly used in the treatment of HBV/HIV co-infected patients in our study, were the main molecule associated with the potential emergence of ADAPVEM during treatment. This was confirmed by Sevin et al., who detected ADAPVEM in 15.4% of chronic HBV patients on 3TC (Sevin and al., 2019).

The results of the study showed that surveillance of HBV strains resistant to NRTIs should be a priority in Ivory Coast in order to improve the management of people infected with HBV.

CONCLUSION

This study has provided important data on the mechanism of HBV escape to NRTIs and immune systems in Côte d'Ivoire. ADAPVEMs strains were identified for the first time in HBV-HIV co-infected patients in Ivory Coast, with an estimated prevalence of 13% in our study. Our study is an important reference for health authorities, as it could contribute to the monitoring of the evolution of HBV antiviral resistance and to preventive immunization strategies in the context of global HIV care in Ivory Coast.

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Ethical approval

National Life Sciences and Health Sciences Ethics Committee (CNESVS ; N/Réf : 137-18/MSHP/CNESVS-km).

Conflict of Interest

The authors do not report any financial or personal links with other persons or organizations, which could have a negative impact on the content of this publication and/or claim copyright on this publication.

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