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Prevalence and molecular characterization of hepatitis B virus infection in HIV-infected children in Senegal

Authors

Rayana Maryse Toyé ^{1,2,6*},
Gora Lô^{2,5},
Halimatou Diop-Ndiaye ^{1,2},
Abdoul Magib Cissé ³,
Anna Julienne Selbé Ndiaye ^{1,2},
Khady Kébé-Fall ¹,
Aboubakri Dramé ¹,
Damien Cohen ⁶,
Flor Helene Pujol ⁷,
Souleymane Mboup ²,
Cheikh Saad Boye ¹,
Isabelle Chemin ⁶,
Gabrièle Laborde-Balen ⁴,
Bernard Taverne ⁴,
Coumba Touré-Kane ^{1,2}

Affiliations

¹ Laboratoire de Bactériologie-Virologie (LBV), CHU Aristide Le Dantec, Dakar, Sénégal

² Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation (Iressef), BP 7325, 20000 Diamniadio, Sénégal

³ Service de pédiatrie. Établissement Public de Santé (EPS) de Mbour, UFR Sciences de la santé de Thiès, Sénégal,

⁴ Centre régional de recherche et de formation à la prise en charge clinique de Fann (CRCF), Dakar, Sénégal,

⁵ Centre Médical Inter-Armées (CMIA), Dakar, Sénégal

⁶ Institut national de la santé et de la recherche médicale (Inserm) U1052, CRCL, 151 cours Albert Thomas, 69003 Lyon, France

⁷ Laboratorio de Virología Molecular, CMBC, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas 1020A, Venezuela

* Corresponding author

Abstract

Background and aims

Sub-Saharan Africa (SSA) is the region with the most patients co-infected with the human immunodeficiency virus (HIV) and the hepatitis B virus (HBV) worldwide. However, few studies have focused on SSA children who are at a higher risk of developing a chronic infection than adults. Furthermore, children on first-line antiretroviral therapy (ART) including low genetic barrier drugs may develop both HBV and HIV resistance mutations. The aim of this work was to document HIV-HBV co-infection and to characterize the HBV isolates in children in Senegal.

Methods

This is a retrospective study of 613 children infected with HIV on ART or not. Dried blood spot (DBS) specimens were used to detect hepatitis B surface antigen (HBsAg) with a rapid diagnostic test (RDT). Confirmation of HBsAg status and hepatitis B e antigen (HBeAg) detection was performed on an automated platform using the chemiluminescence assay technology. HBV viral DNA was quantified by real-time polymerase chain reaction (PCR) and the preS1/preS2/HBsAg region was genotyped by nested PCR followed by sequencing using the Sanger technique.

Results

The prevalence of HIV-HBV co-infection was 4.1% (25/613). The median age of co-infected children was 13 years (2 years-16 years) and 40% (10/25) were girls. Almost all 19/20 (95%) were infected with HIV-1 and 79% (19/24) were treated with 3TC-based triple combination ART. The median duration of time on ART was 15 months (3 months-80 months). More than half of the children 53% (9/17) were experiencing HIV virologic failure and 75% (6/8) had at least one HIV-related RAM. Of the six children with resistance, none of the three administered treatments were effective on HIV. Of the 25 co-infected children, 82% (18/22) were HBeAg-positive, while the median HBV viral load (VL) was 6.20 log₁₀ IU/mL (24/25 patients), and 62,5% (10/16) of the children had a persistent HBV viremia. Combination of ART was the only factor associated with HBV viremia persistence. Amplification was successful in 15 out of 16 patients (rate of 94%), and the ensuing phylogenetic analysis revealed that eight strains (53%) belonged to genotype A and seven (47%) to genotype E. HBV-related 3TC RAMs were uncovered in 20% of these patients (3/15). HBsAg escape mutations were found in 20% of the children (3/15).

Conclusions

Our results showed a high level of drug resistance mutations to both HIV and HBV, a significant level of HBsAg escape mutations, HBV DNA persistence and HIV virologic failure in co-infected children in Senegal. The HBV genotypes found were A and E.

Keywords

HIV, HBV, children, Senegal, genotypes, resistance mutations.

Abbreviations

3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AIDS, acquired immunodeficiency syndrome; AZT, azidothymidine; CHB, chronic hepatitis B; CNLS, Conseil National de Lutte contre le Sida; DBS, dried blood spot; EFV, efavirenz; EPI, Expanded Program on Immunization; FTC, emtricitabine; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; Ig, immunoglobulin; K2P, Kimura 2 parameters; LPV/r, lopinavir/ritonavir; MTCT, mother-to-child transmission; NJ, Neighbor Joining; nt, nucleotide; NVP, nevirapine; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PMTCT, prevention of mother-to-child transmission; RAM, resistance-associated mutation; RDT, rapid diagnostic-test; RT, reverse transcription; SSA, Sub-Saharan Africa; TDF, tenofovir disoproxil fumarate; UNAIDS, Joint United Nations Program on HIV/AIDS; VL, viral load; WHO, World Health Organization.

Introduction

Both human immunodeficiency virus (HIV) and hepatitis B virus (HBV) are major health issues worldwide. According to the World Health Organization (WHO), Sub-Saharan Africa (SSA) alone encompasses 27 of the 36 million HIV-infected adults and children worldwide, and approximately 6.1% (70 million) of the African population is infected with HBV [1]. Furthermore, although 7.4% of HIV-infected patients are co-infected with HBV worldwide [1], this figure rises to 71-74% in SSA (the highest worldwide) [2,3]. Co-infection is well-known to aggravate the prognosis of chronic hepatitis B (CHB), and poses a particular challenge in children as the disease progresses rapidly towards chronicity leading to an increased risk, over time, of death by cirrhosis or hepatocellular carcinoma (HCC) [1,4–7]. In addition, children are highly vulnerable to HIV infection and display antiretroviral therapy (ART) failure, especially in resource-limited settings [8]. However, few studies have focused on co-infected SSA children.

According to the data obtained by the Joint United Nations Program on HIV/AIDS (UNAIDS) in 2019, 1.7 million children are infected with HIV worldwide [9]. In addition,

according to the WHO, 5-8% of SSA children harbor HBV [2]. Mother-to-child transmission (MTCT) of the viruses can be antenatal, perinatal or horizontal [5] and is still an important public health issue. In Senegal, according to the Conseil National de Lutte contre le Sida (CNLS), approximately 5,000 children are infected with HIV [10]. Most of these children are on first-line ART including lamivudine (3TC) and/or other low genetic barrier molecules. The reverse transcription (RT) step of HBV is prone to error owing to the lack of proofreading ability of the polymerase [5]. Ten subgroups of HBV have been described (genotypes A to J) with a distinct geographical distribution, three genotypes of which are primarily found in Africa (A, D and E) [5,11,12]. Numerous studies have reported a predominance of genotype E in West Africa [13] and in Senegal [14,15]. Finally, HBV genotype has been shown to play a major role in disease progression and response to treatment [16,17]. The error-prone step also explains the appearance of “quasi-species” in patients and possibly drug resistance variants under nucleoside analog treatment. These mutations reduce the success of existing treatment strategies [11]. Thus, in cases of HIV-HBV pediatric co-infection with treatments including low genetic barrier drugs, these children may develop resistance mutations.

A recent study in children born to HIV-infected mothers in Dakar, capital of Senegal, reported a significant prevalence of HBsAg carriage (3%) [18]. These results prompted us to conduct the present study to document the rate of HIV and HBV co-infection and to characterize the HBV strains in Senegalese children in other regions outside Dakar.

Patients and methods

Study population

This is a retrospective study, ancillary to the EnPRISE study that aimed at evaluating the efficacy of ART in Senegalese children [19]. Samples were collected between March and June 2015. The National Ethics Committee (n° 377/MSAS/DPRS/CNERS) validated this study.

The samples were collected in health centers and regional hospitals in all regions of Senegal, except in Dakar in which a similar study had previously been conducted. Samples were sent to the Laboratoire de Bactériologie-Virologie (LBV) of the University Teaching Hospital A. Le Dantec where the EnPRISE study analyzes were performed. They were then transferred to the Centre Médical Inter-Armées laboratory and then to the molecular biology platform of the Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formations (Iressef) for the purpose of the study.

The study population consisted of dried blood spot (DBS) specimens of 613 HIV-infected children (out of the 666 analyzed in EnPRISE), followed at 72 pediatric management sites,

treated or not with ART and included in the EnPRISE study. Most of these children were administered triple combination therapy mainly AZT/3TC/NVP.

HBV serology

For each sample, the three remaining blood spots were eluted for at least two hours in 600 microliters (μL) of phosphate buffered saline (PBS) and the eluates were used for assays. All analyzes were performed according to the manufacturers' instructions.

Immuno-chromatographic detection of HBsAg was performed using the Determine TM HBsAg RDT (Alere TM Inc., Waltham Massachusetts, USA). The Architect® i1000SR platform (Abbott Diagnostics, North Ryde, NSW, 2113 Australia) was used to confirm HBsAg status and to detect HBeAg in co-infected individuals. This technique uses chemiluminescent microparticle assay technology. HBV VL was determined in co-infected patients by real-time PCR using the Amplicx® automated platform (Biosynex, 67038 Strasbourg, France). The detection threshold of the device is 26 copies/mL.

HBV DNA extraction, amplification and sequencing

HBV viral DNA was extracted from 200 μL eluted DBS from the co-infected patients with a VL greater than three \log_{10} IU/mL using the NucliSENS easyMAG commercial kit (bioMérieux, Inc., 100 Rodolphe Street, Durham NC 27712 USA). This is an automated magnetic extraction technique based on the BOOM technology, which uses silica particles. The final elution volume was 50 μL and these extracts served as a template for the PCR.

The HBV S gene was amplified using five μL of the extracted DNA with the HBPr1 and HBPr135 primers (outer PCR) followed by a nested PCR using HBPr2 and HBPr94 to generate a fragment of about 1,197 nucleotides (nt). DNA in the 45 μL reaction mix was amplified over 40 cycles with denaturing step at 94°C for 30s, annealing at 50°C for 30s and elongation at 72°C for 30s [12]. The second round PCR products were enzymatically purified using ExoSAP-ITTM PCR Product Cleanup (Affymetrix Inc., 3450 Central Expressway, Santa Clara, CA 95051 USA) and sequenced.

The amplicons were sequenced by the Sanger method on an automated DNA sequencer ABI 3130 (Applied Biosystems, 50 Lincoln Centre Drive Foster City, CA 94404 USA) using five primers (HBPr2, HBPr3, HBPr14, HBPr94 and HBPr134) covering the preS1/preS2/HBsAg region [12].

Data analysis

The resulting sequences were assembled and edited with the SeqMan TM software (DNASTAR, Inc., USA); the consensus sequences obtained were then submitted to the HBVseq (<https://hivdb.stanford.edu/HBV/HBVseq/development/HBVseq.html>) and HBVdb

(<https://hbvdb.ibcp.fr/HBVdb/>) databases for genotype determination and to search for possible mutations. The Seaview version 4.5.4 platform enabled sequence alignment using the muscle software, estimation of genetic distance using the Kimura 2 parameters (K2P) method and construction of the phylogenetic tree using the Neighbor Joining (NJ) method (1000 bootstrap replicas). Reference sequences from the different genotypes and sub-genotypes (only for genotype A) were included in the analysis, as well as closely related sequences obtained by BLAST analysis. The sequences were also submitted to the Genafor (<https://hbv.geno2pheno.org/>) database to search for HBsAg escape mutations. Associations between the variables were tested using a Fisher's exact test. A p-value greater than or equal to 0.05 was considered statistically significant.

Results

A total of 613 DBS from HIV-infected children were analyzed. Twenty-five were positive for HBsAg, representing a global prevalence of 4.1%.

Characteristics of the co-infected children

The characteristics of the 25 co-infected children are shown in **Table I**.

The median age of co-infected children was 13 years (2 years-16 years) and 75% (15/20) were aged ten years or more at the time of the study, thus born before 2005 when HBV vaccine was not implemented in the Expanded Program on Immunization (EPI) in Senegal. Ten children (40%) were girls. Almost all of the patients 96% (24/25) were infected with HIV-1 and were treated with ART combination, mainly 3TC-based (79%) or TDF-based (21%) therapies, except for one untreated child. The median duration of time on ART was 15 months (3 months-80 months) and 71% (17/24) were treated for at least six months.

The physicians estimated that 83% (20/24) of the children had a regular follow-up and 71% (15/21) a good adherence. There was a significant correlation between the regularity of follow-up and adherence to ART ($p < 0.01$). Among the 22 children with available information, only one child had received prevention of mother-to-child transmission (PMTCT) care.

HIV virology and resistance data (obtained from the EnPRISE study results)

HIV RNA results showed that only 47% (8/17) of children treated for at least six months had an undetectable VL. There was no correlation between VL and duration on ART ($p = 1$). The median HIV VL was 3.2 log₁₀ IU/mL (2.2 – 4.8). More than half of the children 53% (9/17) were experiencing virologic failure (VL > 3 log₁₀ IU/mL).

Among the eight children for whom HIV genotyping was done, 75% (6/8) had at least one RAM. The HIV-related 3TC/FTC, AZT, NVP, TDF and EFV were found in 100% (6/6), 83% (5/6), 83% (5/6), 50% (3/6) (though only one had taken it) and 17% (1/6) of the co-infected children with RAMs. Of the six children with resistance, none of the three administered treatments were effective. The 3TC/FTC RAM found was M184V. All of the patients with HIV-related RAMs had received 3TC-based therapy, except one who was receiving TDF/FTC/EFV. There was no correlation between HIV RAM and combination of ART ($p = 0.46$). All six children had a VL greater than three \log_{10} IU/mL. No correlation was found between HIV RAM and HIV VL ($p = 1$). They were all on ART for at least six months. No correlation between HIV RAM and duration on ART ($p = 1$). In terms of genetic diversity, CRF02_AG was the predominant form 88% (7/8).

HBeAg prevalence and HBV viral load

The HBeAg marker was successfully tested in 22 patients, leading to a prevalence of 82% (18/22) (Table I).

HBV DNA results showed that only 29% (5/17) of children treated for at least six months had an undetectable VL, three of which were under TDF-based therapy. The median HBV VL in 24 co-infected patients was 6.20 \log_{10} IU/mL, with a VL greater than six \log_{10} IU/mL in 50% (12/24) of children and an undetectable VL in 25% (6/24) of children (Table I). The VL was greater than three \log_{10} IU/mL in 16 of the children. However, VL was undetectable in six children despite them being positive for HBsAg and HBeAg in three cases.

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236
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Table I. Characteristics of the 25 co-infected children

Characteristic	Number	Percentage (%)
Sex		
F	10/25	40
M	15/25	60
Sex ratio	1.5	
Age groups (years)		
[2 – 10[5/20	25
[10 – 16]	15/20 ¹	75
Median age (min-max)	13 (2-16)	
Average age ± SD	10.8 ± 4.4	
Regularity of follow up		
Regular	20/24	83
Not regular	4/24	17
Adherence to ART		
Good	15/21	71
Average	2/21	10
Bad	4/21	19
Access to PMTCT		
Yes	1/22	5
No	21/22	95
HBeAg-positive	18/22 ¹	82
HBV VL (log₁₀ IU/mL)		
<1.31	6/24	25
[1.31 – 3]	2/24	8
[4 – 6]	4/24	17
>6	12/24 ²	50
Combination of ART		
AZT+3TC+NVP	16/24	67
AZT+3TC+EFV	1/24	4
AZT+3TC+LPV/r	1/24	4
ABC+3TC+NVP	1/24	4
TDF+3TC+EFV	2/24	8
TDF+3TC+LPV/r	1/24	4
TDF+FTC+EFV	2/24	8
PCR positive	15/16	94
HBV genotype		
A	8/15	53
E	7/15	47
HIV profile		
HIV-1	19/20	95
HIV-2	1/20	5
HIV VL (log₁₀ IU/mL)		
<3	12/25	48
>3	13/25	52
HIV genotypes performed	8/13	62
RAMs		
HBV-related	3/15	20
HIV-related	6/8	75
HBsAg escape mutation	3/15	20

¹Three co-infected children could not be tested for HBeAg for technical reasons.

²One co-infected child could not be tested for VL for technical reasons.

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241 **HBV genotype distribution**

242 Among the 25 co-infected children, 16 were eligible for genotyping as their VL was greater
243 than three log₁₀ IU/mL, 15 of which were successfully amplified and sequenced,

corresponding to an amplification rate of 94%. The characteristics of those 16 children are shown in **Table II**.

The molecular analysis of HBV sequences found in the study showed that eight (53%) were genotype A and seven (47%) genotype E. Two sequences, one genotype A and one genotype E, were excluded from the phylogenetic analysis because they were too short, 860 nt and 732 nt respectively. The 13 remaining sequences were trimmed to have the same size for alignment purposes. The final sequences were 1108 nt long for genotype A and 1105 nt for genotype E. Partial preS1/preS2/S gene phylogenetic analysis of the 13 Senegalese HBV sequences with the references from other countries is shown in **Figure 1**. All Senegalese HBV A sequences were closely related to the Cuban HBV A4 isolate. HBV sequence data generated in this study have been deposited into the GenBank database and were attributed accession numbers MT010581-MT010595.

Resistance-associated mutations (RAMs)

The submission of the sequences to the databases showed that three samples had at least one HBV-related 3TC RAM, with an overall resistance rate of 20% (3/15). The RAMs found were L180M, M204V/I and S202N. Two mutations occurred in genotype A and one in genotype E. There was no correlation between HBV RAM and HBV genotype ($p = 1$). No HBV-related TDF RAM was found. All of the patients with RAMs had received 3TC-based therapy and had an HBV VL of at least five \log_{10} IU/mL. No correlation was found between HBV RAM and HBV VL ($p = 1$).

HBsAg escape mutations were detected in three patients, with a prevalence of 20% (3/15) in the co-infected children. The mutations found were 120P/120T, 129H and 133L. They were all vaccine and therapy escape mutations. Two occurred in genotype A and one in genotype E. The patients with HBsAg escape mutants were all treated with ART combination AZT/3TC/NVP and had an HBV VL greater than six \log_{10} IU/mL.

The higher HBV VL (7.8 \log_{10} IU/mL) was found in a male patient aged 14, under triple combination ART including AZT+3TC+NVP for 44 months, with a low-intermediate HIV VL (3.2 \log_{10} IU/mL); HBeAg-positive and genotype A, presenting resistance mutation to both HIV and HBV.

Factors associated with HBV persistence

Among the 16 children with an HBV VL greater than three \log_{10} IU/mL, ten (62,5%) were treated for at least six months. The only factor associated with persistent HBV viremia was the combination of ART ($p = 0.047$).

277 **Table II.** Detailed characteristics of the 16 co-infected children tested for molecular analysis

Patient	Sex	Age	DBS sampling date	Regularity of follow up	Adherence to ART	Access to PMTCT	Combination of ART	3TC based ART	TDF based ART	Duration of ART (months)	HIV profile	HIV RNA (IU/mL)	HBV DNA (IU/mL)	HBeAg	HBV PCR	HBV genotype	HIV related RAM	HBV related RAM	HBsAg escape mutation
1	M	14	19/03/2015	Yes	Good	ND	AZT+3TC+NVP	Yes	No	44	HIV-1	1.50 x 10 ³	7.00 x 10 ⁷	Pos	Pos	A	Yes	Yes	No
2	M	13	19/03/2015	No	Bad	No	AZT+3TC+NVP	Yes	No	29	HIV-1	2.70 x 10 ⁴	2.00 x 10 ⁷	Pos	Pos	A	No	No	No
3	M	12	24/03/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	35	HIV-1	< 800	1.66 x 10 ⁵	NT	Pos	A	NT	Yes	Yes
4	M	4.5	19/03/2015	ND	Good	No	AZT+3TC+NVP	Yes	No	18	HIV-1	< 800	1.10 x 10 ⁶	Pos	Pos	A	NT	No	Yes
5	M	ND	25/03/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	19	HIV-1	< 800	5.80 x 10 ⁶	Neg	Pos	E	NT	No	No
6	F	16	25/03/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	80	HIV-1	2.40 x 10 ⁴	3.90 x 10 ⁷	Pos	Pos	A	Yes	No	No
7	F	14	21/04/2015	Yes	ND	No	TDF+3TC+EFV	Yes	Yes	14	HIV-1	<800	1.89 x 10 ⁴	Pos	Pos	A	NT	No	No
8	F	11	21/04/2015	Yes	ND	No	AZT+3TC+NVP	Yes	No	13	HIV-1	1.20 x 10 ⁴	1.50 x 10 ⁶	Pos	Pos	A	Yes	No	No
9	F	10	21/04/2015	Yes	Average	No	AZT+3TC+NVP	Yes	No	5	ND	< 800	2.50 x 10 ⁶	Pos	Pos	E	NT	No	No
10	M	5	21/04/2015	No	Bad	No	AZT+3TC+NVP	Yes	No	3	HIV-1	< 800	1.30 x 10 ⁶	Pos	Pos	E	NT	Yes	No
11	M	2.4	21/04/2015	No	Bad	No	AZT+3TC+LPV/r	Yes	No	3	HIV-1	3.30 x 10 ³	6.00 x 10 ⁷	Pos	Pos	E	NT	No	No
12	M	ND	08/06/2015	Yes	Good	Yes	TDF+3TC+LPV/r	Yes	Yes	5	HIV-2	5.80 x 10 ³	5.60 x 10 ⁶	Pos	Pos	A	NT	No	No
13	F	16	10/06/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	17	HIV-1	< 800	1.90 x 10 ⁵	Pos	Pos	E	NT	No	No
14	M	10	10/06/2015	Yes	Good	No	AZT+3TC+NVP	Yes	No	4	ND	< 800	3.50 x 10 ⁶	Pos	Pos	E	NT	No	Yes
15	M	13	10/06/2015	Yes	Average	No	AZT+3TC+NVP	Yes	No	40	HIV-1	4.30 x 10 ⁴	1.29 x 10 ⁴	Pos	Neg	NT	Yes	NT	NT
16	M	13	10/06/2015	No	NA	No	NA	NA	NA	NA	HIV-1	6.90 x 10 ⁴	1.60 x 10 ⁵	Pos	Pos	E	NT	No	No

278 F, female; M, male; NA, not applicable; neg, negative; ND, not documented; NT, not tested; pos, positive.

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Discussion

The aim of this study was to determine the rate of HIV-HBV co-infection and to characterize the associated HBV isolates in children in Senegal.

In our study, the overall prevalence of HBsAg was 4.1% (25/613). These results are greater than those of preliminary studies in Senegalese children recruited in three of the main children's hospitals [20] and in HIV infected children in Tanzania that documented a HBsAg prevalence of 1.1% (3/295) and 1.2% (2/167) [21], respectively. This discrepancy may be due to the fact that the majority of children in the latter Senegalese study were vaccinated against HBV. However, the vaccine is only administered to children older than six weeks, leaving a gap for potential MTCT, which would explain the positive cases observed despite the vaccine. A dose administered at birth, as recommended by the WHO [1], could lead to an even greater reduction in this prevalence, if not an elimination. Regarding the Tanzanian study, the observed difference may be due to the lower prevalence of HBV of 6% in the general population [22] compared to the prevalence of 11% in Senegal [20].

Although HBV vaccine was implemented at around the same time in these countries, a higher HBsAg prevalence than in our study was found in HIV-infected children in Ivory Coast [23], Nigeria [24], [25] and Zambia [26], 12.1% (34/280), 7.8% (31/395), 7.7% (12/155) and 10.5% (43/411), respectively.

In terms of HIV results, more than half of the children 53% (9/17) were experiencing virologic failure. These results are consistent with those obtained in HIV-infected children in Senegal 56% (70/125) [8]. Moreover, 75% (6/8) had at least one RAM. In the latter Senegalese study, a higher rate of 92% (48/52) was found.

In addition, the immunization status of children in our cohort was unknown, while in most studies, children had received at least one dose or all three doses of the HBV vaccine [20,27]. However, in our study, the majority of HBsAg-positive children 75% (15/20) were aged ten years or more, thus born before 2005 when HBV vaccine was not implemented in the EPI in Senegal. We can therefore assume that they were not immune. In this context, it would be urgent to organize HBV screening campaigns in people born before the implementation of the vaccine for preventive or therapeutic management if necessary.

Regarding mothers, their HBsAg status or any other HBV marker was not documented in our study; we have thus not been able to establish whether this is MTCT of the infection. However, our results suggest HIV-HBV co-infection of mothers. A previous study showed that all children infected with HBV were born to mothers carrying HBeAg [27], which was

corroborated by Senegalese [18] and South African [28] studies in which 89% (25/28) and 11% of mothers of these children were HBsAg-positive. In addition, in Senegal, a previous study estimated a prevalence of HBsAg carriage in pregnant women around 11% [29]. Moreover, only 5% (1/22) of the children had access to PMTCT in our study. These data suggest a vertical transmission of the infection.

In our study, the prevalence of HBeAg was 82% (18/22). Similar results were obtained in HIV-infected children in the Ivory Coast, 82% (28/34) [23].

Moreover, 53% (8/15) of strains tested were genotype A and 47% (7/15) genotype E. These results are similar to those found in adult patients in Senegal [30], displaying 55% (17/31) of genotype A and 42% (13/31) of E. However, several studies have reported different genotype E ratios of 70.1% (47/67), 70% (19/27), 75% (47/63), 72% (23/32) and 92.1% (58/63), in Australia (in children from SSA) [31], in Senegal in adult patients [32], [15], [14] and in Ghana [6], respectively. Some studies demonstrated that patients with genotype A tended to be younger [14]. This could explain the higher rate of genotype A in our young population than in the adult population of the same region. Moreover, a recent study in The Gambia showed that genotype A is a significant risk factor for fibrosis [17] often associated with the development of HCC. In addition, all Senegalese HBV A sequences were closely related to the Cuban HBV A4 isolate. The A4 isolate from the Cuban study was related to an isolate from The Gambia, suggesting a probable African origin [33].

HBsAg vaccine and therapy (immunoglobulin (Ig)) escape mutations including 120P/120T, 129H and 133L were detected in three patients, with a prevalence of 20% (3/15) in the co-infected children. A study in Ghana reported a lower vaccine escape mutation rate of 11% (7/63) and an immunoprophylaxis escape mutation rate of 100% [6].

In our study, the HBV drug resistance mutation rate was 20% (3/15), while a higher rate of 33.3% (21/63) was found in Ghana [6] and 75% (9/12) in Thailand [34]. These results could be due to the fact that in this latter case, adolescents were on 3TC monotherapy. Furthermore, 67% (2/3) of children with at least one 3TC resistance mutation were genotype A and 33% (1/3) genotype E, whereas different results were reported in adult patients in Ghana with a ratio of 95.2% (20/21) of genotype E [6].

The HBV-related 3TC RAMs found in the present study were L180M, M204V/I and S202N, corroborating former studies that found the same mutations [6]. Moreover, as in our own study, none of the patients receiving TDF displayed HBV-related RAMs. Our present study thus suggests that the use of 3TC as the only HBV-active molecule in HIV-HBV patients is an

ineffective treatment plan. In addition, our results showed that the only factor associated with persistent HBV viremia was the combination of ART.

The most recent treatment guidelines recommend the use of TDF in treatment regimens for both HIV and HBV carriers and HIV-HBV co-infected patients [1] and this may be most useful in the SSA context and easily implementable.

Conclusion

Our study shows that pediatric HIV-HBV co-infection remains a significant health issue. Better management strategies for HIV-infected children are necessary, including regular monitoring for the early diagnosis of treatment failure, systematic detection of co-infections and setting-up of appropriate therapeutic combinations (high genetic barrier ART when available) to prevent the selection of resistance mutations in this sensitive population. These measures are urgently needed to achieve both the WHO and UNAIDS targets for the global elimination of viral hepatitis and acquired immunodeficiency syndrome (AIDS) epidemic by 2030 [1,35].

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Authors' contribution

HDN, CTK and GL: conceptualization and supervision of the study; KKF, AD, AMC, GLB and BT: providing samples and data from patients; GL, AJN and RMT: performing experiments; RMT, IC, FHP and DC: phylogenetic analysis; RMT: drafting of the manuscript. RMT, IC, FHP, CTK, HDN, SM and CSB: critical reading of the manuscript; All co-authors approved the final version of the paper.

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