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Original article

Epidemiological aspects and molecular characterization of the hepatitis B virus among blood donors in Lubumbashi, Democratic Republic of Congo

Aspects épidémiologiques et caractérisation moléculaire du virus de l'hépatite B chez les donneurs de sang à Lubumbashi, République démocratique du Congo

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ABSTRACT

Objectives. – The strains of HBV circulating among blood donors in Lubumbashi, Democratic Republic of Congo (DRC), are not yet characterized. The purpose of this study was to determine seroprevalence, changes in biochemical parameters during HBV infection and molecular characterization of HBV in blood donors in Lubumbashi.
Methods. – The detection of HBsAg was carried out by rapid diagnostic test then confirmed by the Liaison XL® Quant HBsAg technique. PCR targeting the *P* gene was carried out on LightCycler® 96 and genotyping by the sequencing technique on ABI 3500.
Results. – The seroprevalence was 7.9%. The genotypes E (53.1%), A (41.8%), A3/E (3.8%), A1/E (1.3%) and some drug resistance mutations were identified. Disturbances of HDL-cholesterol, direct bilirubin, transaminases (ASAT and ALAT), PAL, GGT and albumin have been observed in HBsAg positive blood donors.
Conclusion. – The results of our study indicated that Lubumbashi is in a region with high endemicity for HBV and report for the first time HBV of genotypes A, E, A1/E and A3/E. They highlight the need to implement strategies to improve transfusion safety in blood transfusion centers and hospital blood banks in Lubumbashi in order to reduce HBV infection in recipients. They could also contribute to the implementation of treatment strategies and the development of mapping of circulating HBV genotypes in the DRC.

Mots clés :
Séroprévalence
Génotypes
VHB
Résistance
Donneur de sang

RÉSUMÉ

Objectifs. – Les souches du virus de l'hépatite B (VHB), circulant parmi les donneurs de sang à Lubumbashi, en République démocratique du Congo (RDC), ne sont pas encore caractérisées. Le but de cette étude était de déterminer la séroprévalence et les changements des paramètres biochimiques au cours de l'infection par le VHB et la caractérisation moléculaire du VHB chez les donneurs de sang à Lubumbashi.
Méthodes. – La détection de l'AgHBs a été réalisée par le test de diagnostic rapide, puis confirmée par la technique Liaison XL® Quant HBsAg. La PCR ciblant le gène *P* a été réalisée sur LightCycler® 96 et le génotypage par la technique de séquençage sur ABI 3500.

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Résultats. – La séroprévalence était de 7,9 %. Les génotypes E (53,1 %), A (41,8 %), A3/E (3,8 %), A1/E (1,3 %) et certaines mutations de résistance aux médicaments ont été identifiées. Des perturbations du cholestérol HDL, de la bilirubine directe, des transaminases (ASAT et ALAT), de la PAL, de la GGT et de l'albumine ont été observées chez des donneurs de sang AgHBs positif.
Conclusion. – Les résultats de notre étude avaient indiqué que Lubumbashi se trouve dans une région de forte endémicité pour le VHB et rapportent, pour la première fois, le VHB des génotypes A, E, A1/E et A3/E. Ils soulignent la nécessité de mettre en œuvre des stratégies pour améliorer la sécurité transfusionnelle dans les centres de transfusion sanguine et les banques de sang des hôpitaux de Lubumbashi, afin de réduire l'infection par le VHB chez les receveurs. Ils pourraient également contribuer à la mise en œuvre de stratégies de traitement au développement de la cartographie des génotypes circulants en RDC.

1. Introduction

Hepatitis B virus (HBV) infection is a public health problem worldwide. According to WHO estimates, around 350 million people worldwide are chronic carriers of the virus [1,2] which is annually responsible for 600,000 deaths [3]. It would be the tenth cause of death and the third cause of cancer in the world [4,5]. HBV is a DNA virus belonging to the family *Hepadnaviridae* and the genus *Orthohepadnaviridae*. HBV is subdivided into 10 genotypes (A to J) and around forty sub-genotypes, which have different biological properties and a different geographic distribution. It has been reported that the HBV genotype might influence the course of the disease, the response to antiviral treatment and possibly vaccination against the virus [5,6].

Blood transfusions save lives and improve health. However, they present significant risks of both immunological and allergic reactions and also risks of transmission of infectious agents such as HBV, le HCV, le VIH, syphilis and malaria.

In sub-Saharan Africa, despite transfusion safety measures, 12.5% of transfused patients are at risk of post-transfusional hepatitis [7] and the seroprevalence of HBV among blood donors in the DRC is estimated at 5% [8].

Transmission of viral hepatitis B by transfusion is well documented and this remains a major public health concern in low-income countries like the DRC, because of inadequacies in national transfusion programs, including scarce financial resources and inadequate infrastructure equipment and reliable detection methods for an efficient laboratory screening.

With respect to the transfusion risk, the selection of blood donors and the biological qualification of blood donations by reliable biological tests before any transfusion are not systematically performed in the various hospital centers of the DRC [9]. The majority of blood donations are of the replacement type (family or paid donors), however, different studies indicate a high prevalence of viral markers in this category of blood donors [6,10].

On the other hand, in hospitals of the DRC where these biological tests are carried out before any transfusion, the residual transfusion risk of hepatitis B remains important because of several factors including the human technical errors, the presence of a variant not recognized by certain reagents, a false seronegative infectious donation in a chronic carrier or a donation made by a subject very recently infected (serological window). Moreover, to date, in the provincial blood transfusion center of Lubumbashi and more particularly in the blood banks of *Hôpital* Jason Sendwe and *Cliniques universitaires* de Lubumbashi, only whole blood is delivered to patients, and technologies for inactivating infectious agents on whole blood are not achievable in Lubumbashi. Leukoreduction, however, of great interest in reducing infectious agents such as cytomegalovirus and parvovirus is not carried out in Lubumbashi.

Therefore, in DRC, and particularly in Lubumbashi, the safe transfusion is a major challenge [2]. The WHO has set a goal of eliminating viral hepatitis B and C by 2030 [3]. Thus, for regions like sub-Saharan Africa including the DRC, this challenge requires among other things a perfect understanding of local epidemiology, risk factors and transmission mechanisms.

Very little work has been carried out so far on the epidemiological profile of HBV among blood donors in Lubumbashi [9,11,12]. Furthermore, according our knowledge, the mapping of circulating HBV genotypes among blood donors has not been studied to date in Lubumbashi. The objectives of this study were: (i) to obtain updated data on the prevalence of viral hepatitis B among blood donors in Lubumbashi; (ii) to determine the biochemical profile of blood donors infected with HBV; and (iii) to study the distribution of HBV genotypes and drug resistance mutations among blood donors in Lubumbashi.

2. Materials and methods

2.1. Site, type and study period

This was a cross-sectional descriptive study of the prevalence of HBsAg in blood donors carried out from November 2017 to December 2019 in 2 referral hospitals, the *Hôpital* Jason Sendwe and the *Cliniques universitaires* de Lubumbashi.

2.2. Population and parameters studied

Our study included all blood donors in apparent good health, both genders, aged from 18 to 65, with a hemoglobin level $\geq$ 12 g/dL. Individuals with a history of blood transfusion, pregnant women, women during menstruation, women during breastfeeding and paid donors were excluded from our study.

The parameters studied were socio-demographic characteristics recorded in a pre-established epidemiological survey form, biochemical parameters and molecular characterization of HBV.

The minimum sample size was determined using the single population proportion formula, based on the previous prevalence of HBsAg positive blood donors in Lubumbashi (DRC) estimated at 6.8% [10]. Accordingly, the minimum calculated sample size was 311. However, the effective sample size used in the frame of this study was 1512, as we recruited 1512 blood donors during the period of the study.

2.3. Screening for viral hepatitis B

The detection of HBsAg was first carried out using "One Step Hepatitis B surface Antigen Test® Strip (Accurate, China)" as a rapid diagnostic test (RDT). Then, the HBsAg positive samples were confirmed by the Liaison XL®Quant HBsAg assay (Diasorin, Italy) at the Microbiology Laboratory of *Institut de recherche expérimentale et clinique* (IREC) of *Université Catholique* de Louvain (UCLouvain) in

Table 1
qPCR primers and probes.

Oligonucleotides	Sequence (5' -> 3')	Tm [°C]
Primers		
HBVq-150 (Primer 1)	GGACCCCTGCTCGTGTTACA (20)	61.4
HBVq-151 (Primer 2)	GAGAGAAGTCCACCMCGAGTCTAGA (25)	65.4
Probes		
HBV-G243 (Probe 1)	TGTTGACAARAATCCTCACAATACCRCAGA (30)	64.0
HBV-G244 (Probe 2)	CGTTGACAARAATCCTCACAATACCRCAGA (30)	65.4

Table 2
Sequences of the sequencing primers.

Sequences (5' -> 3')		Tm [°C]
HBV-S1	5′ GγT GGA TGT GTC TGC GGC GT 3' (20 pb)	62.4
HBV-AS2	5′ CCA GTG GGG GTT GCR TCA GC 3' (20 pb)	64.5
HBV-I	5′ TGT ATT CCC ATC CCA TCA TC 3' (20 bp)	55.3
HBV-S3	5′CAT CCT GCT GCT ATG CCT CA 3' (20 bp)	59.4
HBV-AS4	5′RGC AAC GGG GTA AAG GTT 3' (18 bp)	54.8

Brussels, Belgium. Only positive samples both on RDT and on the Liaison XL assay were included in this study.

2.4. Biochemical evaluation

The biochemical parameters were evaluated using commercial kits by the spectrophotometric method on the Cyan Smart automated analyzer (Cypress Diagnostics, Belgium). These parameters included total cholesterol (T-CL), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), alanine-aminotransferase (ALAT), aspartate-aminotransferase (ASAT), alkaline phosphatase (ALP), gamma glutamyl-transferase (γ-GT), lactate dehydrogenase (LDH), total bilirubin (T-B), direct bilirubin (D-B), indirect bilirubin (I-B), albumin (ALB) and C reactive protein (CRP).

2.5. Molecular characterization of viral hepatitis B

2.5.1. HBV viral load determination

Total nucleic acids were extracted from 200 μL of serum using the Nucelospin Blood kit (Macherey-Nagel, Germany) according to the manufacturer's instructions, eluted in 100 μL of elution buffer and stored at −80 °C until use.

The viral load of HBV was quantified by real-time PCR (qPCR) on the Lightcycler® 96 instrument (Roche diagnostics, Germany).

The probes were labeled with Fam and the amplification protocol was as follows: a 10 minutes denaturation step at 95 °C followed by 45 cycles consisting of 10 seconds at 95 °C (denaturation), 20 seconds at 58.4 °C (annealing and extension) and 1 second at 37 °C (cooling step). The quantification curve has been calibrated against the WHO standard and results were reported in IU/mL. The range of quantification was 10^2 IU/mL to 10^8 IU/mL. We used the following pairs of primers and probes (Table 1).

2.5.2. HBV sequencing

The extracted HBV DNA was amplified to obtain amplicons of 838 base pairs (bp) from the overlap region of the *P* gene, coding for the polymerase and of the *S* gene coding for the HBV surface proteins. The primers used for the PCR and the sequencing are listed in Table 2. Three primers and BigDye Terminator v3.1 cycle sequencing Ready Reaction kit were used to prepare sequencing reaction mixtures according to the manufacturer's recommendations (Life Technologies, Carlsbad, CA). The amplified DNA was purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), confirmed on 1% agarose gel and engaged in the sequencing reaction. Thereafter, sequencing reaction was carried out on thermocycler as follows: 1 minute denaturation step at 96 °C followed by 30 cycles consisting of 10 seconds at 96 °C (denaturation), 5 seconds at 50 °C (annealing) and 4 minutes at 60 °C (extension), a cooling step at 10 °C was added until the removal of reaction tubes. The nucleotide sequence was obtained by capillary electrophoresis on ABI PRISM 3500 DNA analyzer (Applied Biosystems, CA, USA). This analysis allowed to determine simultaneously genotyping and drug resistance mutation with a detection limit for HBV DNA of 1000 IU/mL.

2.5.3. Analysis of HBV genotyping and drug resistance

The obtained sequences were aligned using the software "Geneious version 11.1 (Biomatters, Auckland, New Zealand). We identified the genotype by performing a "Nucleotide Blast" of the consensus sequence using National Center for Biotechnology Information (NCBI) software. HBV drug resistance mutations and genotypes were identified by the online HBVseq (Stanford University, UK) and geno2pheno (Max Planck Institut Informatic, Germany) softwares.

The phylogenetic analysis of HBV genotype was determined by the alignment of the sequences with respect to the reference HBV sequences recovered from GenBank (NCBI). The phylogenetic tree was built by the neighbor-joining method on Geneious software according to the Tamoura–Nei model.

2.6. Data processing and analysis

The collected data were encoded, processed and analyzed using Microsoft Excel 2010 software and EPI INFO™ version 7.2.4.0 (USA). The mean and standard deviation were calculated. The Chi^2 test or Fischer's Exact test when necessary were used to find the association between the sociodemographic characteristics and the positivity of the HBsAg, and of the other parameters analyzed. The differences were considered statistically significant for $P < 0.05$.

2.7. Ethical approval

Free and informed consent was obtained from each blood donor. This study had obtained the approval of the medical ethics committee of the University of Lubumbashi, under the number: UNILU/CEM/095/2017.

3. Results

3.1. Sociodemographic characteristics and HBsAg seroprevalence

During the study period, 1512 blood donors were included and the sociodemographic characteristics are listed in Table 3.

The seroprevalence rate of HBsAg in blood donors in this study was 7.9% (120 HBsAg positive blood donors). Table 4 presents the correlations between the seroprevalence of HBsAg and the sociodemographic characteristics of blood donors.

Table 3
Sociodemographic characteristics of blood donors.

Variable	Total number (n = 1512)	Percentage (%)
Gender		
Male	1081	71.5
Female	431	28.5
Age (years)		
[18–29]	389	25.7
[30–44]	675	44.6
[45–65]	448	29.6
Marital status		
Married	721	47.7
Single	764	50.5
Divorced	27	1.8
Occupation		
Liberal profession	545	36
Government employees	528	34.9
Students	314	20.8
Unemployed	125	8.3
Categories of donors		
Unpaid and volunteers		
Regulars	108	7.1
Irregulars	286	18.9
Family	1118	74.0

3.2. Modifications of biochemical parameters in HBsAg positive blood donors

The biochemical parameters were evaluated in HBsAg positive blood donors and in healthy blood donors (control group). The results of these analyses are shown in Table 5.

3.3. Molecular characterisation

Out of 120 HBsAg positive samples, 79 (65.8%) with a viral load greater than 1000 IU/mL were eligible for HBV genotyping. The mean HBV viral load in HBsAg positive blood donors was $4.6 \pm 3.3 \log_{10}$ IU/mL. Of the 79 analysed samples, the distribution of identified genotypes was as follows: 42 genotype E (53.1%), 33 genotype A (41.8%), 3 recombinant A3/E genotypes (3.8%) and 1 recombinant A1/E genotype (1.3%). The majority of HBV strains of genotype A were related to the A1 sub-genotype (85%) and only 4 strains (12%) and one strain (3%) were related to the A2 and A8 sub-genotypes respectively (Table 6). The phylogenetic analysis showed that the majority of identified HBV subgenotype A1 strains (n = 21) formed a subgroup close to the South Africa Republic strains whereas other 5 HBV subgenotype A1 were close to the strains from Rwanda. The 6 remaining HBV strains (subgenotype A2) were close to the subgenotype A2 from Morocco. With respect

Table 4
Seroprevalence of HBsAg in blood donors according to their socio-demographic characteristics.

Variable	n = 1512	AgHBs +		
		N (%)	OR (IC95%)	P
Gender				
Male	1081	91 (8.4%)	2.05 (0.81–3.55)	0.19
Female	431	29 (6.7%)	0.96 (0.50–1.66)	
Age (years)				
[18–29]	389	20 (5.1%)	0.0 (0.0–13.2)	0.11
[30–44]	675	67 (9.9%)	2,21 (0.13–3.11)	
[45–65]	448	33 (7.4%)	3.49 (0.24–4.41)	
Civil status				
Married	721	57 (7.9%)	1.2 (0.8–4.3)	0.62
Single	764	61 (8.0%)	1.4 (0.7–4.6)	
Divorced	27	2 (7.4%)	1.3 (0.6–4.8)	
Occupation				
Liberal profession	545	57 (10.5%)	2.5 (0.7–3.8)	0.22
Government employees	528	44 (8.3%)	2.4 (0.8–3.1)	
Students	314	13 (4.1%)	1.7 (0.3–2.3)	
Unemployees	125	6 (4.8%)	1.4 (0.1–2.6)	
Categories of donors				
Regular volunteers	108	2 (1.9%)	1	0.00
Irregular volunteers	286	13 (4.5%)	1.2 (0.5–1.9)	
Family	1118	105 (9.4%)	3.47 (0.17–4.18)	
HBV vaccination	121	0 (0%)		

Table 5
Comparison between HBsAg positive blood donors and HBsAg negative blood donors according the biochemical parameters.

Parameters	HBsAg + (n = 46)	HBsAg – (n = 46)	OR (CI 95%)	P
T-CL (< 200 mg/dL)	191.5 ± 2.1	174.2 ± 5.8	0.1 (0.05–1.26)	0.46
HDL-C (> 45 mg/dL)	32.2 ± 2.7	45.2 ± 3.7	3.3 (1.61–5.17)	0.00
LDL-C (< 130 mg/dL)	149.7 ± 6.1	141.2 ± 9.4	0.1 (0.03–1.24)	0.18
TG (< 130 mg/dL)	166.2 ± 4.7	135.2 ± 2.4	3.1 (1.11–4.45)	0.01
T-Bil (< 12 mg/L)	15.3 ± 2.3	12.7 ± 2.8	1	0.24
D-Bil (< 2 mg/L)	4.9 ± 2.2	1.7 ± 1.4	2.9 (1.08–3.71)	0.01
I-Bil I (< 10 mg/L)	10.6 ± 3.3	11.2 ± 2.8	1	0.14
ALAT (8–35 UI/L)	54.2 ± 4.1	19.7 ± 6.3	3.9 (1.15–5.56)	0.00
ASAT (8–30 UI/L)	37.4 ± 5.1	24.8 ± 4.7	2.5 (1.64–3.16)	0.02
ALP (30–100 UI/L)	119.2 ± 1.3	48.4 ± 7.5	3.4 (2.01–4.71)	0.00
γ GT (15–60 UI/L)	87.1 ± 3.4	39.8 ± 6.1	2.7 (0.95–3.72)	0.01
LDH (190–400 UI/L)	292.6 ± 3.8	221.5 ± 6.5	1.6 (0.19–2.16)	0.00
Alb (35–50 g/L)	31.5 ± 4.2	39.6 ± 3.6	2.4 (1.04–3.46)	0.02
CRP (< 6 mg/L)	5.6 ± 2.3	4.6 ± 2.9	1.1 (0.47–2.64)	0.14

OR: odds ratio; CI: confidence interval; P: probability.

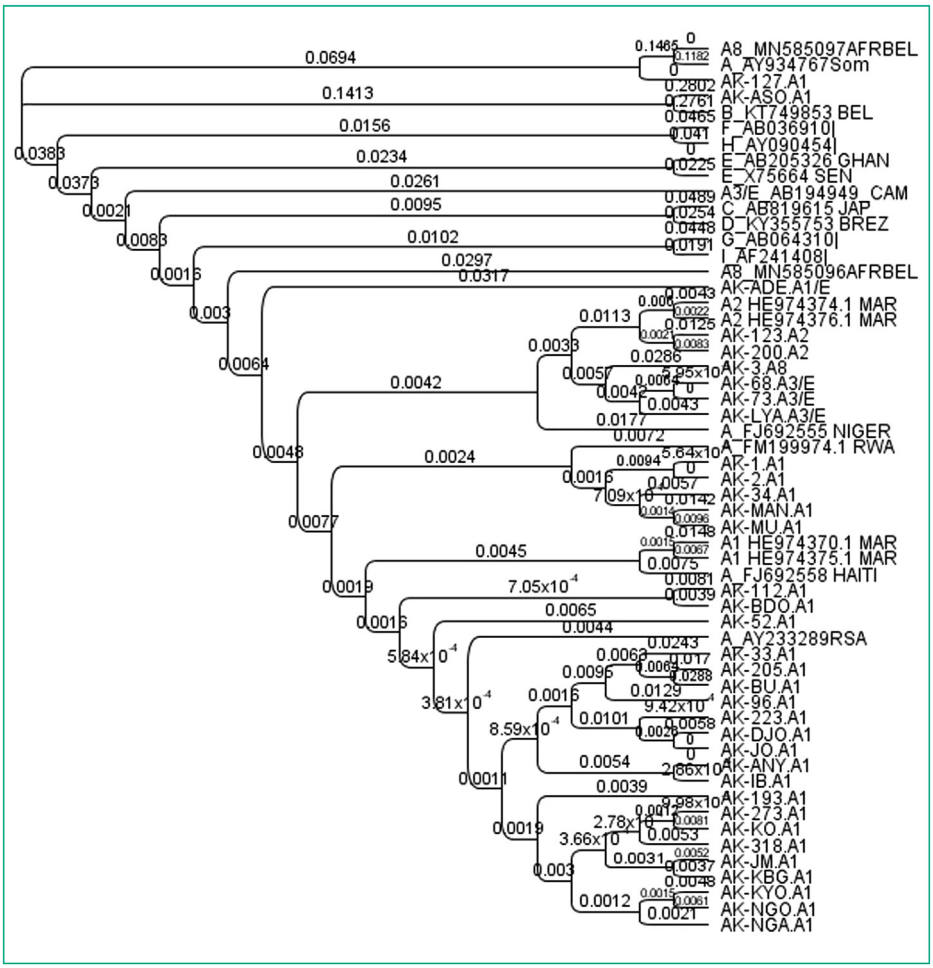

Fig. 1. Phylogenetic analysis of HBV genotype and sub-genotypes A, recombinants (A3/E, A1/E) distribution among positive HBV blood donors in Lubumbashi. This phylogenetic tree is derived from an alignment of a set of 37 HBV DNA sequences. Sequences from clinical samples are identified with the initials "AK", while the reference sequences are identified by NCBI accession number.

Table 6
Detected genotypes and sub-genotypes of HBV in the study blood donors.

Genotypes & sub-genotypes	*n* = 79	Viral load (Log_{10} UI/mL)
A		
A1	28 (35,4%)	4.6 ± 1.7
A2	4 (5,1%)	6.3 ± 2.8
A8	1 (1,3%)	5.5
E	42 (53,1%)	4.7 ± 1.9
Recombinant A3/E	3 (3,8%)	5.7 ± 1.7
Recombinat A1/E	1 (1,3%)	7.4
Total	79 (100%)	Mean = 4.6 ± 3.3

to the single identified subgenotype A8, it was close to 2 novel HBV subgenotype A8 strains recently identified in 2020 in Belgium from Congolese patients. Of the 51 identified HBV genotype E strains, 2 were linked to those found in the Central Africa. The remaining HBV genotype E strains were close to the strains from Madagascar (Figs. 1 and 2).

The mean viral load was high in blood donors with recombinant A3/E genotypes (5.7 ± 1.9 log10 IU/mL). Sequence analysis showed drug resistance mutations in 7.6% of identified genotypes (Table 7).

4. Discussion

During the study period, 1512 blood donors were identified, the vast majority of them were male (71.5%). Sociodemographic characteristics associated with the carriage of HBsAg were family donations and irregular volunteer blood donors.

As mentioned in the studies of Bukasa et al. [13] and Bassandja et al. [6], these 2 categories of blood donors have been associated for years and in several regions of the world with a high risk of transmission of blood-borne infectious agents. The predominance of family donations is linked to the African beliefs that blood donations from family members are of better quality than those from anonymous donors. However, family donors can be exposed to multiple risk factors for contamination such as drug addiction, tattooing, unprotected sex or the multiplicity of sexual partners.

Our results revealed that blood donors in Lubumbashi had a HBsAg seroprevalence of 7.9% (120 positive HBsAg out of 1512 recruited blood donors). This prevalence was close to that observed by Kakisingi et al. [9] in Lubumbashi (8.01%). However, it is slightly lower than that obtained by Kabamba et al. [11] (9.3%) and significantly higher than those observed by Kabinda et al. [14] in Lubumbashi (2.7%). Mba Eko et al. [15] and Osei et al. [16] reported HBsAg prevalence rates of 7.28% in Gabon and 7.5% in Ghana respectively similar rates to that observed in our study. Several factors may explain the differences in the prevalence rates of HBV observed in blood donors such as the socio-economic status and the education level, the health's system, the vaccination coverage, the performance of the screening tests for blood-borne microorganisms and the quality of the selection of blood donors [3].

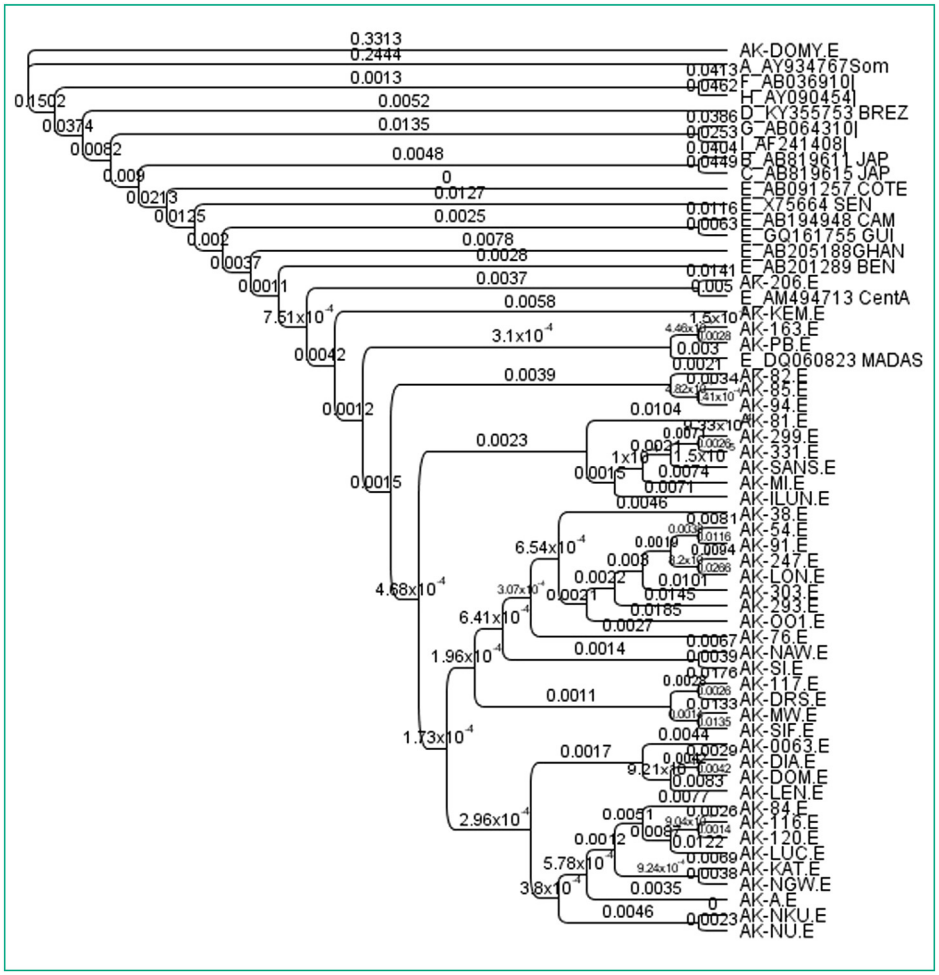

Fig. 2. Phylogenetic analysis of HBV genotype E distribution among positive HBV blood donors in Lubumbashi. This phylogenetic tree is derived from an alignment of a set of 37 HBV DNA sequences. Sequences from clinical samples are identified with the initials "AK", while the reference sequences are identified by NCBI accession number.

Family blood donors were the significantly HBV infected category (87.5%; $P < 0.05$). This observation was shared by many authors including Kyalubile et al. [10] and Osei et al. [16]. Therefore, awareness campaigns aiming to avoid the use of family donations and encouraging voluntary donations should be intensified in DRC.

Moreover, no vaccinated blood donors were tested positive for HBsAg. As some studies indicate [17], vaccination against HBV is the most effective means of protection against infection with HBV.

The biochemical evaluation showed an increased level of HDL-cholesterol, triglycerides, direct bilirubin, enzymes (ALAT, ASAT, ALP, γ-GT) and a decrease of albumin. Our results were in agreement with those reported by Ashraf–Uz–Zaman et al. [18] and Atheer et al. [19]. We have observed a decrease in albumin in HBsAg positive blood donors which could be compatible with an alteration of the hepatic cells from which it is mainly synthesized. On the other hand, even if CRP is the first acute phase protein, its level is not increased in viral hepatitis as we have observed in the context of this study.

Based on the results of this study, it appears that viral hepatitis B is accompanied by changes in biochemical parameters objectifying liver dysfunction.

Table 7
Mutations and resistance to antiviral drugs.

G	Number	Sub-type (*n*)	Mutants	Resistance mutations	Antiviral drugs	
	n (%)		*n* (%)	Types	Resistance	Sensitivity
A	37 (46,8)	A1 (28)	2 (2.5)	202 T, 250L, 202I, 181 T, 173 E	LAM, ETV, LdT	ADV, TDF
		A2 (4)	1 (1.3)	250L, 202H, 202 N, 202R, 181D, 184S	LAM, ETV, TDF, LdT	ADV
		A1/E (1)	–	–	–	LAM, ADV, ETV, TDF, LdT
		A3/E (3)	–	–	–	
		A8 (1)	–	–	–	
E	42 (53,2)	–	3 (3.8)	181 T, 202 T, 180 M, 204I, 184 M, 173E, 173 K, 173 M, 202H, 202P, 202Q, 202 N, 236H	LAM, ADV, ETV, TDF, LdT	–
Total	79 (100)		6 (7.6)			

G: genotype; *n*: number; LAM: Lamivudine; ETV: Entecavir; ADV: Adefovir; LdT: Telbuvidine; TDF: Tenofovir.

HBV genotyping is reported for the first time in Lubumbashi (South-Eastern part of DRC) with genotype E (53.1%), genotype A (41.8%) and the recombinant genotypes A3/E (3.8%) and A1/E (1.3%) are in this study described for the first time in the DRC. Our results were in agreement with those reported by Atipo–Ibara et al. [20] and Fopa et al. [21] who indicated that the majority of blood donors were infected with genotype E and a minority with genotype A respectively in Congo Brazzaville and in Cameroon.

Our results are not in line with those of Shindano et al. [8] who identified in majority the genotype A in a study carried out in Bukavu (DRC). Indeed, in Africa, genotype A is predominant in the South-East, genotype D in the North and genotype E in the West [5]. In the DRC, few studies reported a predominance of HBV genotype A in the eastern part and genotype E in the western part [8,22]. The predominance of genotype E in blood donors in Lubumbashi could be due to migrations of different populations, including those from Kinshasa and Cameroon. It must be outlined that genotype E is found almost exclusively in Africa with some concerns about the effectiveness of the HBV vaccine [23].

The majority of genotype A found in the current study belonged to the subgroup A1, also found in South Africa and Rwanda which are two neighboring countries of the DRC. It has been reported that the African subgenotype A1 is a particularly aggressive conferring higher risk of hepatocellular carcinoma in black African males [23]. Moreover, we reported for the first time the subgenotype A8 in a blood donor of Lubumbashi. This is a novel subgenotype of HBV characterized recently in two Congolese patients living in Belgium [24]. The finding of novel HBV subgenotype in high endemic area highlights the need of using efficient screening tests in Lubumbashi.

In our study, we have identified recombinant genotypes A3/E and A1/E not yet described in the DRC. The group of donors infected with genotypes A1/E, A2 and A3/E had a high viral DNA level (5.7 to 7.4 log10 IU/mL).

Few mutations (7.6%) were revealed in this study causing resistance to Lamivudine, Telbivudine, Adefovir, Entecavir and Tenofovir in different genotypes. The results of the current study are surprising since blood donors are thought not having been previously treated by any antiviral drug. In addition, the selective immune pressure against HBV might result in the HBV mutations.

Our results are not in agreement with those of Shindano et al. [8] who identified only 2 mutations with no impact on antiviral efficacy.

Our study had a limitation related to the studied population consisting only of blood donors. It should not be used to estimate the prevalence of HBV in the general population. Therefore, in the future, a HBV prevalence study should be carried out on individuals from various backgrounds.

5. Conclusion

Our results showed an HBV seroprevalence of 7.9%. They highlight the need to implement in Lubumbashi strategies aiming to improve transfusion safety in recipients, notably the suppression of family donations, the use of more effective screening and diagnostic tests, the use of separated red blood cell concentrates and the application of pathogen inactivation technologies on labile blood derivates. As vaccination is the most effective protection against HBV, it would be essential according to the WHO recommendations to implement the vaccination of new born infants and all people at risk of becoming infected with HBV. The results of our study also indicate for the first time in Lubumbashi a high prevalence of HBV genotypes E and A, a minority of the recombinant genotypes A3/E and A1/E, including the detection of some drug resistance mutations. These results could help not only in the implementation of treatment strategies but also contribute to the development of the mapping of HBV genotypes in the DRC. Finally, viral hepatitis B is accompanied by disturbances in the biochemical parameters contributing to the assessment of the degree of liver damage.

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Disclosure of interest

The authors declare that they have no competing interest.

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