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Molecular characteristics of hepatitis B virus among students and pregnant women in Chad

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Abstract

Background Hepatitis B virus (HBV) infection remains a global public health problem claiming about 1, 1 million lives among the several hundred million people infected with the virus globally. However, low- and middle-income countries including those in sub-Saharan Africa carry the highest burden but limited knowledge of the pathogen. This calls for studies to characterise the circulating genotypes of the virus in Sub-Saharan Africa. This study, sought to determine the molecular characteristics of HBV circulating among pregnant women and students in Chad.

Methods Venous blood samples were collected from pregnant women and students in the capital of Chad between April and August 2021. Whole blood samples were spun at 2500xg at 4 °C for 10 min to separate the cellular parts from the sera. The resulting sera were tested for Hepatitis B surface antigen (HBsAg) using enzyme-linked immunosorbent assay (ELISA). HBsAg positivity was confirmed using the Abbott Architect i1000SR analyser. HBV-DNA viral load detection was performed for HBsAg positive sera. HBV-DNA viral load was determined in HBsAg positive sera samples. The S gene was amplified by nested PCR followed by visualisation of the resulting amplicons under UV transillumination after gel electrophoresis. The amplicons were sequenced using the Sanger technique and subjected to phylogenetic and mutational analyses.

Results A total of 101 HBsAg-positive participants (recruited among students and pregnant women) were included in the study. The mean age was 25 years and 51.49% were males. Viral load was measured in 53 participants, among whom 27 (50.94%) had a viremia higher than 2000 IU/ml. The constructed phylogeny using 31 samples based of the HBV S gene showed that all strains belonged to the E genotype and evolutionary related to HBV from Cameroon, Central African Republic, Burkina Faso, Sudan and Ghana. The comparative mutational analysis identified low intragroup genetic diversity (0.004%) between Chadian strains with only two loci (codon positions 126 and 141) having nonsynonymous mutations including (Y126H, Y126N, Y126C, L140I, Y141S, Y141R, Y141H, Y141F).

Conclusions The study shows the presence and circulation of HBV genotype E in Chad. The Chadian strains, compared to other genotype E strains from surrounding countries have very low genetic variability. The presence of immune escape mutations in the HBV S gene could be responsible for escape vaccine strains and thus reduce the efficacy of vaccination. Additionally, the presence of the transmembrane domain in the HBV S gene could alter the antigenicity of HBsAg, contributing to screening failure by standard tests (HBsAg negative despite active infection) or reduce the efficacy of drugs that target HBsAg. These results highlight the need to evaluate hepatitis B vaccination coverage and efficiency in Chad.

Keywords Genotype, Hepatitis B virus, Students, Pregnant women, Chad, Africa

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Introduction

Hepatitis B is a viral liver infection caused by the hepatitis B virus (HBV), a DNA virus belonging to the Hepadnaviridae family. It represents a major global public health concern, particularly in high-endemic regions such as sub-Saharan Africa, Southeast Asia, and the Western Pacific. According to the World Health Organization (WHO), approximately 254 million people were living with chronic hepatitis B in 2022, with nearly 1.1 million annual deaths, mainly due to cirrhosis and hepatocellular carcinoma (HCC) [1]. The prevalence varies significantly by region; exceeding 8% in high-endemic areas (notably in Central and West Africa), prevalence below 2% in Western countries [2]. Although the World Health Organisation (WHO) has set itself the goal of eliminating viral hepatitis by 2030 [3], many African countries, including Chad, continue to experience a high prevalence of hepatitis B. In Chad, HBV prevalence was estimated at 12.4% in the general population [4], 14.87% in students [5] and 7.2% in pregnant women [6]. To address this, Chad has adopted several strategies in line with the World Health Organization (WHO) recommendations to eliminate hepatitis B as a public health threat by 2030. A major step forward was the introduction of the hepatitis B vaccine into the Expanded Programme on Immunization (EPI) in 2008, which was administered in three doses to infants at 6, 10, and 14 weeks of age [7]. The country is preparing to introduce a first dose of vaccine at birth to reinforce the prevention of mother-to-child transmission [8]. Currently, screening is conducted in major towns such as N'Djamena, Moundou, and Sarh. However, despite efforts by the government and international organizations, diagnostic tests remain expensive. Consequently, few infected individuals have access to screening and treatment due to the high costs of diagnostic tests and medications, as well as inadequate laboratory facilities [8].

HBV is a member of the Hepadnaviridae family with a double stranded Deoxyribonuclease acid (DNA) circular genome of about 3.2kbp encoding four genes: polymerase (P), surface (S; pre-S1 and pre-S2), pre-core/core (C) and X protein [9]. The S gene of the hepatitis B virus corresponds to the part of the gene that codes for the HBV surface antigen, known as HBsAg (hepatitis B surface antigen). It plays a key role in diagnosis, immune response and vaccination [10]. HBV replicates with its own reverse transcriptase, which is encoded by an antigenomic intermediate RNA sequence [11]. The lack of polymerase repair activity leads to mutations in the viral genome. As a result, HBV has an estimated mutation rate of 7.9×10^{-5} nucleotides per site per year, which is about 10 times higher than other DNA viruses [12].

Currently, 10 HBV genotypes (A-J) with distinct geographical distribution have been described [13]. Albeit both genotypes A and E are circulating in sub-Saharan Africa, genotype A is much older [14] and highly variable, with molecular signatures associated with higher risk of HCC [15]. On the other hand, genotype E was more recently introduced during the past 200 years into the general African population [16] and has much significantly lower variability [17], genotype C associated with more severe disease than genotype B [18]. Genotype D appears to be associated with a higher risk of developing HCC and a higher mortality rate after liver. In Africa, HBV genotype D is the most common genotype in North Africa, while genotypes A and E predominate in sub-Saharan Africa [19]. The genotype G circulates in South America, Canada, United States, Europe, Turkey, South Africa, Japan and Vietnam. Genotype H is found in Mexico and Central America; and genotype I was first identified in Vietnam and Laos in south-east Asia, and has more recently been reported in the eastern part of India and in China [20], the latest genotype of HBV, genotype J, has been reported in the Ryukyu Islands in Japan [21]. In addition to differences in geographical distribution, there is increasing evidence that different HBV genotypes or subtypes have variable levels of virulence such as risk of developing chronic infection, the rate of hepatitis B surface antigen (HBsAg) seroconversion, the severity of liver disease, the response to antiviral treatment (interferon) [22], and the risk of hepatocellular carcinoma(HCC) [23]. Consequently, HBV genotyping may be of great importance in guiding treatment and management of liver disease. Knowledge of HBV genotypes has proven useful in understanding disease progression and response to antiviral therapy [24].

Although HBV infection is a high-risk factor for HCC, the clinical picture following exposure to HBV is highly variable. Genetic and environmental factors may also play an important role in modulating susceptibility and progression of liver disease [25]. In Africa, 83 million people are chronic carriers of HBV [26] and the highest endemicity is found in sub-Saharan Africa with a prevalence of HBsAg greater than 10% [27]. Chad is surrounded by highly endemic neighbouring countries, dominated by HBV genotypes A and E [28]. Although numerous epidemiological studies have established the prevalence and relationship between HBV and its associated factors, this is the first study to genotype HBV in Chad.

This study sought to determine the molecular characteristics of HBV circulating in Chad among pregnant women (associated with vertical transmission) [29] and students in tertiary institutions mostly associated with heightened transmission [5].

Methods and materials

Study design and settings

This cross-sectional multicentre study was conducted to determine the molecular characteristics of HBV circulating in Chad. The study was conducted between April and August 2021, spanning 8 gynaecology and obstetrics departments and 2 universities in the capital. The capital was chosen for its high population with 9 Demographic weight [30].

Study population and sampling technique

This study included pregnant women and students. The minimum sample size for students was estimated using the Schwartz formula:

$$n = \frac{z^2 pq}{i^2}$$

In the absence of prior studies among university students in Chad, a conservative prevalence estimate of 50% was used to calculate the sample size. A z-score of 1.96 corresponding to a 95% confidence level ($\alpha = 0.05$) and a relative precision of 10% were applied, resulting in a minimum required sample of 422 students. Ultimately, 457 students were surveyed and provided biological samples.

The final sample was proportionally allocated according to the student population in each faculty: Faculty of Languages, Literature, Arts, and Communication (75 students), Faculty of Educational Sciences (80 students), Faculty of Exact and Applied Sciences (50 students), Faculty of Economics and Management Sciences (88 students), Faculty of Human and Social Sciences (75 students), and Emi Koussi University (50 students).

The same methodology was applied to the group of pregnant women. Since no similar study had been conducted among pregnant women in Chad, a prevalence estimate of 50%, a z-score of 1.96 (corresponding to a 95% confidence level, $\alpha = 0.05$), and a relative precision of 10% were used. This yielded a minimum required sample size of 422 pregnant women. Ultimately, 458 pregnant women were enrolled, interviewed, and provided biological samples.

On the expected annual number of pregnant women attending the gynaecology and obstetrics departments of the selected hospitals. The sample was distributed proportionally among the following maternity and health care centres: Gynaecology and Obstetrics Department of Assiam Vantou Hospital (100 participants), Mother and Child Hospital (58), Guinebor Hospital (50), Notre Dame des Apôtres Hospital (50), Ardeptiman Health Centre (50), Goudji Health Centre (50), N'gueli Health Centre (50), and Boutalbagar Health Centre (50).

Pregnant women attending antenatal care with prenatal consultation and students who provided informed consent were enrolled. Participants were recruited consecutively, regardless of age, sex, other sociodemographic characteristics, or risk factors, as previously described [5, 6]. A total of 915 participants were enrolled, including 458 pregnant women and 457 students.

Of these, 33 pregnant women and 68 students tested positive for HBsAg (Fig. 1). After 5 ml blood was drawn from each participant, the tubes were sent to the laboratory of the National Reference hospital. All samples were centrifuged at 3000 rpm for 15 min and sera were stored at −80 °C in the biobank, before being sent to the "Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation" (IRESSEF) in Senegal for molecular testing.

HBV measurements

Qualitative detection of HBsAg was performed using a ALERE Determine HBsAg test, providing sensitivity and specificity of 95.3% and 93.3% which is based on the principle of immunochromatography [31], according to the manufacturer's instructions. Positive results were confirmed using the Abbott Architect i1000SR analyzer (Abbott Diagnostics, Abbott Park, IL, USA). DNA was extracted from HBV-confirmed samples (200 uL) using the MagMAXTM Viral/Pathogen II Nucleic Acid Kit (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) on KingFisher™ Flex automated system from Thermo Scientific™, according to the manufacturer's instructions. DNA was eluted with 50 µl of elution buffer and stored at −20 °C until use. Real-time quantitative polymerase chain reaction (qPCR) was performed on Amplix-NG-48 using a GeneProof Hepatitis B Virus (HBV) PCR Kit, the GeneProof HBV PCR kit which targets the P gene. The kit contains an internal control, PCR inhibition and DNA extraction quality controls. A negative control (RNAse Free Water) and a positive control (Cal A) was included in each series. which provides excellent sensitivity up to 13.9 IU/ml and 100% diagnostic sensitivity [32], according to the manufacturer's instruction (GeneProof a.s; Brno, Czech Republic).

Amplification and sequencing of the S gene

Sequencing was performed on samples with viral load $\geq 10^3$ IU/ml to ensure obtaining sequences of good quality for downstream analysis. Nested PCR was used to amplify the HBV S gene using 5 µL of the extracted DNA with the HBPr1(5'-GGGTCACCATATTCTTGGG-3') and HBPr135 (5'-CA(A/G)AGACAAAAGAAA ATTGG-3') as outer PCR primer-pairs and HBPr2(5'-GAACAAGAGCTACAGCATGGG-3') and HBPr94 (5'-GGTA (A/T) AAAGGGACTCA(C/A) GATG-3') as

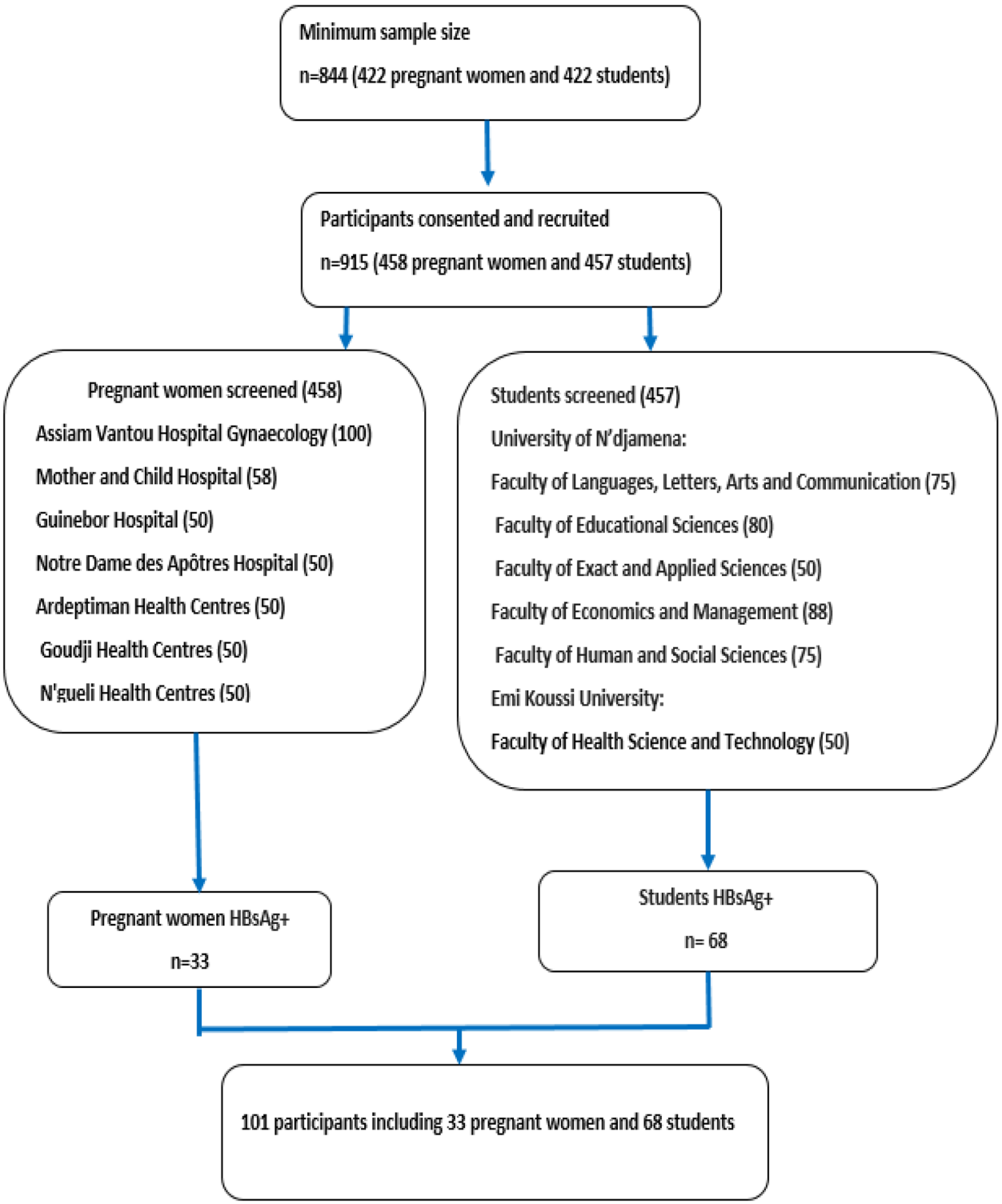

Fig. 1 Recruitment flowchart

inner PCR primer-pairs to generate a fragment of about 1197 nucleotides (nt). DNA in the 45 µL reaction mix was amplified over 40 cycles with denaturation step at 94 °C for 30 s, annealing at 50 °C for 30 s and elongation at 72 °C for 30 s [33]. PCR products were analysed by gel electrophoresis on 1% agarose gels in TBE 1X buffer

and visualized using the iBright CL750 imaging system [34]. 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 Applied Biosystems SeqStudio Genetic Analyzer (Applied Biosystems, 50 Lincoln Centre Drive Foster City, CA 94404, USA) using the BigDye Terminator and five primers (HBPr2(5'-GAACAAGAGCTA CAGCATGGG-3'), HBPr3 (5'-GGTTGTTGGTTTTCT TGTTGGTTTT-3'), HBPr14 (5'-GGCTTTGGGGCG CACTCCCAT-3'), HBPr94(5'-GGTA(A/T) AAAGGG ACTCA(C/A) GATG-3') and HBPr134(5'-TGCTGCTAT GCCTCATCTTC-3') covering the preS1/preS2/HBsAg region [33]. The sequencing PCR program was carried out as 25 cycles of 94 °C for 30 s, 50 °C for 30 s and finally 60 °C for 4 min.

HBV genotypes

After assembly and editing of sequences using the SeqManTM software (DNASTAR, Inc., USA), the nucleotide sequences were concatenated and aligned using ClustalW in MEGA11 [35]. HBV mutations and genotypes were determined using the HBVseq [36].

Phylogenetic analysis

The phylogenetic analysis was inferred by using the Maximum Likelihood method and Kimura 2-parameter model [37]. The tree with the highest log likelihood (−3583.87) as shown (Fig. 3) was used. The percentage of trees in which the associated taxa clustered together is shown above the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value in MEGA11 [35].

Genetic distance

An analysis of the number of base substitutions per site between nucleotide sequences (genetic distance (d)) was performed using the second version of the synonymous and non-synonymous analysis program SNAP v2.1 [38].

GenBank

The HBV sequence data generated in this study have been deposited into the GenBank database with assigned accession numbers PP262125-PP262128; PP266011-PP266018 and PP317898-PP317916.

Statistical analyses

Data were analysed using Stata 12. A descriptive analysis was carried out to summarise the socio-demographic characteristics of the participants included in the study. Qualitative variables (such as gender, marital status and occupation) were expressed as numbers (n) and percentages (%). Quantitative variables (such as age or viral load) were described using means ± standard deviations (SD) for normal distributions, or medians and interquartile ranges (IQR) when the data were not normally distributed.

Results

Study population characteristics

This study enrolled 101 participants including 33 pregnant women and 68 students who were HBsAg seropositive out of the total 458 and 457 pregnant women and students screened respectively. The mean age of participants was 25 years (range 18–59 years). Socio-demographic characteristics of study participants are shown in Table 1. In summary, 51.49% (52/101) of the study population were men. Fifty-one (50.50%) were single and 72 (71.29%) were students (Table 1).

HBV DNA detection and quantification

HBV DNA was detected in 53 (52.4%) HBsAg positive samples by quantitative PCR with a median viral load of 3005.35 IU/ml (IQR: 148.928571 IU/ml). Among them, 26 (49.05%) had HBV VL under 2000 IU/ml (median = 148.928571 IU/ml; IQR: 69.5535714 IU/ml) and 27 (50.94%) had HBV VL greater than 2000 IU/ml (median = 1,287,500 IU/ml; IQR: 28,928.5714 IU/ml). Seven pregnant women had a viral load < 2000 (median = 91.9642857 IU/ml; IRQ = 48.0357143 IU/ml) and ten had a viral load > 2000 (median = 848,214.286 IU/ml; IRQ = 6531.25 IU/ml). Nineteen students had a viral load < 2000 (median = 149.285714 IU/ml; IRQ

Table 1 Socio-demographic characteristics of study participants

Variable	Category	frequency (percentage)
Group Age (*N* = 101)	Mean Age = 25 ± 5 years	
	18–24	64(63.37%)
	25–34	31(30.69%)
	≥ 35	6(5.94%)
Sex (*N* = 101)	Male	52(51.49%)
	Female	49(48.51%)
Marital status (*N* = 101)	Single	51(50.50%)
	Married	50(49.50%)
Occupation (*N* = 101)	Housewife	15(14.85%)
	Student and Pupil	75(74.26%)
	Liberal profession	4(3.96%)
	Government employees	7(6.93%)

= 73.5714286 IU/ml) and seventeen of these students had a viral load > 2000 (median = 11,142,857.1 IU/ml; IRQ = 39,642.8571 IU/ml) (Fig. 2).

Phylogenetic analysis

Molecular characterization of detected virus strains

Among the 53 samples with detectable viral load, the S gene of 31 samples were successfully sequenced and genotyped using the standford HBVSeq database as genotype E. However, one sample (TC CSBO 26 HBV) failed the phylogenetic analysis. As shown below (Fig. 3), most of the 30 genotype E samples from Chad (22/30), which passed the phylogenetic analysis clustered together as a monophyletic group. An additional 4 samples clustered together with strains from Senegal, Ghana and Sudan whereas a single sample clustered with peculiar E genotype strains from

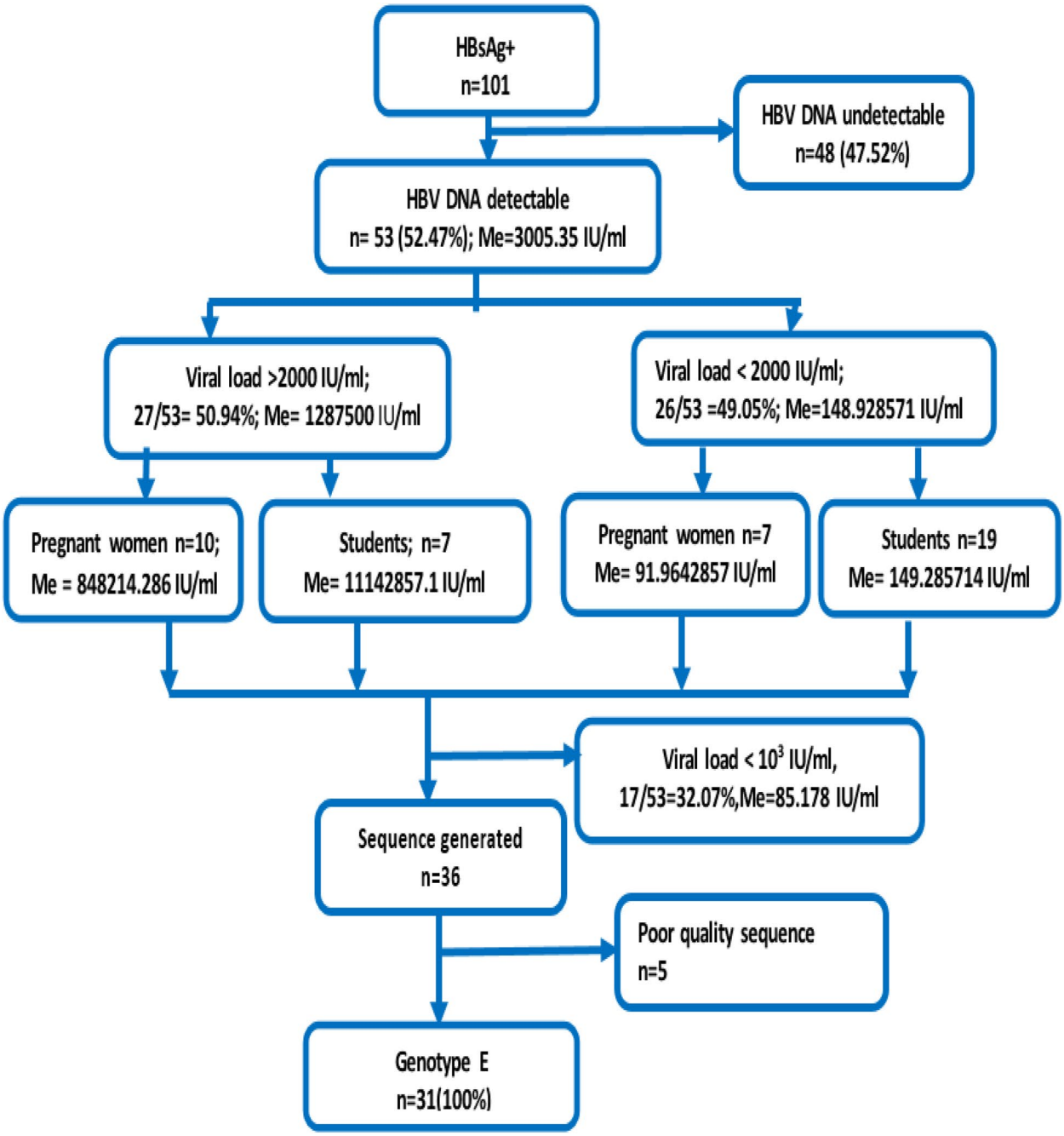

Fig. 2 Flow chart of study participants

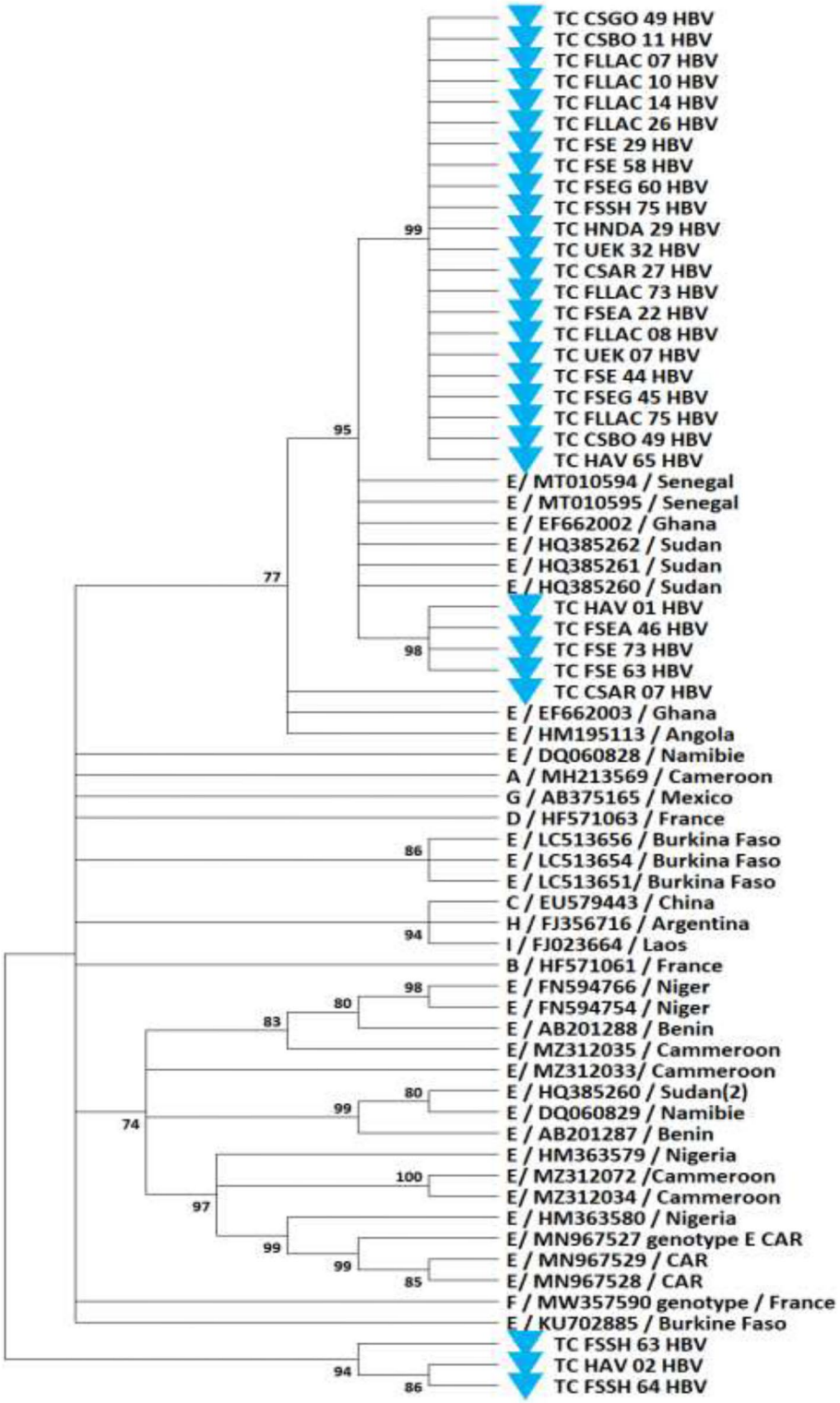

Fig. 3 Phylogeny of HBV from Chad based on S gene sequence analysis. (Samples from Chad are in blue)

Namibia, Angola and Ghana. The remaining 3 samples resolved as single branch close to the root of the tree.

Mutations of HBV S gene

Of the 31 samples genotyped, 93.35% ($n = 29/31$) had amino acid substitutions predominantly among transmembrane structural domains as well as those associated with immune escape as shown below. The HBV immune escape mutations spanned the major hydrophilic region (MHR) and a-determinant domain (codons 124–149) of the HBV S gene were more frequent (Y126H, Y126N, Y126C, L140I, Y141S, Y141R, Y141H, Y141F), followed by the transmembrane domain mutants (TMD)(H9Y,H9N, H9D,H9Q, H9L, M98I, M98I, M98R, M98R, G152R, G152E, R153W, R153H, R153Q, R153H, R153C, R153K) as shown below (Fig. 4).

Genetic diversity of HBV genotype E strains

The averages global genetic distances for all pairwise comparisons were: synonymous distances (ds) 0.0176, nonsynonymous distances (dn) 0.0085 and the ratio dn/ds was 0.48 which shows that the viruses are highly conserved and under purifying selection. As shown below (Fig. 5). the dn/ds of Chadian samples was 0.4, which was lower than that of the HBV/E reference sequences (0.55). Similar intra-group purifying selection was found in among HBV from Cameroon (0.4), Benin (0.39), Sudan (0.5), Ghana (0.5), Niger (0.7), Nigeria (0.49), Angola (0.2), Senegal (0.3), Central African Republic (0.4) and Burkina Faso (0.5).

Discussion

This study determined the molecular characteristics of HBV circulating among pregnant women and students in Chad. Our study found genotype E (HBV/E) as the only strain infecting all the participants irrespective of gender, age or cohort. There are limited studies about HBV genotypes in Chad, but our data show the predominance of genotype E in this country [39] and calls for further studies using a larger sample size among participants from other parts of the country. The strong genetic similarity

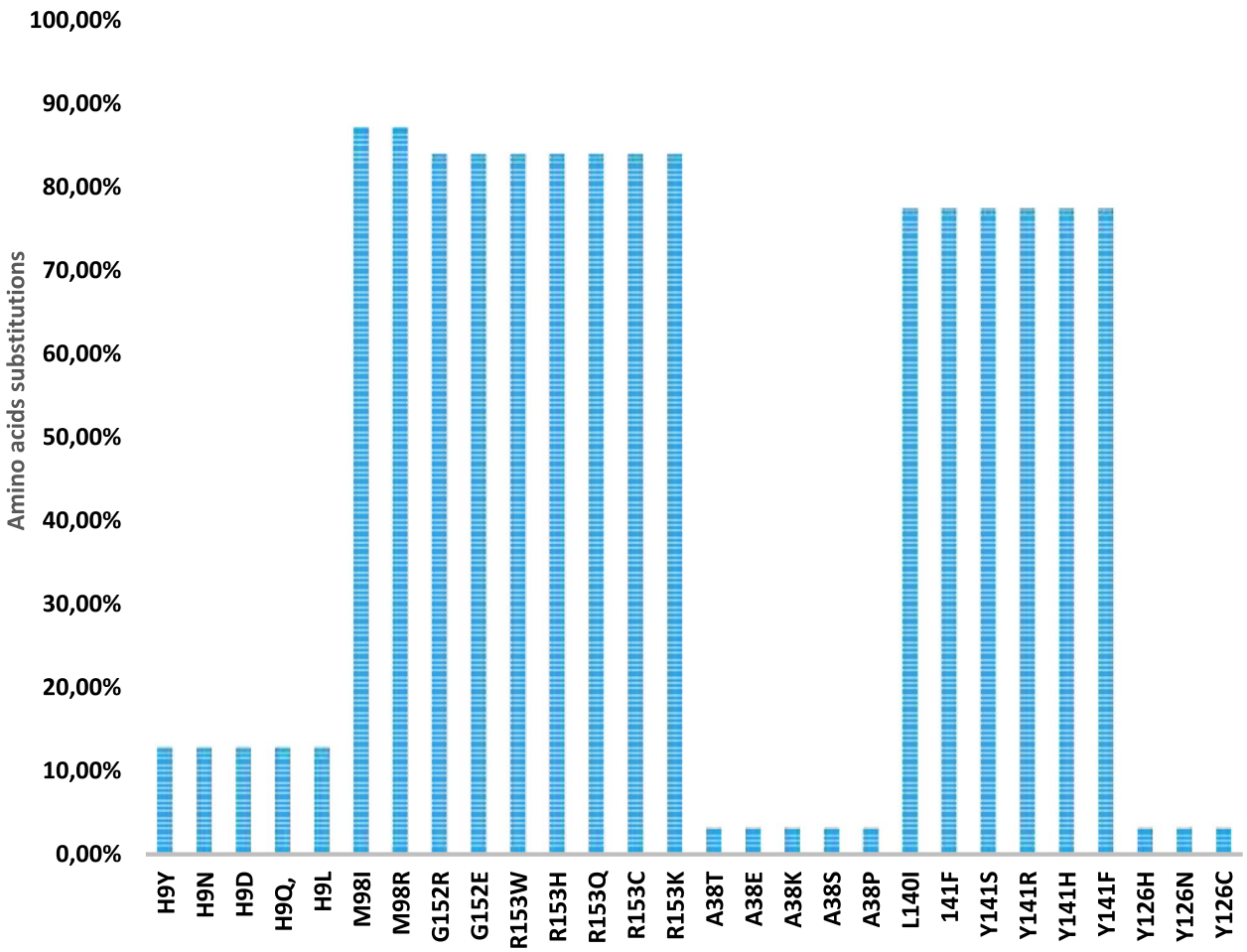

Fig. 4 Amino acids substitutions in HBV S protein

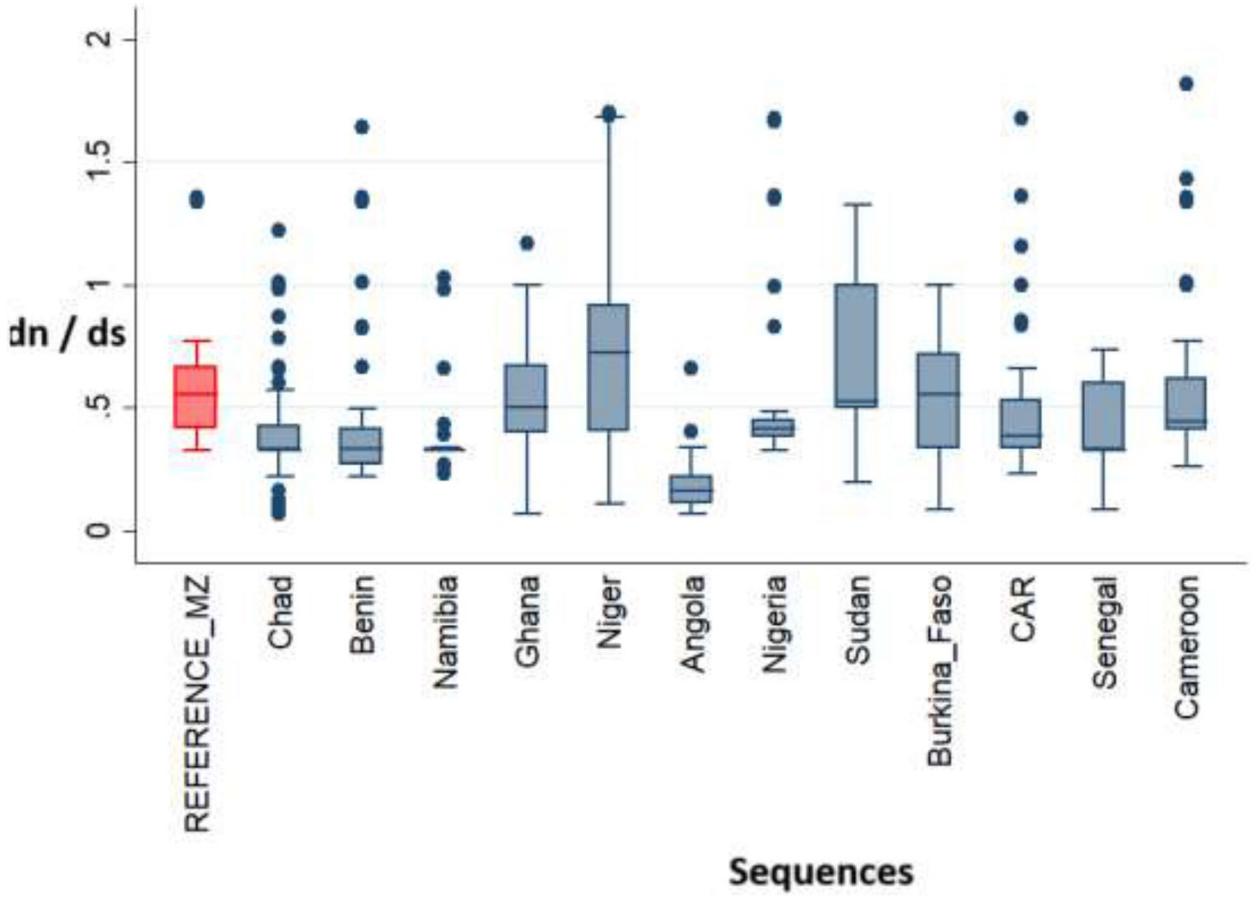

Fig. 5 Genetic distances of reference sequence MZ312033 genotype E to all isolates recovered in this study and isolates in Africa

between the Chadian sequences and those from other sub-Sahara African countries such as Senegal [33], Ghana [40], and Sudan [41] supports the hypothesis of regional circulation of genotype E with limited diversification [42]. The close clustering of these sequences, supported by high bootstrap values (≥ 95), reflects the low genetic variability of genotype E. While this low diversity reduces phylogenetic resolution, it facilitates the identification of region-specific strains. These findings highlight the need for sustained molecular surveillance to monitor HBV evolution in the region and to inform tailored strategies for diagnosis, vaccination, and control efforts. Even though genotype E in Sub-Saharan Africa is associated with low sequence variability, its spread has not yet been explained [43].

The participants of this study had high viral loads, similar to a study in Sudan which reported, that people infected with genotype E have generally a high viral loads [44], and thus at high risk of transmission. It has been also suggested that current HBV birth dose vaccine might offer poorer protection against MTCT HBV genotype E infection which could be due to the associated higher viral load [45]. However, the approximately 48% of HBsAg-positive samples with undetectable HBV DNA by PCR, could be due to very low viral loads below assay detection limits resulting from low replicating integrated viral elements or specific chronic infection state like the inactive carrier state or PCR target mismatch issues with local genotypes which could pose a diagnostic challenge [46, 47]. This highlights the need for local research into understanding the genomic diversity of the HBV to help development more sensitive diagnostics for local use.

Among the amino acid substitutions, 29/31 (93.35% were HBV immune escape mutations spanning the major hydrophilic region in the a-determinant domain (Y126H, Y126N, Y126C, L140I, Y141F, Y141S, Y141R, Y141H, Y141F), the main target for neutralising anti-HBs antibodies produced after natural infection or vaccination. This is consistent with previous studies reporting a predominance of immune escape mutants associated with genotype E [48]. However, the prevalence of HBV MHR in our study is higher than that reported in Nigeria [49]. In fact, most of the strains from our study (79.31%) had a leucine at position 140, which is known to affect HBsAg

recognition. This position140 is within an HLA class 2 epitope comprising amino acids within codons 139–146 associated with anti-HB antibody production following HBV vaccination [48]. Thus, such mutations may increase the immune escape potential of these strains to the humoral response after vaccination.

Hepatitis B virus immune escape mutants can be selected by host immune pressure resulting from vaccination or the administration of hepatitis B immunoglobulin [50], or they can emerge as an indirect consequence of antiviral drug resistance mutations within the overlapping polymerase gene which subsequently alter HBsAg antigenicity [51]. For a resource limited country such as Chad, this process can be exacerbated by factors such as incomplete vaccination, suboptimal diagnostics, high HBV endemicity and lack of regular genomic surveillance. Therefore, the detection of HBV immune escape mutants (IEMs) among pregnant women and students in Ghana could imply an increased risk of mother-to-child transmission despite standard immune-prophylaxis (as IEMs may not be neutralized by hepatitis B immunoglobulin or vaccine-induced antibodies in newborns) and a higher potential for breakthrough infections in vaccinated student populations (reducing vaccine effectiveness), thereby challenging current prevention strategies and diagnostic accuracy [50, 52].

Numerous studies have reported that universal vaccination with HBV vaccines (most of which are derived from genotype A) is highly effective, providing effective prevention of HBV infection and contributing to a reduction in the prevalence of chronic HBV infection, as well as a reduction in HBV-induced mortality and incidence of HCC [53, 54]. However, recent studies have reported reduced HB vaccine efficacy against different HBV genotypes due to potential vaccine escape [55, 56].

The detection of mutations in the transmembrane domain of the HBV S gene of HBV from our study participants raises significant concerns regarding both diagnostic accuracy and vaccine efficacy because the transmembrane region play a critical role in the proper folding, secretion, and antigenicity of the HBsAg. Mutations in these regions can alter conformation of the resulting peptide, potentially leading to reduced recognition by commercial diagnostic assays and contributing to occult HBV infections (HBsAg-negative despite active infection) [57]. These findings highlight the need for molecular surveillance and the potential revision of diagnostic strategies to account, particularly in endemic regions like Chad.

The current study revealed low genetic variability of HBV/E strains within and between groups. The mean genetic diversity was low (< 0.01) and comparable to those from Cameroon, Sudan, Burkina Faso, Niger, Namibia and Ghana but below those from Central Africa Republic and Angola consistent (> 0.01) with report from a previous study which compared HBV/E from different parts of the Africa [58]. However, the clustering of majority of the strains (22/30) as monophyletic branch of the ML-tree indicate clonal expansion of a single HBV strain which points to an ongoing transmission of HBV in the study areas.

In the present study, 50.94% of participants had a viral load greater than 2,000 IU/mL, which is an important clinical threshold in the WHO guidelines for the management of chronic hepatitis B infection. According to the WHO 2024 guidelines, antiviral treatment should be considered in individuals with persistent HBV DNA levels above 2,000 IU/mL when associated with elevated ALT levels or evidence of liver damage, even in the absence of cirrhosis [59]. Albeit asymptomatic at the time of diagnosis and thus at high risk of transmitting infection to children (mother to child transmission, MTCT), family, social and sexual contacts. There is also evidence that high viral load is a factor associated with an increased risk of cirrhosis and hepatocellular carcinoma in HBV carriers [60]. These finding highlights the importance of hepatitis B screening, early treatment, and birth dose vaccination to prevent MTCT [61]. Our findings indicate that over half of the participants may be at increased risk and potentially eligible for treatment, highlighting the need for comprehensive clinical assessment including liver function tests and fibrosis evaluation. These results reinforce the importance of early viral load monitoring and suggest that many HBV-infected individuals in this population may benefit from timely antiviral therapy in line with WHO recommendations aimed at reducing HBV-related morbidity and mortality.

Limitations

Our study is limited by the fact that we did not have clinical data on our participants which could have help us to evaluate the relationship between viral load, genetic diversity and clinical phenotypes. Another limitation of this study is the fact that we only sequenced a single genetic element as a complete genome sequencing may yield better results in terms of mutations and phylogenetic relationships. Another important limitation was the failure to test for other HBV serological markers to confirm HBV infection and immunization. Moreso, this study was a cross-sectional one with no follow up activity to monitor potential vertical transmission from the HBV-infected pregnant women due to limited funding. Thus, we could not examine vertical transmissibility of the HBV genotypes with immune escape mutations and its potential implication for diagnosis.

Conclusion

This study confirms the predominance of genotype E in Chadian students and pregnant women, with a low genetic variability of HBV/E strains and suggests potential ongoing transmission of HBV/E, the serotype associated with higher viral loads with potential for immune escape. Our findings also indicate important HBV immune escape mutations, spanning the major hydrophilic region, and highlights the need to evaluate hepatitis B vaccination coverage, efficiency, and therapies targeting the HBsAg.

Abbreviations

HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
PCR	Polymerase chain reaction
rt	Reverse transcriptase
(P)	Polymerase
WHO	World Health Organization

Authors' contributions

ND, FLF, AMM, and NCT, conceived the project; ND oversaw the data collection; ND, DB, GL, NL, MD, OD and IDO analysed and interpreted the quantitative data, and, NL, GL, MD, OD and IDO and AS, NKK, NAD, DD processed the sample. ND drafted the manuscript which was reviewed and validated by all authors who gave FLF the authority for publication.

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Data availability

The HBV sequence data generated in this study have been deposited into the GenBank database with assigned accession numbers PP262125-PP262128; PP266011-PP266018 and PP317898-PP317916.

Declarations

Ethics approval and consent to participate

The study with its protocols were reviewed and approved by the National Bioethics Committee of Chad (N° 201/PR/MESRI/DG/CNBT/2020, 8 November 2020) and the UCAD Research Ethics Committee (CER/UCAD/AD/MSN/050/2020, 7 December 2021). Following an explanation of the study in language understood by participants, an informed consent was obtained from participants before inclusion in the study. All procedures of the study were performed in accordance with relevant guidelines and regulations guided by the ethically approved protocols.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. WHO sounds alarm on viral hepatitis infections claiming 3500 lives each day. Available from: https://www.who.int/news/item/09-04-2024-who-sounds-alarm-on-viral-hepatitis-infections-claiming-3500-lives-each-day. Cited 14 March 2025.
2. Sonderup MW, Spearman CW. HBV elimination in Africa—Current status and challenges. Clin Liver Dis (Hoboken). 2024;23(1):e0166.
3. Combating hepatitis B and C to reach elimination by 2030. Available from: https://www.who.int/publications/i/item/combating-hepatitis-b-and-c-to-reach-elimination-by-2030. Cited 22 Apr 2025.
4. GBD 2019 Hepatitis B Collaborators. Global, regional, and national burden of hepatitis B, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet Gastroenterol Hepatol. 2022;7(9):796-829.
5. Debsikréo N, Mankréo BL, Moukénet A, Ouangkake M, Mara N, Moussa AM, et al. Prevalence of hepatitis B virus infection and its associated factors among students in N'Djamena, Chad. PLoS ONE. 2024;19(4):e0273589.
6. Debsikréo N, Mankréo BL, Moukenet A, Ndiaye AJS, Diouf NL, Lo G, et al. Prevalence of Hepatitis B and Associated Factors among Pregnant Women in N'djamena. Chad Fortune Journal of Health Sciences. 2023;6(3):253–62.
7. Ferrinho P, Dramé M, Tumusiime P. Perceptions of the usefulness of external support to immunization coverage in Chad: an analysis of the GAVI-Alliance cash-based support. Pan Afr Med J. 2013;15:44.
8. Journée mondiale contre l'Hépatite : l'OMS lance un appel pour des actions de lutte et de prévention permanentes | OMS | Bureau régional pour l'Afrique. 2022. Available from: https://www.afro.who.int/fr/countries/chad/news/journee-mondiale-contre-lhepatite-loms-lance-un-appel-pour-des-actions-de-lutte-et-de-prevention. Cited 6 May 2025.
9. Summers J, O'Connell A, Millman I. Genome of hepatitis B virus: restriction enzyme cleavage and structure of DNA extracted from Dane particles. Proc Natl Acad Sci U S A. 1975;72(11):4597–601.
10. Lazarevic I, Banko A, Miljanovic D, Cupic M. Hepatitis B Surface Antigen Isoforms: Their Clinical Implications, Utilisation in Diagnosis, Prevention and New Antiviral Strategies. Pathogens. 2024;13(1):46.
11. Olenginski LT, Kasprzak WK, Bergonzo C, Shapiro BA, Dayie TK. Conformational Dynamics of the Hepatitis B Virus Pre-genomic RNA on Multiple Time Scales: Implications for Viral Replication. J Mol Biol. 2022;434(18):167633.
12. Osiowy C, Giles E, Tanaka Y, Mizokami M, Minuk GY. Molecular Evolution of Hepatitis B Virus over 25 Years. J Virol. 2006;80(21):10307–14.
13. Liu Z, Zhang Y, Xu M, Li X, Zhang Z. Distribution of hepatitis B virus genotypes and subgenotypes. Medicine (Baltimore). 2021;100(50): e27941.
14. Andernach IE, Nolte C, Pape JW, Muller CP. Slave Trade and Hepatitis B Virus Genotypes and Subgenotypes in Haiti and Africa. Emerg Infect Dis août. 2009;15(8):1222–8.
15. Toyé RM, Cohen D, Pujol FH, Sow-Sall A, Lô G, Hoshino K, et al. Hepatitis B Virus Genotype Study in West Africa Reveals an Expanding Clade of Subgenotype A4. Microorganisms. 2021;9(3):623.
16. Andernach IE, Hübschen JM, Muller CP. Hepatitis B virus: the genotype E puzzle. Rev Med Virol. 2009;19(4):231–40.
17. Singh J, Dickens C, Pahal V, Kumar R, Chaudhary R, Kramvis A, et al. First Report of Genotype E of Hepatitis B Virus in an Indian Population. Intervirology. 2009;52(5):235–8.
18. Li J, Li J, Chen S, Xu W, Zhang J, Tong S. Clinical isolates of hepatitis B virus genotype C have higher in vitro transmission efficiency than genotype B isolates. J Med Virol. 2023;95(6): e28879.
19. Hübschen JM, Andernach IE, Muller CP. Hepatitis B virus genotype E variability in Africa. J Clin Virol. 2008;43(4):376–80.
20. Velkov S, Ott JJ, Protzer U, Michler T. The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. Genes (Basel). 2018;9(10):495.
21. Tatematsu K, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, et al. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype. J J Virol. 2009;83(20):10538–47.

22. Elizalde MM, Mojsiejczuk L, Speroni M, Bouzas B, Tadey L, Mammana L, et al. Molecular and biological characterization of hepatitis B virus subgenotype F1b clusters: Unraveling its role in hepatocarcinogenesis. Front Microbiol. 2022;13: 946703.
23. Kaur SP, Talat A, Karimi-Sari H, Grees A, Chen HW, Lau DTY, et al. Hepatocellular Carcinoma in Hepatitis B Virus-Infected Patients and the Role of Hepatitis B Surface Antigen (HBsAg). J Clin Med. 2022;11(4). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8878376/. Cited 3 Jun 2024.
24. Sunbul M. Hepatitis B virus genotypes: Global distribution and clinical importance. World J Gastroenterol. 2014;20(18):5427–34.
25. Ramos-Lopez O, Martinez-Lopez E, Roman S, Fierro NA, Panduro A. Genetic, metabolic and environmental factors involved in the development of liver cirrhosis in Mexico. World J Gastroenterol. 2015;21(41):11552–66.
26. CDC. Viral Hepatitis. Global Viral Hepatitis. Available from: https://www.cdc.gov/hepatitis/global/index.html. Cited 18 Apr 2025.
27. Razavi H. Global Epidemiology of Viral Hepatitis. Gastroenterol Clin North Am. 2020;49(2):179–89.
28. Kafeero HM, Ndagire D, Ocama P, Kato CD, Wampande E, Walusansa A, et al. Mapping hepatitis B virus genotypes on the African continent from 1997 to 2021: a systematic review with meta-analysis. Sci Rep. 2023;13(1):5723.
29. Buti M, Gane E, Seto WK, Chan HLY, Chuang WL, Stepanova T, et al. Tenofovir alafenamide versus tenofovir disoproxil fumarate for the treatment of patients with HBeAg-negative chronic hepatitis B virus infection: a randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol. 2016;1(3):196–206.
30. INSEED-TCHAD - POPULATION. Available from: https://www.inseed.td/index.php/thematiques/statistique-demographique/population. Cited 3 June 2024.
31. Njai HF, Shimakawa Y, Sanneh B, Ferguson L, Ndow G, Mendy M, et al. Validation of rapid point-of-care (POC) tests for detection of hepatitis B surface antigen in field and laboratory settings in the Gambia. Western Africa J Clin Microbiol. 2015;53(4):1156–63.
32. Aurea s.r.o VA. GeneProof Hepatitis B Virus (HBV) PCR testing. Available from: https://www.geneproof.com/geneproof-hepatitis-b-virus-hbv-pcr-kit/p1093. Cited 27 June 2023.
33. Toyé RM, Lô G, Diop-Ndiaye H, Cissé AM, Ndiaye AJS, Kébé-Fall K, et al. Prevalence and molecular characterization of hepatitis B virus infection in HIV-infected children in Senegal. Clin Res Hepatol Gastroenterol. 2021;45(2): 101502.
34. iBright™ CL750 Imaging System. Available from: https://www.thermofisher.com/order/catalog/product/A44116. Cited 27 June 2023.
35. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol. 2021;38(7):3022–7.
36. HBVseq: Sequence Analysis. Available from: https://hivdb.stanford.edu/HBV/HBVseq/development/hbvseq.pl?action=showSequenceForm. Cited 27 May 2024.
37. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111–20.
38. SNAP: Synonymous Non-synonymous Analysis Program. Available from: https://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html?sample_input=1. Cited 29 May 2024.
39. Yousif M, Mudawi H, Bakhiet S, Glebe D, Kramvis A. Molecular characterization of hepatitis B virus in liver disease patients and asymptomatic carriers of the virus in Sudan. BMC Infect Dis. 2013;13:328.
40. Candotti D, Danso K, Allain JP. Maternofetal transmission of hepatitis B virus genotype E in Ghana, west Africa. J Gen Virol. 2007;88(Pt 10):2686–95.
41. Mahgoub S, Candotti D, El Ekiaby M, Allain JP. Hepatitis B Virus (HBV) Infection and Recombination between HBV Genotypes D and E in Asymptomatic Blood Donors from Khartoum. Sudan J Clin Microbiol. 2011;49(1):298–306.
42. Mulders MN, Venard V, Njayou M, Edorh AP, Bola Oyefolu AO, Kehinde MO, et al. Low genetic diversity despite hyperendemicity of hepatitis B virus genotype E throughout West Africa. J Infect Dis. 2004;190(2):400–8.
43. Shimakawa Y, Veillon P, Birguel J, Pivert A, Sauvage V, Guillou-Guillemette HL, et al. Residual risk of mother-to-child transmission of hepatitis B virus infection despite timely birth-dose vaccination in Cameroon (ANRS 12303): a single-centre, longitudinal observational study. Lancet Glob Health. 2022;10(4):e521–9.
44. Malagnino V, Salpini R, Maffongelli G, Battisti A, Fabeni L, Piermatteo L, et al. High rates of chronic HBV genotype E infection in a group of migrants in Italy from West Africa: Virological characteristics associated with poor immune clearance. PLoS ONE. 2018;13(3): e0195045.
45. Ahmad AE, Bakari AG, Musa BOP, Mustapha SK, Jamoh BY, Abdullahi IN, et al. Pattern of Prevalent Hepatitis B Virus Genotypes in Zaria. Nigeria Niger Postgrad Med J. 2019;26(2):80–6.
46. Kramvis A. The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics. Rev Med Virol. 2016;26(4):285–303.
47. Raimondo G, Locarnini S, Pollicino T, Levrero M, Zoulim F, Lok AS, et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J Hepatol. 2019;71(2):397–408.
48. Schuenke KW, Cook RG, Rich RR. Binding specificity of a class II-restricted hepatitis B epitope by DR molecules from responder and nonresponder vaccine recipients. Hum Immunol. 1998;59(12):783–93.
49. Anejo-Okopi J, Okeke E, Davwar PM, Onwuamah C, Onywera H, Omaiye P, et al. Molecular detection of hepatitis B virus genotype E with immune escape mutations in chronic hepatitis B patients on long-term antiviral therapy in Jos, Nigeria. Afr J Lab Med. 2022;11(1):1677.
50. Romanò L, Paladini S, Galli C, Raimondo G, Pollicino T, Zanetti AR. Hepatitis B vaccination. Hum Vaccin Immunother. 2014;11(1):53–7.
51. Torresi J, Earnest-Silveira L, Deliyannis G, Edgtton K, Zhuang H, Locarnini SA, et al. Reduced Antigenicity of the Hepatitis B Virus HBsAg Protein Arising as a Consequence of Sequence Changes in the Overlapping Polymerase Gene That Are Selected by Lamivudine Therapy. Virology. 2002;293(2):305–13.
52. Kanji JN, Penner RED, Giles E, Goodison K, Martin SR, Marinier E, et al. Horizontal Transmission of Hepatitis B Virus From Mother to Child Due to Immune Escape Despite Immunoprophylaxis. J Pediatr Gastroenterol Nutr. 2019;68(5):e81–4.
53. Chen HL, Chang MH, Ni YH, Hsu HY, Lee PI, Lee CY, et al. Seroepidemiology of hepatitis B virus infection in children: Ten years of mass vaccination in Taiwan. JAMA. 1996;276(11):906–8.
54. Schillie S, Vellozzi C, Reingold A, Harris A, Haber P, Ward JW, et al. Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep. 2018;67(1):1–31.
55. Aono J, Yotsuyanagi H, Miyoshi H, Tsutsumi T, Fujie H, Shintani Y, et al. Amino acid substitutions in the S region of hepatitis B virus in sera from patients with acute hepatitis. Hepatol Res. 2007;37(9):731–9.
56. Stramer SL, Wend U, Candotti D, Foster GA, Hollinger FB, Dodd RY, et al. Nucleic acid testing to detect HBV infection in blood donors. N Engl J Med. 2011;364(3):236–47.
57. Lehmann F, Slanina H, Roderfeld M, Roeb E, Trebicka J, Ziebuhr J, et al. A Novel Insertion in the Hepatitis B Virus Surface Protein Leading to Hyperglycosylation Causes Diagnostic and Immune Escape. Viruses. 2023;15(4):838.
58. Ingasia LAO, Wose Kinge C, Kramvis A. Genotype E: The neglected genotype of hepatitis B virus. World J Hepatol. 2021;13(12):1875–91.
59. Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection. Available from: https://www.who.int/publications/i/item/9789240090903. Cited 4 May 2025.
60. El-Serag HB. Epidemiology of Viral Hepatitis and Hepatocellular Carcinoma. Gastroenterology. 2012;142(6):1264-1273.e1.
61. Matthews PC, Ocama P, Wang S, El-Sayed M, Turkova A, Ford D, et al. Enhancing interventions for prevention of mother-to-child- transmission of hepatitis B virus. JHEP Rep. 2023;5(8): 100777.

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