Prevalence of Hepatitis B Virus Genotypes Among Patients with Liver Disease in Eritrea

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Abstract

Background

Hepatitis B virus (HBV) is a blood-borne hepatotrophic virus and a major causative agent of liver disease. The virus is highly endemic in African countries, and five genotypes (A–E) have been identified. This study aimed to establish the most prevalent genotypes of HBV among liver disease patients from different geographical regions of Eritrea, an East African multi-ethnic country.

Methods

This study included 293 Eritrean liver disease patients who were hepatitis B surface antigen (HBsAg)-positive. Enzyme-linked immunosorbent assay (ELISA)-based serological screening and multiplex-nested PCR using type-specific primer-based genotyping were performed to determine the prevalence of genotypes in the Eritrean population.

Results

The mean (± standard deviation) age was 41.66 ± 13.84 years; 213 (72.7%) patients were males and 80 (27.3%) were females. The median (interquartile range) of HBV DNA and alanine aminotransferase levels were 3.47 (1.66) log IU/ml and 28 (15.3) IU/L, respectively. All patients' sera were HBsAg- and anti-HBc-total positive; 20 (6.8%) were HBeAg-positive/anti-HBe-negative, 242 (82.6%) were HBeAg-negative/anti-HBe-positive, and 31 (10.6%) had neither HBeAg nor anti-HBe according to the ELISA screening test.

Of the 293 patients, only 122 (41.6%) were positive for HBV DNA, 57.38% had a single genotype, and 42.62% had a mixed HBV genotype infection. Irrespective of mode of occurrence, HBV genotype D (n = 26; 21.3%) was the predominant circulating genotype, followed by genotypes C (n = 21; 17.2%), E (n = 19; 15.6%), C/D (n = 16; 13.1%), and C/E (n = 13; 10.7%). Genotypes C/D/E (n = 9; 7.4%), A/D (n = 6; 4.9%), D/E (n = 5; 4.1%), A (n = 3; 2.5%), and B, A/E, B/E, and A/D/C (each with n = 1; 0.8%) were also present.

Conclusion

HBV in Eritrea is comprised of a mixture of genotypes A, B, C, D, and E separately or in combinations. Our findings demonstrated that in Eritrea, the most prevalent HBV genotype in Eritrea is genotype D among theliver disease patients with higher HBeAg positivity. This is the first study of HBV genotyping based on PCR methods in Eritrea.

Background

Hepatitis B virus (HBV) is a double-stranded DNA virus belonging to the family *Hepadnaviridae* [1]. HBV infection is the most frequent cause of liver cirrhosis and hepatocellular carcinoma (HCC) and a cause of chronic infection in more than 240 million individuals worldwide, including 65 million people in Africa [1, 2]. Eritrea is an East African multi-ethnic country with an intermediate HBV seroprevalence as indicated by

HBsAg positivity rates of 2.6–3.2% [3]. Ten genotypes of HBV (A–J) with distinct geographic distributions have been recognized [4–6]. Differences in function and structure among genotypes can influence the severity and clinical outcomes of HBV infection as well as complications associated with differences in response to antiviral therapy [7]. Infection by HBV genotypes A and D is more likely to progress to the chronic phase than infection by genotypes B and C, whereas genotypes A and B have higher rates of HBeAg seroconversion than genotypes C and D [8]. Recent studies observed unusual mixed-genotype HBV infections, suggesting overlapping clinical outcomes [9, 10]. Genotypes A, D, and E circulate in diverse geographical locales in Africa. Genotype A is the dominant genotype in southern, eastern and central Africa, genotype D prevails in northern Africa, and genotype E predominates in western Africa [11].

To the best of our knowledge, no prior study of HBV genotypes has been conducted in Eritrea. Considering Eritrea's proximity to highly endemic countries where diverse genotypes have been reported including Sudan [10, 12, 13], Ethiopia [14], and Kenya [15], establishment of the nature of HBV infection in the country becomes of paramount important. Therefore, the present work aimed to define the most prevalent HBV genotypes among patients with liver disease in Eritrea.

Methodology

Study design and patients

This was a cross-sectional, laboratory-based study. Sera were collected from 293 patients chronically infected with HBV who attended hepatology units and outpatient clinics at Orotta Referral National Teaching Hospital (ONRTH), Halibet Hospital, Sembel Hospital, and the National Health Laboratory (NHL). These are the largest hospitals with highest available diagnostic services in the country. These facilities serve as referral centers to patients coming from different geographical regions of Eritrea, which is divided into six administrative regions (zobas): Maekel, Debub, Anseba, Gash-Barka, Semienawi Keiyh Bahri (SKB), and Debubawi Keiyh Bahri (DKB). The samples were collected between January 2017 and February 2019.

All HBV positive liver disease adult patients who presented to the hospitals for management between January 2017 and February 2019 participated in the study. Patients with acute hepatitis and blood donors who tested positive for HBV surface antigen (HBsAg) were excluded from this study. The patients' sera were collected and stored at -20 °C until the experiment was completed.

Biochemical assays

Levels of liver transaminases (alanine transaminase (ALT) and aspartate transaminase (AST)) were measured using an automated clinical chemistry analyzer (Beckman coulter au480, USA) following the manufacturer's instructions. The upper limit of normal (ULN) values for both ALT and AST were 40 IU/L [13].

Serological evaluation

Sera samples from all patients were re-tested for HBsAg and screened for hepatitis B surface antibody (anti-HBc total). Those with detectable anti-HBc total and HBsAg levels were tested for the hepatitis B 'e' antigen (HBeAg) and the antibody to HBeAg (anti-HBe) using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Fortress Diagnostics, UK), according to the procedures described by the manufacturers of the Anthos labtec ELISA 2001 analyzer (Anthos Labtec, Austria).

HBV viral load quantification

The HBV DNA (viral load) level was assessed by commercial real-time PCR (COBAS AmpliPrep/COBAS TaqMan HBV test, version 2.0, Roche Diagnostics, Germany) for automated specimen processing and amplification, with a lower limit of detection of approximately 20 IU/mL. If the samples had detectable HBV DNA, a quantitative real-time PCR analysis was performed to determine the level of DNA.

HBV DNA extraction

DNA was extracted from 200 µL of the patients' sera according to the manufacturer's instructions using an Analytik Jena DNA mini-extraction kit (Analytik Jena, Germany), eluted with 60 µl of pre-heat RNase-free water, and stored at -20 °C until use.

HBV genotype

The HBV DNA genotyping system was based on multiplex-nested PCR using type-specific primers according to that applied by Naito et al. [16]. The HBV genome was amplified using the universal primers (P1 and S1-2) for the outer primers, followed by two different mixtures containing type-specific inner primers. The PCR primers used in this study are shown in Table 1.

Table 1
Primer sequences used for HBV genotyping in this study

Primers	Sequences*	(Specificity, Polarity, and Position)	Amplicon size (bp)
First round of PCR			
P1	5'-TCA CCA TAT TCT TGG GAA CAA GA-3'	(nt 2823–2845, universal, sense)	1063
S1-2	5'-CGA ACC ACT GAA CAA ATG GC-3'	(nt 685–704, universal, antisense)	
Second round of PCR			
Mix A			
B2	5'-GGC TCM AGT TCM GGA ACA GT-3'	(nt 67–86, types A to E specific, sense)	
BA1R	5'-CTC GCG GAG ATT GAC GAG ATG T-3'	(nt 113–134, type A specific, antisense)	68
BB1R	5'-CAG GTT GGT GAG TGA CTG GAG A-3'	(nt 324–345, type B specific, antisense)	281
BC1R	5'-GGT CCT AGG AAT CCT GAT GTT G-3'	(nt 165–186, type C specific, antisense)	122
Mix B			
BD1	5'-GCC AAC AAG GTA GGA GCT-3'	(nt 2979–2996, type D specific, sense)	119
BE1	5'-CAC CAG AAA TCC AGA TTG GGA CCA-3'	(nt 2955–2978, type E specific, sense)	167
BF1	5'-GYT ACG GTC CAG GGT TAC CA-3'	(nt 3032–3051, type F specific, sense)	97
B2R	5'-GGA GGC GGA TYT GCT GGC AA-3'	(nt 3078–3097, types D to F specific, antisense)	

*An “M” indicates a nucleotide that may be either A or C; a “Y” indicates a nucleotide that may be either C or T nt, nucleotide.

The first-round PCR was performed using a master cycler gradient (Ependorf, Germany) by incubating the samples at 94 °C for 5 minutes, followed by 40 cycles consisting of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and elongation at 72 °C for 2 min. The final elongation step was performed at 72 °C for 5 min.

Two second-round PCRs were performed for each first-round PCR product. Mix A was applied for the identification of genotypes A, B, and C and mix B was applied for the identification of genotypes D, E, and F. Three-microliter aliquots of the first-round PCR product were added to mix A and mix B. The reaction mixture of mix A (iNtRON, Biotechnology, Korea) contained 15 µl of nuclease-free water and 2 µl (10 pmol) of mix A primers whereas the mix B reaction mixture contained 15 µl of nuclease-free water and 2 µl (10 pmol) of mix B primers.

The second-round PCRs underwent 40 cycles with the following parameters: preheating at 95 °C for 5 min, 20 cycles of amplification at 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 30 s, and an additional 20 cycles of 95 °C for 20 s, 62 °C for 20 s, and 72 °C for 30 s. The PCR products were identified by electrophoresis on a 1.5% agarose gel and stained with gel red (1 hour and 10 min at 100 V). The bands were evaluated under a UV light transilluminator (UVP, UK). The size of the product bands was estimated according to the migration pattern of the 50 bp DNA ladder. The expected band sizes of a genotype of Mix A as A (68 bp), B (281 bp), C (122 bp), and Mix B genotype as D (119 bp), E (167 bp), and F (97 bp).

Statistical analysis

SPSS ver. 25 (IBM; Chicago, IL, USA) was used for the statistical analysis, and the values were presented as numbers, percentages, and means ± standard deviations (SD). The statistical analyses and categorical comparisons were performed using the Chi-square test (χ^2). This statistical method was used to detect statistically significant relationships between variables. A two-tailed p-value < 0.05 was considered statistically significant.

Results

Socio-demographic characteristics

Of the 293 patients enrolled in this study, 213 (72.7%) were males and 80 (27.3%) were females. The mean age ± standard deviation (SD) was 41.66 ± 13.84 years with a range of 16 to 78 years. Most of the patients were married (71.7%), followed by single (24.6%) and divorced (3.8%) patients. All administrative regions of Eritrea were represented, with 49.8% of participants from Zoba Maekel, the central region where a large proportion of the Eritrean population resides and where the study was conducted, 21.5% from Debub, 14.3% from Gash Barka, 5.5% from Anseba, 5.5% from SKB, and 3.4% from DKB. Therefore, this study was virtually representative of the population distribution across Eritrea (Table 2).

Table 2
Socio-demographic characteristics of liver disease patients (n = 293).

Variable	Gender*		Total**
	Male n (%)	Female n (%)	n (%)
Age (M = 41.66 ± SD = 13.84, Md = 41.0 ± IQR = 62, Min. = 16, Max. =78)			
Less than 28	42 (71.2)	17 (28.8)	59 (20.1)
29 to 37	44 (69.8)	19 (30.2)	63 (21.5)
38 to 44	34 (64.2)	19 (35.8)	53 (18.1)
45 to 54	43 (74.1)	15 (25.9)	58 (19.8)
55 or above	50 (83.3)	10 (16.7)	60 (20.5)
Occupation			
Government employee	92 (80.0)	23 (20.0)	115 (39.2)
Unemployed	34 (44.7)	42 (55.3)	76 (25.9)
Private workers	74 (89.2)	9 (10.8)	83 (28.3)
Students	13 (68.4)	6 (31.6)	19 (6.5)
Marital Status			
Single	56 (77.8)	16 (22.2)	72 (24.6)
Married	152 (72.4)	58 (27.6)	210 (71.7)
Divorced	5 (45.5)	6 (54.5)	11 (3.8)
Religion			
Muslim	28 (75.7)	9 (24.3)	37 (12.6)
Christian	185 (72.3)	71 (27.7)	256 (87.4)
Zoba			
Anseba	10 (62.5)	6 (37.5)	16 (5.5)
Debub	42 (66.7)	21 (33.3)	63 (21.5)
Gash Barka	32 (76.2)	10 (23.8)	42 (14.3)
Maekel	107 (73.3)	39 (26.7)	146 (49.8)
Semienawi Keiyh Bahri	14 (87.5)	2 (12.5)	16 (5.5)
Debubawi Keiyh Bahri	8 (80.0)	2 (20.0)	10 (3.4)

**Percentage is computed from the row total, **Percentage is computed from total liver patients.*

Variable	Gender*		Total**
	Male n (%)	Female n (%)	n (%)
Hospital			
National Health Laboratory	147 (76.2)	46 (23.8)	193 (65.9)
Orotta Hospital	46 (65.7)	24 (34.3)	70 (23.9)
Halibet Hospital	13 (68.4)	6 (31.6)	19 (6.5)
Sembel Hospital	7 (63.6)	4 (36.4)	11 (3.8)
Total	**213 (72.7%)**	**80 (27.3%)**	**293 (100%)**

**Percentage is computed from the row total, **Percentage is computed from total liver patients.*

HBV serology and viral load

All patients' sera were HBsAg-positive and anti-HBc-positive; 20 (6.8%) were HBeAg-positive/anti-HBe-negative, 242 (82.6%) were HBeAg-negative/anti-HBe-positive, and 31 (10.6%) were negative for both HBeAg and anti-HBe.

The median (interquartile range (IQR) serum levels of ALT and AST were 28 (15.3) and 26 (10.3) IU/L, respectively. The serum ALT levels were significantly higher in the HBeAg-negative samples than in the HBeAg-positive samples ($p < 0.001$).

The HBV DNA viral load was quantified in 122/293 (41.6%) samples, and the median (IQR) was 3.47 (1.66) log IU/ml. An undetectable viral load result was obtained in 171 (58.36%) sera samples. The median viral load of the HBeAg-negative samples [3.3 (1.55) log IU/ml] was significantly higher than that in the HBeAg-positive samples [4.2 (5.12) log IU/ml] ($p < 0.001$). Table 3 summarizes the ALT, AST, and HBV viral load (log IU) results obtained.

Table 3
Summary of ALT and HBV viral load test results

Test parameters		Males, n = 213	Females, n = 80	General, n = 293
ALT (U/L)	**Mean ± SD**	32.44 ± 15.49	35.47 ± 23.79	33.26 ± 18.0
	Median	28.0	28.0	28.0
	Minimum	11.9	17.0	11.9
	Maximum	102.4	150.6	150.6
AST **(U/L)**	**Mean ± SD**	28.76 ± 12.7	37.631 ± 33.9	31.16 ± 20.92
	Median	25.0	29.0	26.0
	Minimum	14.0	13.0	13.0
	Maximum	97.2	200.0	200.0
HBV viral load (log IU)	**Mean ± SD**	3.48 ± 1.08	3.45 ± 1.42	3.74 ± 1.17
	Median	3.49	3.28	3.47
	Minimum	1.30	1.30	1.30
	Maximum	6.68	7.95	7.95

Distribution of HBV genotypes among the study population

This study included 293 samples that were tested by the multiplex nested PCR technique for HBV DNA using type-specific primers (Fig. 1). One hundred and twenty-two samples (41.6%) were positive for HBV DNA, 57.38% of the HBV DNA-positive samples were infected with a single HBV genotype, and the remaining 42.62% had mixed genotype infections (Fig. 2). The mean age of the HBV DNA-positive patients was 40.21 (SD = 12.66) years, and males were pre-dominant (73%).

HBV genotype D (n = 26; 21.3%) was found to be the predominant circulating genotype, followed by genotypes C (n = 21; 17.2%), E (n = 19; 15.6%), C/D (n = 16; 13.1%), and C/E (n = 13; 10.7%). Genotypes C/D/E (n = 9; 7.4%), A/D (n = 6; 4.9%), D/E (n = 5; 4.1%), A (n = 3; 2.5%), and B, A/E, B/E, and A/D/C (each with n = 1; 0.8%) were also present (Table 4).

Table 4
Summary and distribution of HBV genotypes among liver disease patients with HBV in Eritrea

HBV Genotypes	n (%)	Age (M ± SD)	Gender	
			Male, n (%)	Female, n (%)
HBV mono-genotype infection				
Genotype A	3 (2.5)	33 ± 5.292	1 (33.3)	2 (66.7)
Genotype B	1 (0.8)	-	1 (100)	0
Genotype C	21 (17.2)	39.19 ± 10.736	14 (66.7)	7 (33.3)
Genotype D	26 (21.3)	39.77 ± 13.168	17 (64.4)	9 (34.6)
Genotype E	19 (15.6)	37.42 ± 10.052	12 (63.2)	7 (36.7)
HBV mixed-genotype infection				
Genotype A/D	6 (4.9)	32.17 ± 10.815	6 (100)	0 (0.0)
Genotype A/E	1 (0.8)	-	0 (0.0)	1 (3.0)
Genotype B/E	1 (0.8)	-	1 (100)	0 (0.0)
Genotype C/D	16 (13.1)	42.56 ± 11.302	9 (56.3)	7 (21.2)
Genotype C/E	13 (10.7)	42.54 ± 14.339	13 (100)	0 (0.0)
Genotype D/E	5 (4.1)	55.00 ± 11.467	5 (100)	0 (0.0)
Genotype A/D/C	1 (0.8)	-	1 (100)	0 (0.0)
Genotype C/D/E	9 (7.4)	44.11 ± 17.222	9 (100)	0 (0.0)
Total	**122 (100%)**	**40.21 ± 12.660**	**89 (73%)**	**33 (27%)**

(-) Mean and SD cannot be computed because there was only one patient each with genotype B, A/E, B/E, and A/D/C.

Serological and biochemical characteristics of patients (n = 122) with HBV genotypes

All the HBV serological and biochemical characteristics of the 122 liver patients with genotyped HBV are shown in Table 5. The results of the HBV mono-genotype analysis revealed that in the HBeAg-positive patients infected with a single genotype, only genotype D (25.0%) was found (Fig. 1); there were no patients with genotypes A, B, C, or E. The median (IQR) ALT level of genotype D was 30.0 (17.2) IU/L. Moreover, the median (IQR) AST level was 26.0 (7.3) IU/L, and the average viral load in log median (IQR) was 3.39 (2.31) IU/ml. Conversely, the results of the mixed HBV genotype analysis showed (Fig. 3) that in the HBeAg-positive patients infected with multiple HBV genotypes, genotypes C/D (10%) (Fig. 4), C/E (10%), C/D/E (5%), A/D (5%), D/E (5%), and A/E (5%) were found, but there were no patients with

genotypes B/E or A/D/C. The median (IQR) ALT levels of genotypes C/D and C/E were 34.0 (16.8) and 26.0 (15.5) IU/L, respectively. Moreover, the median (IQR) AST levels of genotypes C/D and C/E were 30.0 (10.5) and 26.0 (9.6) IU/L, respectively. For these respective genotypes, the average viral load in log median (IQR) was 2.77 (1.21) and 3.59 (0.75) IU/ml, respectively.

Table 5
Serological and biochemical characteristics of 122 liver patients with genotyped HBV

HBV Genotypes	Serological and Biochemical Characteristics				Total n (%)
	HBeAg Positive (%)	ALT, IU/L Median (IQR)	AST, IU/L Median (IQR)	Viral load Log Median IU/ml (IQR)	
HBV mono-genotype					
Genotype A	0	22.0 (-)	20.0 (-)	3.48 (-)	3 (1.0)
Genotype B	0	-	-	-	1 (0.3)
Genotype C	0	26.0 (11.0)	30.0(11.5)	3.63 (1.46)	21 (6.9)
Genotype D	5 (25.0)	30.0 (17.2)	26.0 (7.3)	3.38 (2.31)	26 (8.5)
Genotype E	0	29.0 (14.6)	29.0 (20.5)	3.46 (1.97)	19 (6.2)
HBV mixed genotypes					
Genotype A/D	1 (5.0)	24.9 (42.9)	21.0 (23.7)	3.59 (3.91)	6 (2.0)
Genotype A/E	1 (5.0)	-	-	-	1 (0.3)
Genotype B/E	0	-	-	-	1 (0.3)
Genotype C/D	2 (10.0)	34.0 (16.8)	30.0 (10.5)	2.77 (1.21)	16 (5.2)
Genotype C/E	2 (10.0)	26.0 (15.5)	26.0 (9.6)	3.59 (0.75)	13 (4.3)
Genotype D/E	1 (5.0)	38.0 (51.2)	29.0 (40.6)	3.51 (2.02)	5 (1.6)
Genotype A/D/C	0	-	-	-	1 (0.3)
Genotype C/D/E	1 (5.0)	29.0 (22)	22.5 (8.6)	4.09 (1.40)	9 (3.0)

(-) Median and IQR cannot be computed because there was only one patient each with genotypes B, A/E, B/E, and A/D/C.

Distribution of HBV genotypes in six geographical zobas

The distribution of different HBV genotypes was described in the different zobas of Eritrea (Table 6). The majority of patients (45.1%) were from Maekel, followed by Debub (20.2%), Gash Barka (18%), Anseba (8.2%), SKB (4.9%), and DKB (3.3%).

Table 6
Distribution of different HBV genotypes in different zobas of Eritrea

HBV Genotypes	Zoba						N
	Anseba	Debub	Gash Barka	Maekel	SKB	DKB	
	(n = 10), %	(n = 25), %	(n = 22), %	(n = 55), %	(n = 6), %	(n = 4), %	
HBV mono-genotype infection							
Genotype A	0	1 (4%)	0	2 (3.6%)	0	0	3
Genotype B	0	0	0	1 (1.8%)	0	0	1
Genotype C	5 (50%)	4 (16%)	4 (18.2%)	7 (12.7%)	1 (16.7%)	0	21
Genotype D	1 (10%)	4 (16%)	6 (27.3%)	13 (23.6%)	1 (16.7%)	1 (25%)	26
Genotype E	1 (10%)	4 (16%)	3 (13.6%)	8 (14.5%)	1 (16.7%)	2 (50%)	19
HBV mixed-genotype infection							
Genotype A/D	0	1 (4%)	0	4 (7.3%)	1 (16.7%)	0	6
Genotype A/E	0	1 (4%)	0	0	0	0	1
Genotype B/E	0	1 (4%)	0	0	0	0	1
Genotype C/D	1 (10%)	4 (16%)	4 (18.2%)	7 (12.7%)	0	0	16
Genotype C/E	0	1 (4%)	3 (13.6%)	8 (14.5%)	0	1 (25%)	13
Genotype D/E	0	1 (4%)	1 (4.5%)	2 (3.6%)	1 (16.7%)	0	5
Genotype A/D/C	0	0	1 (4.5%)	0	0	0	1
Genotype C/D/E	2 (20%)	3 (12%)	0	3 (5.5%)	1 (16.7%)	0	9

Among the mono-HBV genotype infections in Eritrea, genotype D (Fig. 1) was predominant in Maekel (23.6%) and Gash Barka (27.3%). HBV genotype C was predominant in Anseba (50%), genotype E was predominant in DKB (50%), and HBV genotypes C, D, and E were equally predominant in Debub (16%) and SKB (16.7%) (Table 6).

Among the mixed-HBV genotype infections, genotype C/D (Fig. 3) was predominant in Gash Barka (18.2%) and Debub (16%) whereas HBV genotype C/E was predominant in Maekel (14.5%) and DKB (25%). Conversely, HBV genotype C/D/E was predominant in Anseba (20%) whereas genotypes D/E and C/D/E were equally predominant in SKB (16.7%) (Table 6).

Discussion

Hepatitis B virus (HBV), a highly contagious infectious disease being as a major threat in developing countries. Hence, this study focuses on assessing the prevalence of HBV genotype in Eritrea. This molecular genotyping of HBV was first of its kind in Eritrea using a PCR-based method, and no data about the genotypes and mutants of HBV in patients with liver disease were previously reported.

Different HBV genotyping methods have been developed including sequencing, INNO-LiPA, restriction fragment polymorphism, multiplex PCR, serotyping, oligonucleotide microarray chips, reverse dot blot, restriction fragment mass polymorphism, invader assay, and real-time PCR. However, the sensitivity, specificity, expense and time requirements differ among these methods [17]. In this study, we focused on six major genotypes (A–F) among our patients using the multiplex nested PCR technique developed by Naito et al. [16]. This method appears to have higher sensitivity for detecting mixed genotypes, and it is simple and cost-effective for large population studies with a high accuracy rate of 93% [17].

The genotypes of HBV were examined in 293 patients, and samples were successfully genotyped in 122 (41.6%) patients. This genotype success rate was comparable with the findings obtained using different genotype methods in various countries, such as United Arab Emirates, Nigeria, Egypt, India, Cote d'Ivoire and Iraq, in which the genotype success rates were 95%, 83.6%, 71.4%, 69.7%, 68.7%, and 60.5%, respectively [18–22]. These findings confirmed the variability of different methods of HBV-DNA detection in relation to HBsAg positivity, and that the variability could be influenced by whether patients have chronic infection [23].

The most important finding in our results was that single-genotype HBV infection (57.38%) was more common than mixed-genotype infection (42.62%). This is in concordance with findings from Taiwan, in which genotype B infection was most common [24]. Our finding of single-genotype predominance also agrees with a study in Egypt using INNO-LiPA, which reported that 87% of patients harboured single-genotype infection, most commonly genotype D [25]. However, these findings contrast with those from Iraq and Nigeria, in which mixed-genotype infections were documented in 75 and 82.6% of patients, respectively [19, 26].

The identification of five HBV genotypes (A–E) in this study corroborates their higher prevalence in certain geographical regions in Africa [2, 4, 27]. Irrespective of the occurrence of mixed or single infection, our study identified that genotype D had the highest prevalence, followed by genotypes C, E, C/D, B, A/E, B/E, and A/D/C. However, in terms of single-genotype infection, genotype D was the most prevalent, followed by genotypes C, E, A, and B. On the other hand, concerning mixed-genotype HBV infection, genotype C/D infection predominated, followed by genotypes C/E, C/D/E, A/D, D/E, A/E, B/E, and A/D/C.

We found that the genotypes of HBV in Eritrea conform to those described in the region. Our results of this study concur with previous findings in neighbouring countries, including the predominance of HBV genotype D in Sudan [12, 13] and Egypt [28]. Globally, mixed-genotype HBV infection has been reported to predominant in different regions [10]. Eritrea's geographical location in the horn of Africa with close proximity to Asia through the Red Sea could explain the nature of distribution of HBV genotypes in the country. The observation of genotype C infection alone or together with genotype D, especially in people hailing from coastal areas of Eritrea, may be attributable to frequent migration and contact with people from regions such as the Middle East because of commercial activities. In addition, mixed-genotype infection was noted in patients from different geographical areas of Eritrea, in line with observations of infection by genotypes A, D and E, including single and mixed infections, in Sudan [10]. In addition, the observation of mixed-genotype infection in this country could be linked to the significant number of refugees returning from neighbouring and distant countries [29].

The clinical impact of HBV genotype D has not been studied extensively. Emerging evidence suggests that patients with genotype D infection may develop fulminant hepatitis at high frequency [30]. The prevalence of different HBV genotypes in our study subjects provides a basis to compare different parameters in a stratified manner for various genotypes (Table 5). Patients infected with HBV genotype C exhibited a higher viral load (3.63 log IU/ml) than that reported in one study [31] but this was not true when compared with a second report [14]. When assessing HBeAg seropositivity among isolated genotypes, a significantly higher frequency of HBeAg positivity was noted among reported genotypes. In a similar study, Yousif et al. found higher rates of HBeAg positivity in patients with liver disease associated with HBV genotype D [13]. Conversely, Mahgoub et al. observed higher HBeAg positivity rates in blood donors infected with HBV genotype E [32].

The distribution of HBV genotypes within the country was assessed using the location where the patients hailed from. Concerning single-genotype infection, genotype D was predominant in Zoba Maekel (23.6%) and Gash Barka (27.3%). Among mixed HBV genotypes, genotype C/D infection was predominant in Gash Barka (18.2%) and Debub (16%), whereas HBV genotype C/E infection was predominant in Maekel (14.5%) and DKB (25%). Similarly, the genotype distribution among various subgroups in various countries, including Eritrea, was reported by Scotto et al. [33], and Asaad et al. described the genotypic distribution of HBV in the Saudi population [34].

Knowing the predominant HBV genotype in specific areas is important for assessing diagnostic capabilities and vaccine efficacy [1]. Our study narrows the existing gaps in HBV molecular research in

Eritrea. All of our isolates were obtained from patients with chronic HBV infection in hospital settings. Generally, patients with liver disease who are identified as reactive for HBV infection during the study were not monitored or evaluated concerning the exact stage of their liver disease such as cirrhosis or hepatocellular carcinoma (HCC). Hence, we suggest that HBV genotyping of patients be studied in correlation with the clinical progression of liver disease to provide a clear clinical picture and molecular epidemiology. Furthermore, future studies must evaluate the clinical relevance, treatment response, and rates of co-infection, which may affect disease outcome.

Conclusion

In conclusion, this study portrays the overall prevalence of HBV genotypes among Eritrean liver disease patients infected with HBV who seek medical attention at the hospital setting. In mono-HBV genotype infections, genotype D was the most prevalent genotype. In mixed-HBV genotype infections, genotype C/D was the most prevalent among the study region. The high prevalence of genotype D was similar to most of the previous studies, including those in Saudi Arabia [34]. This results in poor outcomes in clinical management. In the future, clinical trials and treatment regimens should be postulated individually based on the genotype to effectively manage chronic HBV infection. To that ends, a prospective nation-wide population study of HBV genotype distribution and clinical outcome is recommended.

Abbreviations

ALT
Alanine amino transferase;
AST
Aspartate transaminase;
DKB
Debubawi Keiyh Bahri;
DNA
Deoxyribonuclease;
ELISA
Enzyme-linked immunosorbent assay;
HBcAb
Hepatitis B core antibody;
HBeAb
Hepatitis B e antibody;
HBeAg
Hepatitis B e antigen;
HBsAb
hepatitis B surface antibody;

HBsAg
Hepatitis B surface antigen;
HBV
Hepatitis B virus
HCC
Hepatocellular carcinoma;
IQR
Interquartile range;
M
Mean;
Max
Maximum;
Md
Median;
Min
Minimum;
PCR
Polymerase chain reaction;
SD
Standard deviation;
SKB
Semienawi Keiyh Bahri;

Declarations

Ethics approval and consent to participate:

This study was approved by the ethics committee of the Orotta College of Medicine and Health Sciences and the health facility management division of the Ministry of Health. All patients provided written informed consent to participate in this study.

Consent for Publication:

Since, it is case report. Consent for publication is not applicable here. Personal or identifying information of study participants is not disclosed in any form in this paper.

Availability of data and materials

All datasets used for this study are available from corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' contributions

MEH, SMR, YS, IME and FT conceived and designed the study. MEH, SMR, YP and MW analyzed the data and revised the paper. MEH and SMR wrote the manuscript. All authors read and approved the final manuscript.

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Figures

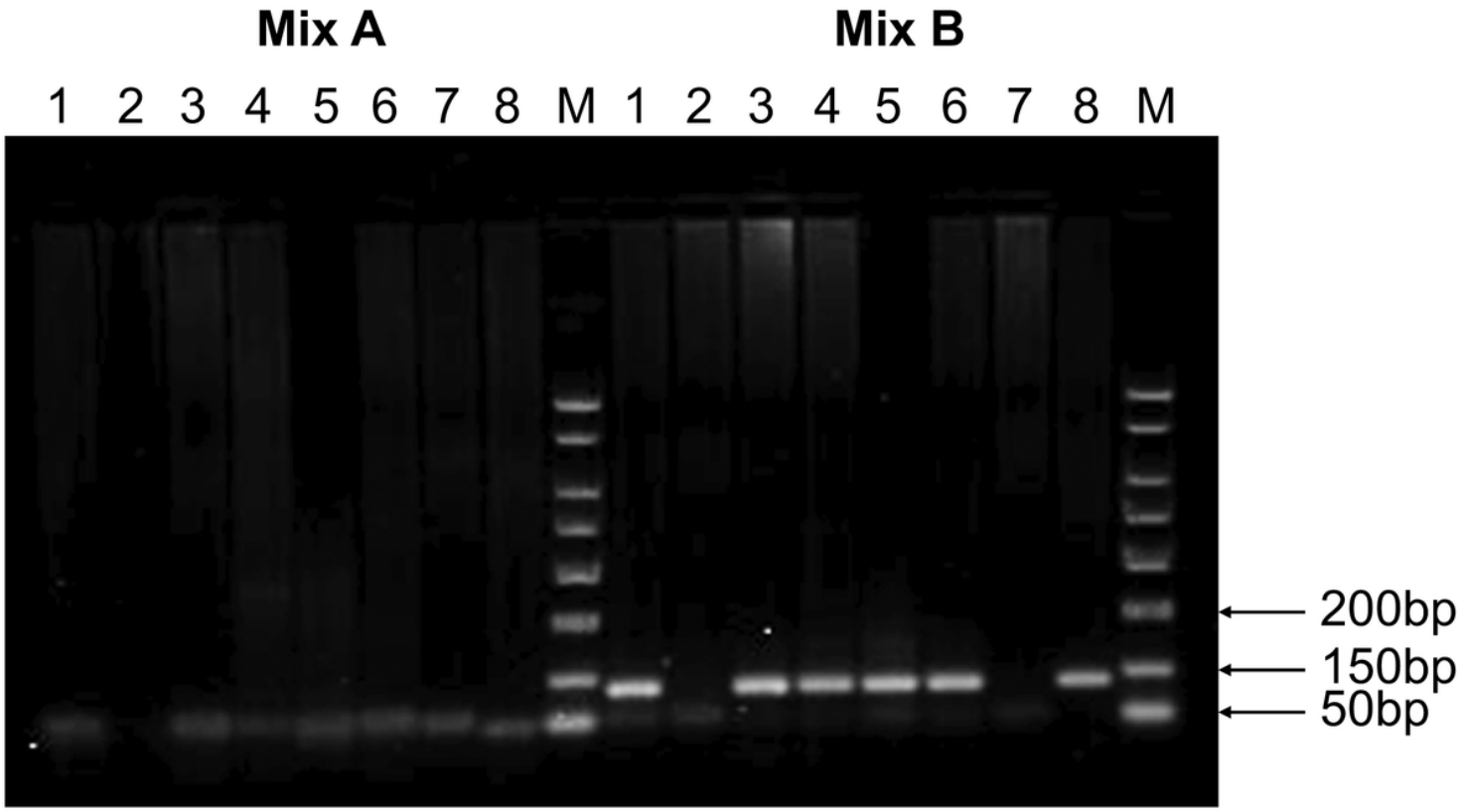

Figure 1

Second-round PCR products showing a single genotype (D, 119 bp). M, molecular size standards.

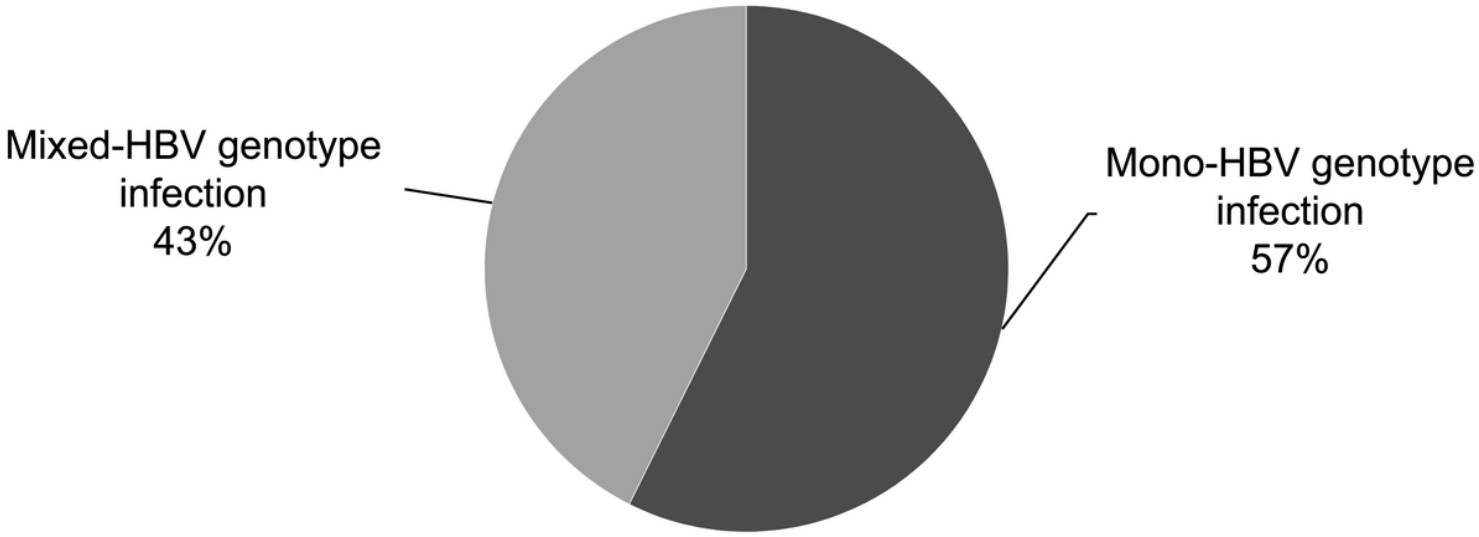

Figure 2

Proportion of mono- and mixed genotype infections in HBV DNA-positive liver disease patients in Eritrea.

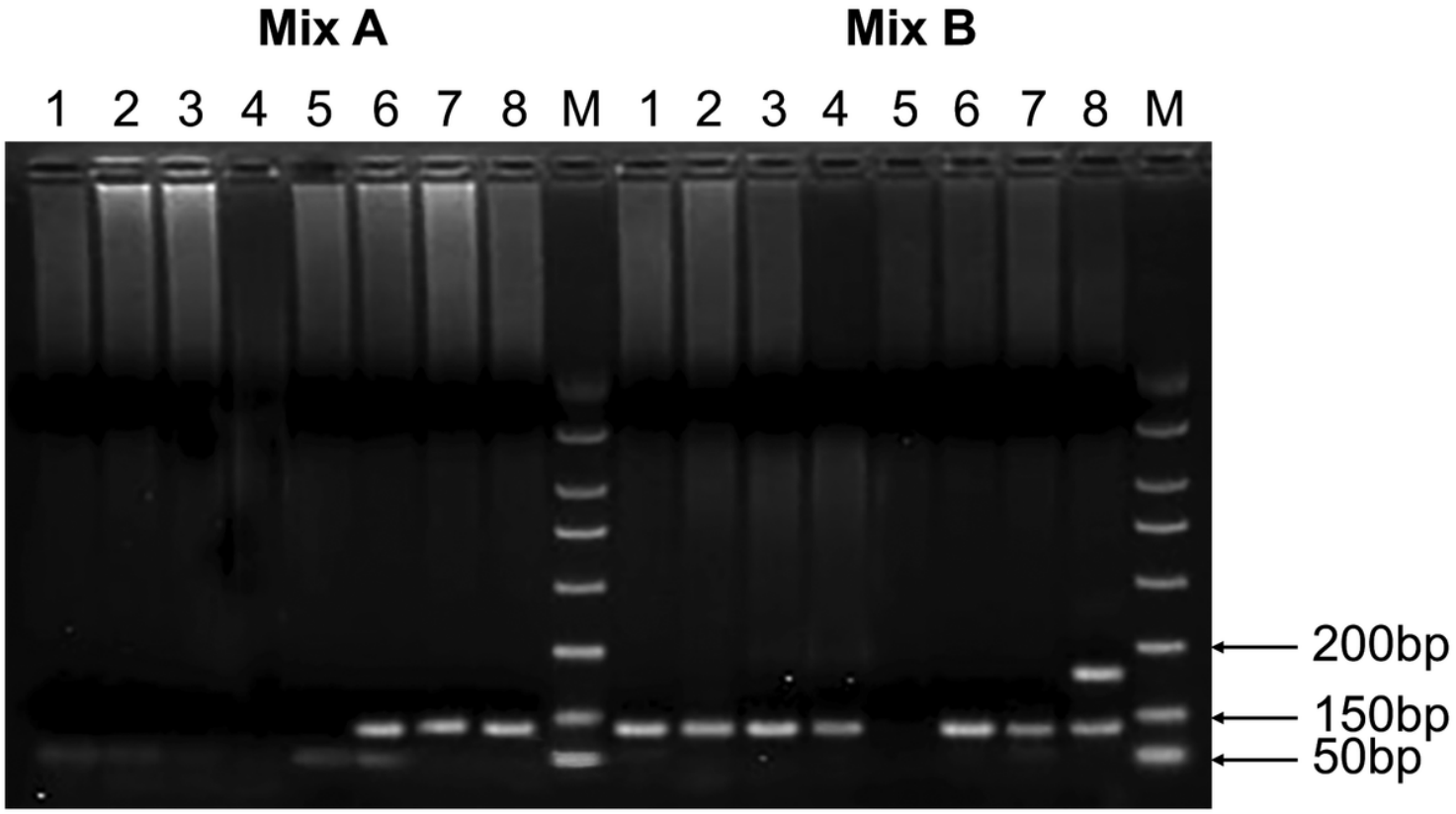

Figure 3

Second-round PCR showing the mixed genotype of C/D, mixed genotype of C/D/E.

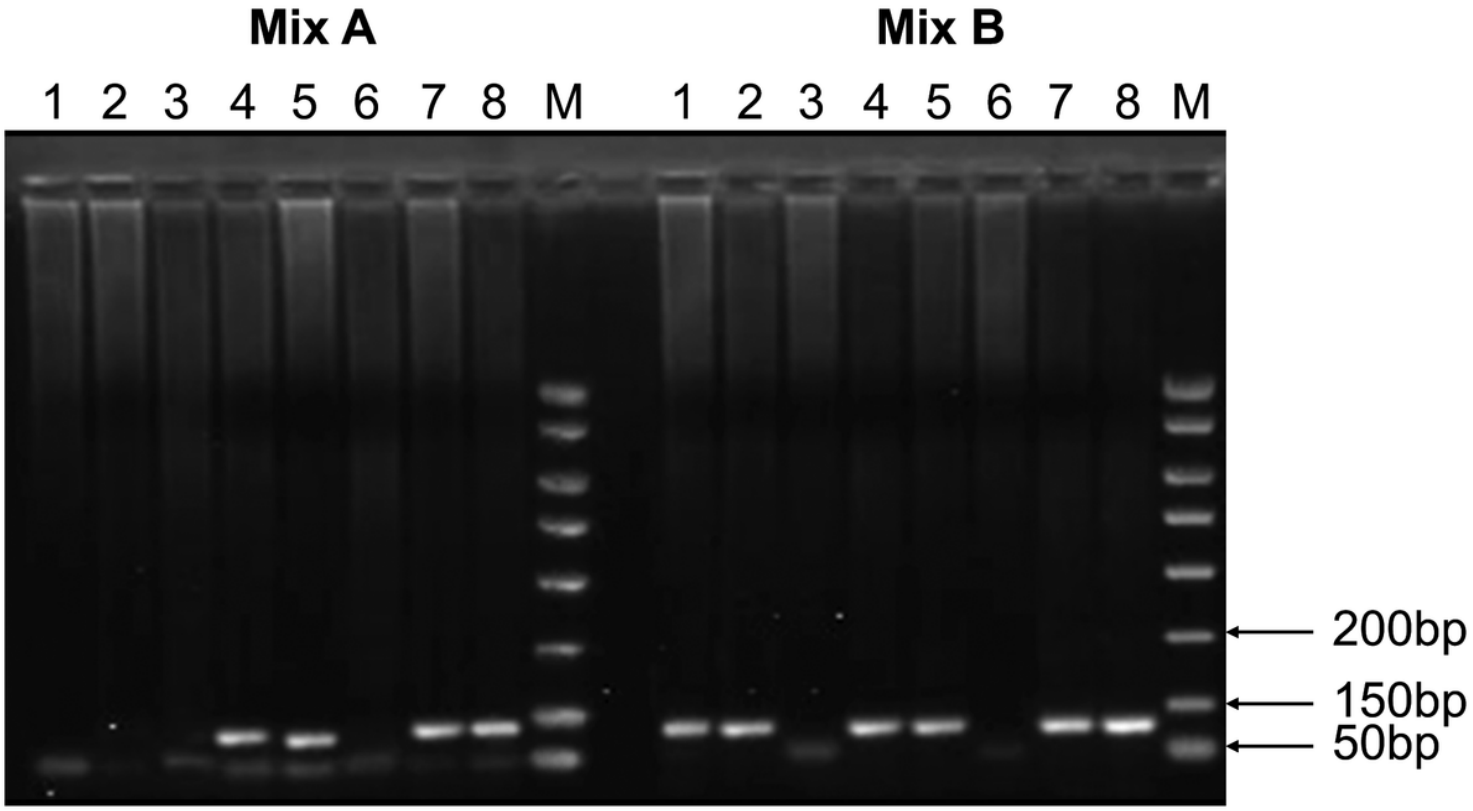

Figure 4

Second-round PCR products showing the mixed genotype of C/D and single genotype of D.

Supplementary Files

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