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The association of hepatitis B virus genotypes with liver cirrhosis in HBV-infected Sudanese patients

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ABSTRACT

Background: The genome of hepatitis B virus (HBV) includes genetic variations arranged into ten genotypes, eight well-known genotypes labeled from A to H, plus two new genotypes called I and J. Several studies suggest that the variations between HBV genotypes are closely associated with disease progression, treatment, and clinical outcome. This study aimed to associate HBV genotypes with liver cirrhosis among HBV-infected patients in Sudan.

Methods: Ninety sera were collected from HBV-infected patients; 30(33.3%) of the participants were suffering from liver cirrhosis, and the rest were non-cirrhotic HBV patients as the control group. HBV genotypes were determined by PCR, and HBV viral load was estimated by real-time PCR. The concentration of AST and ALT was measured by a fully automated chemistry analyzer.

Results: Genotype D is the most frequent genotype **(94.4%)** in this study population, followed by genotypes B (52.2%), A (20%), and E (1.1%). Genotypes C, F, G, H, and I were not detected. Mixed genotypes were detected in most (60%) participants. Genotype D was the most frequent genotype **(93.3%)** among the cirrhotic group, as well as showing the highest level of viral loads, AST, and ALT.

Conclusion: Genotype D is more associated with severe manifestations because it showed the highest levels of viral load, AST, and ALT. Genotypes B and B+D mixed infections are more probably associated with the development of liver cirrhosis in HBV infected patients, and genotypes B and D are more frequent among patients with liver cirrhosis regardless of whether they are a single genotype or mixed with other genotypes.

Key words: HBV, genotypes, Liver cirrhosis

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Introduction

Hepatitis B virus is a *hepadnavirus* that belongs to the genus *Orthohepadnavirus* of the *Hepadnaviridae* family (1). The virus is an enveloped icosahedral nucleocapsid virion containing the length of 3200 base pairs (bp) of a circular, partially double-stranded deoxyribonucleic acid (DNA) genome. The two unequal strands of the DNA genome consist of the short strand (S strand), which is the positive sense, and the long strand (L strand), which is a negative sense or non-coding and complementary

to viral messenger ribonucleic acid (mRNA) (2, 3). The viral genome contains four genes (C, P, S, and X) or four open reading frames (ORFs) that encode seven proteins that include structural proteins of the virion surface and core, a small transcriptional trans-activator (X), and a large polymerase (2). The C gene encodes HBcAg and hepatitis B e antigen (HBeAg), the P gene encodes DNA polymerase, which also acts as reverse transcriptase, and the S gene encodes HBsAg. This gene is divided into three sections; pre-S1, pre-S2, and S sequences, and the size of the final polypeptides of these sequences is varied according to the number of build-up sequences into large (pre-S1+pre-S2+S), middle (pre-S2+S), and small (S) (2, 3, 4). The last X gene encodes for hepatitis B virus X protein (HBx), which may be involved in the oncogenesis prosperities of the virus because it can inactivate the p53 tumor suppressor protein besides its role as an activator of viral RNA transcription (2).

Genotypes

The genetic variations of HBV were arranged into ten genotypes, eight well-known genotypes labeled from A to H, plus two new genotypes called I and J (5, 6, 7). Genotypes A, D, and G have global distributions, whereas genotypes B and C are commonly found in East and Southeast Asia, genotype E is found in West Africa, while genotypes F and H are in Central and South America (5). Usually, there is a 10–13%, or at least an 8–9% difference in nucleotide sequences between each genotype, except for the genome of genotype F, which diverges by 14% (5, 8, 9). There are at least 26 subtypes with a difference of 4-8% in the genome sequences (8).Type A has three subtypes (A1, A2, and A3), Aa (A1), which is predominant in Africa and Asia, and subtype Ae (A2), which is found in Europe and the United States. Genotype B is found in Asia and is divided into Ba and Bj. Ba (B1) is further sub-typed into (B2, B3, B4, and B5) whereas Bj is found in Japan. Type C was divided into six subgroups (C1-C6): type Cs (C1) is found in Southeast Asia, Ce (C2) in East Asia, C3 in New Caledonia and Polynesia, C4 in Australia, C5 and C6 in the Philippines. Genotype D is globally distributed and subdivided into nine sub-genotypes (D1-D9) (7). Genotype F was sub-grouped into four subtypes (F1–F4) with further sub-typing of F1 into F1a and F1b (9, 10, 11). The variations between HBV genotypes are closely associated with disease progression, treatment, and clinical outcome (12).

Materials and Methods

This study was conducted at *Ibn Sina* specialized hospital and Alzaiem Alazhari University in Khartoum State during the period from June 2016 to June 2017. Ninety blood samples were collected from HBV-infected patients, and their infections were confirmed by being positive for HBsAg using ELISA (*Fortress Diagnostics).* The population was divided into two categories: Category A includes HBV-positive patients with liver cirrhosis, and they were set as a case group in the study population. Category B contains HBV carriers, acutely infected patients, and

recently infected individuals. All participants in category B had no liver cirrhosis, and they were used as the control group.

Ethical considerations: The ethical considerations and conformity of individuals in this study were considered with the approval of the ethical committees of Alzaiem Alazhari University and Ibn Sina Specialized Hospital, in addition to the documented agreements included in the questionnaire and signed by the participants.

DNA extraction: HBV DNA was extracted from all specimens using the G-spinTM Total DNA Extraction Kit from Invitrogen. HBV viral load was estimated by real-time *PCR (iNtRON Biotechnology Incorporation*). The concentration of AST and ALT was measured by a fully automated chemistry analyzer (*Mindray Bs120*) and *BioSystem* reagent kits.

Determination of HBV genotypes: HBV genotypes were determined by the PCR-Based Genotyping method, which is a rapid and specific genotyping system for HBV that corresponds to six major genotypes (A–F) by PCR using type-specific primers.In the first PCR, primer set P1/S1-2 was used to generate a 1103 bp product; in the second PCR, primer mix A contains B2/BA1R (68 bp) for genotype A, B2/BB1R (281 bp) for genotype B, B2/BC1R (122 bp) for genotype C; primer mix B contains BD1/B2R (119 bp) for genotype D, BE1/B2R (167 bp) for genotype E, and BF1/B2R (97 bp) for genotype F (13).

Table (1): Specific primers sequences use for HBV genotypes

Primer	Sequence (5′-3′)	Nucleotide Position	Specificity
P1 (sense)	TCA CCA TAT TCT TGG GAA CAA GA	2817–2839	Common
S1-2 (antisense)	CGA ACC ACT GAA CAA ATG GC	704–684	Common
B2 (sense)	GGC TCM AGT TCM GGA ACA GT	67–86	types A–E
BA1R (antisense)	CTC GCG GAG ATT GAC GAG ATG T	113–134	type A
BB1R (antisense)	CAG GTT GGT GAG TGA CTG GAG A	324–345	type B
BC1R (antisense)	GGT CCT AGG AAT CCT GAT GTT G	165–186	type C
BD1 (sense)	GCC AAC AAG GTA GGA GCT	2979–2996	type D
BE1 (sense)	CAC CAG AAA TCC AGA TTG GGA CCA	2955–2978	type E
BF1 (sense)	GYT ACG GTC CAG GGT TCA CA	3032–3051	type F
B2R (antisense)	GGA GGC GGA TYT GCT GGC AA	3078–3097	types D–F

Method

Forty µl of the first PCR mixture were prepared, containing 50 ng of each outer primer (P1/S1-2), 200 micromolar (µ*M*) of each of the dNTPs, 1 U of Takara Ex Taq DNA polymerase, and 1 1 × PCR buffer containing 2 millimolar (m*M*) MgCl2. The samples were incubated in a thermocycler at 95°C for 2 min; 40 cycles of 94°C for 20 sec, 55°C for 20 sec and 72°C for 30 sec. The second PCR mixture was prepared as

the first PCR (two second-round PCRs are performed for each sample), with the common universal sense primer (B2) and mix A for types A–C, and the common universal antisense primer (B2R) and mix B for types D–F, respectively, together with a 2 µl aliquot of the first PCR product. The second PCR mixture was preheated at 95°C for 2 min, amplified for 20 cycles of 94 94°C for 20 sec, 58°C for 20 sec, 58 °C for 20 sec, and 72°C for 30 sec, and additional 20 cycles of 94°C for 20 sec, 60°C for 20 sec, and 72°C for 30 sec. Each of the two different second-PCR products from one sample was separated by electrophoresis on a 3% agarose gel, stained with ethidium bromide, and evaluated under UV light. Determination of the genotypes of HBV for each sample was done by identifying the genotype-specific DNA bands (13). The size of amplicons was estimated according to the migration pattern of a 50-bp DNA ladder (Pharmacia Biotech).

Results

The participants were divided into two main groups. One group included 30 participants with liver cirrhosis. The second group was composed of 60 HBV positive participants without liver cirrhosis. The final group included 30 HBV carriers, 19 acute hepatitis patients, and 11 newly diagnosed HBV patients.

Out of all ninety patients with HBV infection, genotype D was the most frequently detected genotype and identified in 85 (94.4%) of the specimens, whereas the other genotypes of B, A, and E were detected in 47(52.2%), 18(20%) and 1(1.1%) patients respectively.

As a singular genotype, D was found in 32(35.6%), B in 3(3.3%) and A in 1(1.1%). Mixed genotypes either two or three genotype was detected. The most frequent mixture was B+D, which occurred in 37(41.1%) followed by A+D in 10(11.1%) then A+B+D in 6(6.7%) and finally A+B+E in 1(1.1%) out of the 90 blood samples (Table 1).

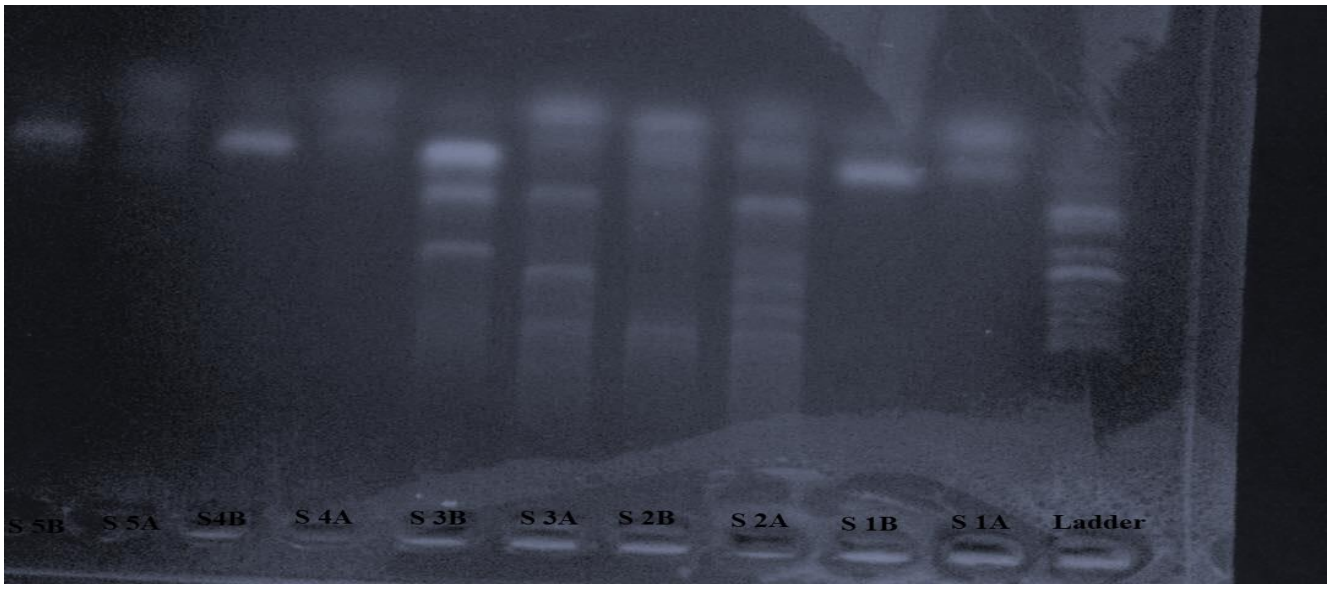
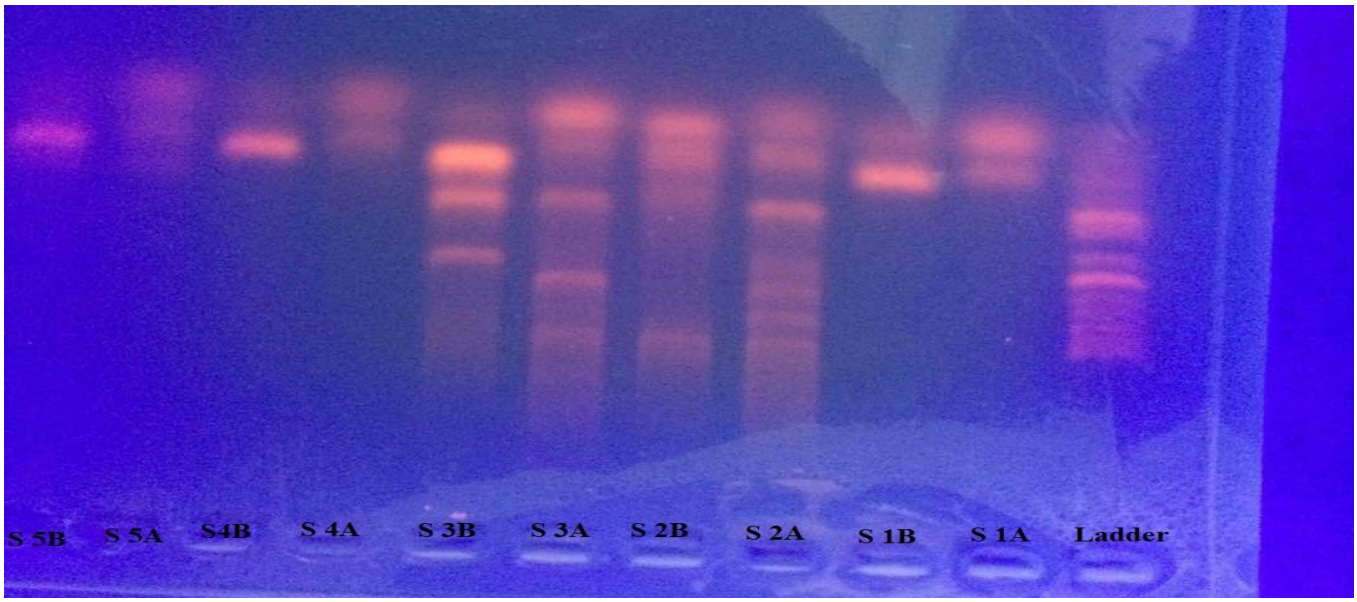


Figure 1: HBV DNA amplification from a plasma sample of liver cirrhosis among HBV infected patients



Figuren2: Characteristic bands of HBV genotypes

Table (2): The frequencies of HBV genotypes according to study groups

Genotypes	Study groups				Total
	HBV infection with liver cirrhosis	**HBV carriers**	**Acute HBV infection**	**Recent HBV infection**	
B+D	18(60.0)	6(20.0)	8(42.1)	5(45.5)	37(41.1)
D	9(30.0)	14(46.7)	4(21.1)	5(45.5)	32(35.6)
A+D	1(3.3)	5(16.7)	4(21.1)	0(0.0)	10(11.1)
A+B+D	0(0.0)	4(13.3)	1(5.3)	1(9.1)	6(6.7)
B	1(3.3)	1(3.3)	1(5.3)	0(0.0)	3(3.3)
A	0(0.0)	0(0.0)	1(5.3)	0(0.0)	1(1.1)
A+B+E	1(3.3)	0(0.0)	0(0.0)	0(0.0)	1(1.1)
Total	30(100.0)	30(100.0)	19(100.0)	11(100.0)	90(100.0)

Genotype D was the most frequent in patients with liver cirrhosis (93.3%) (Table 2), being present alone in 9(30%) patients, in contribution with genotype B in 18(60%) patients, and with genotype A in only one (3.33) patient. (Table 8)

Table (3): The prevalence of HBV genotypes among HBV infection groups with liver cirrhosis

Genotypes	Out of all 90 patients	Out of all 30 cirrhosis patients	Cirrhosis occurrence ratio from all genotypes
D	85(94.4)	28(93.3)	28(32.9)
B	47(52.2)	20(66.7)	20(42.5)
A	18(20)	2(6.7)	2(11.1)
E	1(1.1)	1(3.3)	1(100)

Regarding the patients with acute hepatitis, genotype D was the most frequently identified; it was detected in 17 out of the 19 patients (89.5%). It was found alone in 4(21.1%) patients, together with genotype A in 4 (21.1%) patients, and with A and B in one additional (5.3%) patient. The second most frequent genotype detected was B in 10 (58.8%) patients (Table 1).

Genotype D was the most frequent genotype detected in patients with recent HBV infection (100%), followed by genotype B in 6 out of 11 (54.5%) patients (Table 1). The least detected HBV genotype was type E in only one patient (Table 1).

The most common genotype among HBV carriers was D, which was found in 29 out of 30 (96.7%) patients, singular in 14 (46.7%) patients, mixed with B in 20% of patients, A in 16.7% of patients, and A and B in 13.3% of patients. Genotype B alone was detected in only one (3.3%) patient.

A mixture of B and D genotypes is the most common mixture, occurring in 18 (60%) of HBV infections with liver cirrhosis, 8 (42.1%) of the acute hepatitis B group, 6 (20%) of the HBV carriers, and 5 (45.5%) of the recently infected HBV group of the study population.

A triple mix of genotypes A, B, and D is found as four (13.3%) carrier groups, one (9.1%) recent HBV infection group, and one (5.3%) acute HBV infection group. A, B, and E mixtures are found only in HBV-infected people with liver cirrhosis group as one (3.3%).

There was an insignificant association between genotype D and the development of liver cirrhosis among HBV infected individuals (P value 0.543), and the genotype is less probably associated with liver cirrhosis (odds ratio 0.737/likelihood ratio 0.103) (Table 3).

Table (3) shows that HBV genotype B has an insignificant association with (P value 0.052), despite being statistically more likely to be associated with liver cirrhosis (odd ratio 2.444/likelihood ratio 3.821).

The same conclusion was obtained from the association of both genotypes B and D with the development of liver cirrhosis in HBV infected patients: the two genotypes are more associated with liver cirrhosis (odd ratio 2.10/likelihood ratio 2.705) with an insignificant association (P value 0.078) (Table 3).

Table (4): Associations of HBV genotypes B and D with liver cirrhosis.

Genotypes		Study groups		P value	Odd ratio	Likelihood ratio
		HBV infection with liver cirrhosis (n=30)	**HBV infection without liver cirrhosis (n=60)**			
Genotypes D	Genotypes D	28(93.3)	57(95)	0.543	0.737	0.103
	Other genotypes	2(6.7)	3(5)			
Genotypes B	Genotypes B	20(66.7)	27(45)	0.052	2.444	3.821
	Other genotypes	10(33.3)	33(55)			
Mixed	Mixed Genotypes D+B	18(60)	25(41.7)	0.078	2.10	2.705
	Other genotypes	12(40)	35(58.3)			

Table (4) shows the frequencies of viral load among different genotypes, the highest mean was found in pure genotype B (29781698.00), followed by genotype D (29185647.34), B+D (20148544.59), A+D (54487.50), A+B+D (34908.33), (253875282522.34), A (31400.00),

and the least value recognized in the mixture of A+B+E genotypes (0.0).

This indicates that the genotypes B and D showed the highest viral load regardless of whether they were pure genotypes or mixed with other genotypes, followed by A and E.

Table (5): Statistics of viral load copy/ml among different HBV genotypes

Genotypes	Mean	Std. Deviation	Minimum	Maximum
B	**29781698.00**	51284843.851	94	89000000
D	29185647.34	111960880.726	0	618000000
B+D	20148544.59	74561612.069	0	412000000
A+D	54487.50	55254.528	100	150000
A+B+D	34908.33	67171.854	87	169000
A	31400.00	.	31400	31400
A+B+E	.00	.	0	0

Tables (5) show the distribution of the liver enzymes AST and ALT among different genotypes. AST was high with genotype D (80.953), followed by genotypes B+D (66.703), A+B+E (63.000), A+B+D (50.167), A+D (49.400), A (48.000), and B (37.667). ALT was elevated in genotype D (58.869), followed by genotypes B+D (42.081), A+B+D (38.167), A+D (37.900), A (31.000), A+B+E (28.000), and B (20.667).

Table (6): Statistics of AST IU/L and ALT IU/L among different HBV genotypes.

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
AST IU/L of serum	D	**80.953**	88.233	16.00	345.00
	B+D	66.703	59.904	16.00	300.00
	A+B+E	63.000	.	63.00	63.00
	A+B+D	50.167	28.499	20.00	90.00
	A+D	49.400	24.070	25.00	100.00
	A	48.000	.	48.00	48.00
	B	37.667	21.197	15.00	57.00
ALT IU/L of serum	D	**58.869**	94.171	12.00	520.00
	B+D	42.081	25.902	15.00	105.00
	A+B+D	38.167	14.825	17.00	59.00
	A+D	37.900	11.939	16.00	60.00
	A	31.000	.	31.00	31.00
	A+B+E	28.000	.	28.00	28.00
	B	20.667	11.060	9.00	31.00

These findings illustrate that the liver enzymes AST and ALT were markedly elevated in genotype D, either pure or mixed with other genotypes.

Among the recent HBV-infected subgroup of the study population, genotype D showed the highest viral load, AST, and ALT (Tables 6).

Table (7): Statistics of viral load copy/mL, AST IU/L, and ALT IU/L within the recent HBV-infected group

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
Viral Load copy/ml of serum	B+D	183437.00	197641.529	395	467000
	D	**49028520.00**	67093275.121	26000	125000000
	A+B+D	1470.00	.	1470	1470
AST IU/L of serum	B+D	62.000	26.833	20.00	90.00
	D	**196.000**	133.869	53.00	345.00
	A+B+D	30.000	.	30.00	30.00
ALT IU/L of serum	B+D	42.600	18.636	17.00	68.00
	D	**173.800**	204.287	31.00	520.00
	A+B+D	30.000	.	30.00	30.00

Table (7) shows the statistics of study variables among the acute subgroup of the study population. Genotype D showed the highest viral load and AST, while genotypes B+D showed the highest level of ALT.

Table (8): Statistics on viral load copy/mL, AST, IU, and ALT, IU, and L HBV genotypes in acute HBV-infected patients

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
Viral Load copy/ml of serum	B+D	66328891.50	145084690.466	812	412000000
	D	**154582500.00**	308945000.184	101000	618000000
	A+D	103200.00	39355.135	53800	150000
	A+B+D	36100.00	.	36100	36100
	B	345000.00	.	345000	345000
	A	31400.00	.	31400	31400
AST IU/L of serum	B+D	116.375	95.403	36.00	300.00
	D	**138.250**	95.451	56.00	270.00
	A+D	68.000	26.445	37.00	100.00
	A+B+D	40.000	.	40.00	40.00
	B	41.000	.	41.00	41.00
	A	48.000	.	48.00	48.00
ALT IU/L of serum	B+D	**68.375**	33.619	20.00	105.00
	D	61.250	41.307	20.00	115.00
	A+D	45.000	10.000	40.00	60.00
	A+B+D	38.000	.	38.00	38.00
	B	22.000	.	22.00	22.00
	A	31.000	.	31.00	31.00

Tables (8) show the statistics of study variables among the carriers subgroup of the study population: genotype D showed the highest viral load, and genotypes A+B+D showed the highest concentrations of AST and ALT.

Table (9): Statistics of viral load copy/ml, AST IU/Land ALT IU/L among HBV genotypes within HBV carriers groups

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
Viral Load copy/ml of serum	B+D	5322.17	12531.552	69	30900
	D	**595805.36**	2226055.097	38	8330000
	A+D	7055.00	8658.764	100	20800
	A+B+D	42970.00	84025.364	87	169000
	B	94.00	.	94	94
AST IU/L of serum	B+D	29.667	8.335	21.00	40.00
	D	34.821	15.970	16.00	80.00
	A+D	39.400	12.621	25.00	55.00
	A+B+D	**57.750**	33.270	20.00	90.00
	B	15.000	.	15.00	15.00
ALT IU/L of serum	B+D	25.333	7.941	18.00	40.00
	D	24.200	9.083	12.00	42.00
	A+D	31.600	11.971	16.00	45.00
	A+B+D	**40.250**	18.392	17.00	59.00
	B	9.000	.	9.00	9.00

Tables (9) show the statistics of study variables among the cirrhosis group of the study population: genotype D showed the highest viral load, genotype D showed the highest concentration of AST, and genotype D showed the highest level of ALT.

Table (10): Statistics of viral load copy/ml, ALT IU/L among HBV genotypes within group of HBV infection with liver cirrhosis

Variable	Genotypes	Mean	Std. Deviation	Minimum	Maximum
Viral Load copy/ml of serum	B+D	11884216.67	42060594.409	0	178000000
	D	6902982.22	20623931.328	0	61900000
	A+D	96800.00	.	96800	96800
	B	**89000000.00**	.	89000000	89000000
	A+B+E	.00	.	0	0
AST IU/L of serum	B+D	58.278	43.985	16.00	155.00
	D	**63.333**	58.211	18.00	210.00
	A+D	25.000	.	25.00	25.00
	B	57.000	.	57.00	57.00
	A+B+E	63.000	.	63.00	63.00
ALT IU/L of serum	B+D	35.833	19.731	15.00	90.00
	D	**47.889**	47.179	17.00	170.00
	A+D	41.000	.	41.00	41.00
	B	31.000	.	31.00	31.00
	A+B+E	28.000	.	28.00	28.00

Discussion

The present study shows that 54 (60%) of the ninety tested specimens were mixed-infected with two or three genotypes, detailed as B+D in 37(41.1%), A+D in 10(11.1%), A+B+D in 6(6.7%) and A+B+E in 1(1.1%). These percentages are higher than the findings of other studies done in Sudan, which reported a 13.5% combination of genotype D+E (14), and much higher than the 4.3% D+E of *Shaza* and her group (15) and the 2% D+E mixed genotypes reported

by another Sudanese worker (16).These percentages are higher than the findings of another study done in Sudan, which reported a 13.5% combination of genotype D+E (14), and much higher than the 4.3% D+E of *Shaza* and her group of *Shaza et al* (15) and the 2% D+E mixed genotypes reported by another Sudanese worker. Outside Sudan, our findings were higher than the 15.7% prevalence of mixed infections, especially mixed A/D genotype infections, found in Egypt (17) and the reported 10% of mixed infections in Saudi Arabia (18). A single genotype was detected in 36(40%) out of all ninety participants, the most frequent genotypes were D 23(35.6%), B 3(3.3%) and A 1(1.1%). This finding is not far from the publications of other Sudanese workers; they reported D 46.0%, E 21.6%, and A 18.9% (16); in another study, E 57.5%, D 40.5%, and A22% (15); and the third study reported D 48.%, E 24%, and A 7%(16). Another study reported E 57.5%, D 40.5%, and A2 2% (15), while a third study reported D 48%, E 24%, and A 7% (16). Various findings from various countries have been published, including Egypt, where the prevalence of HBV genotypes was 37.1% for genotype D, 25.7% for genotype B, 10% for genotype A, and 8.6% for genotype C (16). In Côte d'Ivoire, E (87%) and A (13%); in Cameroon, E (67%) and A (33%); in Ghana, E (100%); and in Uganda, A (100%) (19).Our findings agreed with a study conducted in Saudi Arabia and reported that genotype D is the predominant genotype (81.4%), followed by E (5.7%), A (1.4%), and C (1.4%) (18). Other studies in Turkey found that all the HBV detected belonged to genotype D (20, 21). In India, genotype A accounts for 46% of the population, while genotype D accounts for 48%.Whereas the prevalence of HBV genotypes in China was 53% and 41% for genotypes C and B, respectively (23).In France and the United States, HBV genotype A was found in 54% of the specimens, while genotypes B, C, D, and E were found in 4% (12%), 14% (19%), and 1% (24%), respectively.In Poland, genotype A (56%), and genotype D (4%) were found to be (25%).This study shows that genotype D was the most frequent genotype among all the 30 HBV isolates, with liver cirrhosis individuals being detected in 28 (93.3%), genotype B in 20(66.7%), genotype A in 2(6.7%) and finally genotype E in 1(3.3%).Our study reveals that genotypes B and B+D mixed infections are more probably associated with the development of liver cirrhosis in HBV-infected patients, although the associations were insignificant. And genotypes B and D are more frequent among patients with liver cirrhosis, regardless of whether they are pure genotypes or mixed with other genotypes. These differ from the findings of several studies done in China, which reported that the genotype C of HBV is more associated with aggressive disease and the development of liver cirrhosis (26, 27, 28, 29). Other studies found that genotype A of HBV is more associated with liver cirrhosis (30, 31); in addition to that, Kobayashi and his colleagues found genotype A to be associated with milder disease than genotypes B and C (32). These

findings differ due to variation in the predominant genotypes' distribution among their study populations. The highest serum concentration of HBV DNA copies in this study was found in pure genotype D, followed by genotypes B+D, B, A+D (54487.50), A+B+D, A, and the mixture of A+B+E genotypes. Genotype D also showed the highest level of viral load in different subgroups of the study population. This was agreed by Feld and his colleagues, who reported that genotype-D infection has been associated with more advanced disease (33). The present study considers the concentration of liver enzymes among different genotypes. AST was high with genotype D (80.9531) and then genotypes B+D (66.7027), A+B+E (63.0000), A+B+D (50.1667), A+D (49.4000), A (48.0000), and B (37.6667). ALT was elevated in genotype D (58.8688), followed by genotypes B+D (42.0811), A+B+D (38.1667), A+D (37.9000), A (31.0000), A+B+E (28.0000), and B (20.6667). These findings illustrate that the liver enzymes AST and ALT were markedly elevated in genotype D, either pure or mixed with other genotypes.

Conclusion

Genotype D is more associated with severe manifestations because it showed the highest levels of viral load, AST, and ALT. Genotypes B and B+D mixed infections are more probably associated with the development of liver cirrhosis in HBV-infected patients, and genotypes B and D are more frequent among patients with liver cirrhosis regardless of whether they are a single genotype or mixed with other genotypes.

Recommendations

Based on the findings of this study, we recommend additional research to determine the role of different HBV genotypes in the development of liver cirrhosis.

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