

Molecular detection of Hepatitis D virus infection and associated factors among hepatitis B patients in Ghana

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Abstract

Background

Hepatitis D virus (HDV) infection is a growing public health challenge among individuals with chronic hepatitis B virus (HBV) infection. The HDV accelerates liver disease progression and significantly increases the risk of cirrhosis and hepatocellular carcinoma (HCC). Despite this, the epidemiology of HDV in Ghana remains inadequately documented. This study sought to determine the prevalence of HDV and its associated risk factors among HBV infected patients in Ghana.

Methods

A cross-sectional study was adopted, where 152 serum samples were collected from HBV-infected persons attending four selected hospitals. Each sample was screened for HBsAg, and those positive were further tested for HDV. The HDV viral load was estimated using a real time reversed transcription-quantitative polymerase chain reaction (rRT-qPCR) assay and were further sequenced for both genotyping and phylogenetic analysis.

Results

Out of the 152 serum samples, 85 (55.9%) were males and 144 tested positives for Hepatitis B surface antigen (HBsAg). Of the 144 HBsAg positives 2 had detectable HDV-RNA of genotype 1. Age was associated with both HBV and HDV infections ($P < 0.05$). Furthermore, an association was established between multiple sexual partners and HDV infection ($P = 0.030$). The study recorded 9 (5.9%) of the males with HCC.

Conclusion

The study established a 1.4% prevalence of HDV infection by molecular detection. This gives an indication of the virus' presence and circulates within the population. The high prevalence of HBsAg, though expected, suggests an at risk population of contracting the HBV/HDV superinfection. This could present a public health threat since Ghana is endemic for HBV according to a WHO's report. Hepatitis D virus infections present a potential public health challenge and therefore require routine testing of HBV patients in hospitals in Ghana, education and appropriate control and interventions including mass immunization against HBV.

Introduction

Hepatitis B virus (HBV) is a significant public health issue. As a result of the widespread distribution, chronic hepatitis B virus (cHBV) infection has become a leading cause of liver-related deaths. The World Health Organization, in 2019 estimated the prevalence of chronic HBV worldwide to be 296 million and new infections to be 1.5 million [1–4]. These individuals are more likely to develop cirrhosis and hepatocellular carcinoma (HCC) and have a higher mortality rate [5]. In 2021, the total global estimate for hepatitis B surface antigen positives was 262 million, out of which 1,994,000 were newly diagnosed infections [6]. Additionally, Africa reported an estimated total of 69 million, 512 thousand HBsAg positive infections of which 187,000 were new infections [6]. In Ghana, a systematic review done on the prevalence of HBV among the populace reported an estimated high percentage of 14.4% [7]. HBV infections over time could result in HCC. When compounded with HDV co-infection or super-infection, the severity of liver disease and risk of progression to HCC increases significantly [8].

The HDV is a circular, 36 nm-diameter RNA virus that encodes a structural protein known as the hepatitis delta antigen (HDAg) [9]. It requires the hepatitis B surface antigen (HBsAg) to infect an individual as it is unable to infect on its own. Thus, HDV infects people with chronic HBV who are positive for the HBsAg or individuals who are not immune to HBV could get infected through coinfection of HBV and HDV simultaneously. Even though HDV is extremely contagious, many people who are thought to be infected with it go undiagnosed, particularly, high risk groups in endemic locations including injectable drug users. It is advised that HBsAg-positive people be checked for HDV infection [10].

The WHO aims to eradicate viral hepatitis by 2030 and for HDV/HBV superinfection or coinfection to be a thing of the past, more effort is needed in the diagnosis and screening of HDV [11], especially, in developing countries and more importantly sub-Saharan Africa. Additionally, in Ghana there is not much research done on HBV/HDV superinfections or coinfections, making information on severity of HDV infection very scanty. There is the need for more research to generate enough data on HDV for policy makers to integrate HDV screening with the HBV test particularly for chronic cases. We therefore sought to determine the prevalence and the risk factors associated with HDV infections among HBV patients in Ghana.

Methods

Study design and sites

This was a cross-sectional purposive study conducted in four hospitals in Accra between January-May 2024. The study sites were the Greater Accra Regional hospital, University of Ghana hospital, Korle Bu Teaching hospital and Maamobi General hospital.

Study population and ethical considerations

The study enrolled HBV-infected individuals receiving care at the above-mentioned hospitals in Accra with no age limit. Prior to the inception of the study, ethical approvals were obtained from the Ghana Health Service Ethics Review committee (GHS-ERC:036/06/23) and the Korle Bu Teaching Hospital Scientific and Technical Committee (STC) and Institutional Review Committee (KBTH-STC/IRB/000142/2023). Before enrollment, written informed consent was obtained from the participants.

Sample and data collection

The sample size was calculated using Daniel's equation based on a 10% minimum HDV prevalence among HBV infected persons, reported elsewhere [12] and a confidence level of 95%. An additional 10% was included to account for possible non-response resulting in the total sample size of 152 HBV positive persons recruited for this study. A 4ml blood sample was collected from each participant into a serum separated vacutainer, and the samples were transported to the Department of Virology at the Noguchi Memorial Institute for Medical Research (NMIMR) for processing. Alongside the blood samples, demographic data was collected from the participants and captured on a semi-structured questionnaire developed purposely for the study. The samples were centrifuged at 1500 rpm for 10 minutes, and the serum from each sample was collected into sterile labelled cyro-vials. The serum samples were confirmed for HBsAg before storage in the ultra-low (-80° C) freezer prior to the molecular tests for the HDV.

Laboratory Investigations

Hepatitis B surface antigen test

All 152 HBV serum samples were tested for HBsAg using Advanced Quality one step HBsAg test strip from InTec PRODUCTS, INC, China. The kits relative sensitivity and specificity were >99 % and 97.0 % respectively, with accuracy of 98.5 %. The HBsAg tests were conducted in a biosafety level two laboratory under class II biosafety cabinet following manufacturer's protocols. A volume of 80 µl of each serum sample was dispensed unto each new test strip and timed for a maximum of 15 minutes.

Interpretation of results was done within 15 minutes for HBsAg positive samples and 15 minutes for HBsAg negative samples after the application of serum on the test strips. All 152 serum samples were tested for the presence of the HBsAg. The HBsAg negative serum samples were excluded from the HDV molecular detection.

Hepatitis D virus detection and quantification of viral copies Nuclei acid extraction

Ribonucleic acid (RNA) was extracted from the HBsAg positives serum samples using the QIAmp viral RNA kit from Qiagen, following the manufacturer's protocol.

Nucleic Acid Amplification and Quantification

The nucleic acid extracts were tested for HDV using real time RT-qPCR HDV detection kit (Bio-premier company, Portugal), using the 7500 Fast Real-time PCR System, applied biosystems by Thermo Fisher Scientific. The nucleic extracts were amplified

alongside the HDV standards with known concentrations. Viral load was computed by comparing the RT-qPCR cutoff figures with those of standards with known concentrations.

HDV Sequencing

Synthesis of cDNA and Amplification

The RNA of the HDV positives was reverse transcribed to complementary DNA (c DNA) using 2 µl Luna-script master mix and 8 µl of the RNA template. The amplification conditions were 1 cycle each at 25° C for 2 minutes, 55° C for 10 minutes and 95° C for 1 minute.

The resulting cDNA was amplified with the primer pair 5'CATGCCGACCCGAAGAGGAAAG3'(889_s R0F) and 5'GAAGGAAAGGCCCTCGAGAACAAGA3'(1289_as R0R), to the 3' ribozyme (R0) region [13] in a reaction containing 6.3µl of Q5 hot-start master mix, 1.10 µl each of the forward and reverse primer and 2.6 µl of nuclease free water. Thermal cycling conditions were: 98°C for 30 seconds, and 35 cycles of 98 °C for 10 seconds, 60 °C for 30 seconds and 72 °C for 30 seconds followed by a final extension at 72 °C for 2 minutes in a 96 well thermal cycler (Veriti, applied biosystems by Thermo Fisher Scientific).

Gel electrophoresis was done for the amplified HDV positive products. The products were resolved by 1.2 % agarose gel, and a band size of approximately 400bp was observed.

Sequencing of HDV amplicons

The PCR product was processed for sequencing by purifying it with Qiagen purification kit using the manufacturer's protocol. Cycle-sequencing was done with BigDye cycle sequencing kit, version 3.1, where the Ghana National Polio laboratory protocol for Sanger sequencing was adopted, as it was done by Attiku *et al.* [13]. The reagents for the cycle sequencing were nuclease free water (6.0 µl), BigDye (1.0 µl), 5X sequencing buffer (1.0 µl) and primer (1.0 µl). The master mix was prepared for each primer set (in this case there were two primers used, the forward and reverse primers as described previously under synthesis of cDNA (HDV RNA) and amplification. However, the total volume for the master mix depended on the number of samples sequenced. The volume of purified cDNA amplicon template used was 2 µl. The master mix with the templates in PCR standard strips were run in a thermal cycler (Veriti, applied biosystems by Thermo Fisher Scientific) at these run settings- (94 °C for 20 seconds, 42 °C for 15 seconds, 0.4 °C /second to 60°C, 60°C for 4 minutes) for 25 cycles. The sequencing product was held at 4 °C after the run in the thermal cycler. After the cycle sequencing, the products were purified with QIAquick PCR purification kit from QIAGEN. The purified HDV products were placed in the genetic analyzer (ABI 3130 XL/ 3500 XL) to generate the sequences. The HDV sequences generated were analyzed with the Sequencher (version 5.4.6) and compared with standard HDV sequences from the Gene bank.

Statistical Analysis of data

Statistical analyses between risk factors (age, sex, multiple sexual partners etc.) and HBV/ HDV infections and other socio-demographics (educational level, economic status, marital status etc.) were performed using Chi square and Fisher's exact tests in STATA version 14.2. A *p*-value < 0.05 was considered statistically significant. Sequencing analysis was conducted using MEGA X software (version 12). Microsoft excel was used to calculate HDV viral copies.

Results

Background characteristics of study Participants

The study involved 152 participants aged between 20 and 74 years, with an average age of 40.7 years. There were 67 (44.1%) female participants with a mean age of 42.9 years and 85 (55.9%) males with an average age of 38.9 years. Korle Bu Teaching Hospital (KBTH) had the highest number of participants involved in the study, followed by University of Ghana Hospital (UGH), Greater Accra Regional Hospital (GARH) and Maamobi General Hospital (MGH). Participants of the age group 30 to 39 years had the highest HBsAg positives of 29.9 percent, with 2 of the males in that age group, being positive with HDV (Table 1).

Table 1: Background characteristics of study participants

Characteristics	Frequency	HBsAg positives	HDV positives
	N=152(%)	n = 144 (%)	n = 2 (%)
Sex			
Males	85 (55.9)	79 (54.9)	2 (2.4)
Females	67 (44.1)	65 (45.1)	
Age			
20-29	32 (21.1)	31 (21.5)	
30-39	43 (28.3)	43 (29.9)	2 (4.7)
40-49	39 (25.7)	33 (22.9)	
50-59	27 (17.8)	26 (18.1)	
≥60	11 (7.3)	11 (7.6)	
Study Sites			
GARH	16 (10.5)	13 (9.0)	1 (6.3)
KBTH	73 (48)	69 (47.9)	
MGH	6 (4)	6 (4.2)	1 (16.7)
UGH	57 (37.5)	56 (38.9)	
Educational level			
No formal education	8 (5.3)	7 (4.9)	
Basic	22 (14.5)	21 (14.6)	
Secondary	45 (29.6)	41 (28.5)	2 (4.4)
Tertiary	77 (50.7)	75 (52.1)	

Note: N , total number of samples; n, number of HBsAg and HDV positive respectively; HBsAg, hepatitis B surface antigen; HDV, hepatitis D virus; GARH, Greater Accra Regional Hospital; KBTH, Korle Bu Teaching Hospital; MGH, Maamobi General Hospital; UGH, University of Ghana Hospital

Prevalence of HDV among HBV patients

Out of the 152 enrolled participants, 144 (94.7%) tested positive for the HBsAg serum marker while 8 (5.3%) tested negative. Two of those who tested positive for HBsAg also tested positive for the HDV, yielding an HDV prevalence of 1.4% amongst the study participants.

Quantified viral copies of HDV

The viral loads for the HDV-positive samples (GARH004 and MGH001) were **calculated using the standard curve** generated from the known HDV standard concentrations (STD C1–C5). Table 2 shows RT-PCR results for HDV RNA quantification. Two samples (GARH004 and MGH001) tested positive, with CT values of 27.17 and 30.84, corresponding to viral loads of approximately 708 and 80 copies/2.5 µl, respectively.

Table 2: Quantification of HDV samples C_T values into viral copies using HDV standards

Standards	CT	Viral copies / 2.5µl	Log 10 CT (x)	Log 10 Copies (y)	Sample ID	CT	Log 10 CT (x)	Log10 copies (y)	Copies (10 ^y)/2.5µl
STD C1	35.99	5	1.5561	0.699	GARH004	27.17	1.4341	2.850	708.0
STD C2	31.54	50	1.4989	1.699	MGH001	30.84	1.4891	1.901	79.6
STD C3	28.15	500	1.4495	2.699					
STD C4	24.38	5000	1.3870	3.699					
STD C5	21.03	50000	1.3228	4.699					

Note: STD C1 to STD C5, Standard viral copies of the hepatitis delta virus from the lowest copies **STD C1** to the highest copies **STD C5**; **CT**, Cycle threshold; **GARH**, Greater Accra Regional hospital; **MGH**, Maamobi General hospital; **ID**, Identification number;

Log 10, logarithm of 10; **CT**, Cycle threshold; **µl**,microliter; **y**, viral copies in log 10 and **x**, CT values in log 10 used to draw a linear graph where the viral copies of samples with ID GARH004 and MGH001 were extrapolated.

The viral copies of the HDV positive samples in 2.5µl was quantified or calculated from a linear graph created with the CT values in logarithm of 10 (x- axis) and the viral copies in logarithm of 10 (y-axis) of the HDV standards in 2.5µl. Table 2. The HBV viral copies of the 2 HDV positive samples were sought for from the hospital records of those patients and compared with their HDV viral copies quantified. Thus, the obtained HBV and HDV viral loads in international units per milliliters (IU) (copies/ml) from the confirmed HDV samples are shown in Table 3.

Table 3: HBV and HDV IU/ml (copies/ml) in confirmed samples

Sample ID	Hepatitis B viral copies	Hepatitis D viral copies (copies/ ml)
GARH004	25IU/ml (140copies/ml)	50,597.37IU/ml (283345 copies/ml)
MGH001	Undetectable	5,690IU/ml (31,864 copies/ml)

Note: **ID**, Identification number; **GARH**, Greater Accra regional hospital; **MGH**, Maamobi General hospital; **IU**, International unit; **ml**, millilitre

Hepatitis delta virus genotype and Phylogeny

We successfully conducted whole genome sequencing for 1 of the two samples that tested positive for HDV with a sample ID HDV-GHA-GARH-004-2024. After the alignment and sequence analyses of the HDV-GHA-GARH-004-2024 (marked with red dot) to selected sequences from the Gene bank, the HDV sample showed similarity and clustered with the reference strains of genotype HDV-1 from Cameroon, Pakistan and Gabon as seen in figure 1.

Factors associated with HBV(HBsAg) and HDV infections

Table 4. shows the association between participants socio-demographic characteristics and HBsAg and HDV infections. Age was associated with both HBsAg and HDV ($P<0.05$). The study site was associated with HDV ($P=0.006$) and Region of Residence was associated with HBsAg ($P=0.005$).

Table 4: Association between socio-demographics and HBsAg and HDV infections

Demographics	Frequency			HBsAg	χ^2	p-value	HDV	Fisher's	p-value
	N=152	%	n (%)				n (%)		
Sex									
Males	85	55.9	79 (54.9)		1.25	0.264	2 (2.4)		0.252
Females	67	44.1	65 (45.1)						
Age									
20-29	32	21.1	31 (21.5)			0.022*			0.042*
30-39	43	28.3	43 (29.9)		11.44		2 (4.7)		
40-49	39	25.7	33 (22.9)						
50-59	27	17.8	26 (18.1)						
>=60	11	7.3	11 (7.6)						
Total			144 (94.7)				2 (1.4)		
Study Sites									
GARH	16	10.5	13 (9.0)			0.055	1 (6.3)		0.006*
KBTH	73	48	69 (47.9)		7.58				
MGH	6	4	6 (4.2)				1 (16.7)		
UGH	57	37.5	56 (38.9)						
Educational level									
No formal education			7 (4.9)			0.37			0.148
	8	5.3							
Basic	22	14.5	21 (14.6)		3.15				
Secondary	45	29.6	41 (28.5)				2 (4.4)		
Tertiary	77	50.7	75 (52.1)						
Economic status									
Employed	106	69.7	99 (68.8)		1.33	0.515	1 (0.9)		0.645
Unemployed	40	26.3	39 (27.1)				1 (2.5)		
Retired	6	4.0	6 (4.2)						
Marital status									
Single	48	31.6	47 (32.6)			0.545	2 (4.2)		0.276
Married	91	59.9	85 (59.0)		2.14				
Divorced	9	5.9	8 (5.6)						
Widow	4	2.6	4 (2.8)						
Region of residence									

Ahafo	1	0.7	1 (0.7)				
Ashanti	1	0.7	1 (0.7)				
Central	5	3.3	5 (3.5)				
Eastern	4	2.6	4 (2.8)				
Greater Accra	131	86.2	120 (83.3)	25.47	0.005*	2 (1.5)	0.107
Oti	2	1.3	1 (0.7)				
Savanna	2	1.3	1 (0.7)				
Upper East	1	0.7	1 (0.7)				
Volta	2	1.3	1 (0.7)				
Western	2	1.3	2 (1.4)				
Western North	1	0.7	1 (0.7)				

Note: **N**, Number of observations; **n**, number of participants with HBsAg and HDV positives; **HBsAg**, Hepatitis B surface antigen, **HDV**, Hepatitis D virus; **χ^2** , Chi square values; *P, values less than the alpha value 0.05

Table 5. presents the analysis of the association between selected behavioral and medical risk factors with hepatitis B surface antigen (HBsAg) and hepatitis D virus (HDV) infections among the 152 participants enrolled in the study.

Participants who reported having multiple sexual partners (42.8%), 91% tested positive for HBsAg, compared to 98% among those with only one or no sexual partner. Although this difference was not statistically significant ($\chi^2 = 3.59$, $p = 0.058$), it likely shows a trend toward increased HBV transmission risk among individuals with multiple sexual partners. Notably, a statistically significant association was observed between having multiple sexual partners and HDV infection ($P = 0.030$). The 2 HDV positive cases were identified among individuals who reported having multiple sexual partners, suggesting that sexual behavior may play a critical role in the transmission of HDV in this cohort.

Table 5: Association between risk factors and HBsAg and HDV infections

Risk factors	Resp.	Frequency		HBsAg	χ^2	p-value	Fisher's		p-value
		N=152	%				n (%)	HDV	
Multiple sex partners									
Yes		65	42.8	59 (91)	3.59	0.058	2 (3.1)		0.030*
No		87	57.2	85 (98)					
Injectable									
Yes		9	5.9	9 (6.25)	0.53	0.466			1.000
No		143	94.1	135(93.7)			2 (1.4)		
Tattoo									
Yes		2	1.3	2 (1.4)	0.11	0.737			1.000
No		150	98.7	142 (98.6)			2 (1.3)		
Blood transfusion									
Yes		22	14.5	21 (14.6)		0.958			1.000
No		129	84.9	122 (84.7)	0.10		2 (1.6)		
Did not know		1	0.7	1 (0.7)					
Shared infected items									
Yes		10	6.6	8 (5.6)		0.097			0.260
No		120	79.0	115 (79.9)	4.67		2 (1.7)		
Did not know		22	14.5	21 (14.6)					

Resp., Response from participants; **N**, Total number of observations; **n**, number of observations for each response under a risk factor; **HBsAg**, Hepatitis B surface antigen; **HDV**, Hepatitis D virus; * P, value less than the alpha value 0.05

Perceived Routes of Hepatitis B Virus Transmission

To assess participants' understanding of HBV transmission, the study administered a semi-structured questionnaire which focused on three major categories of risk factors: healthcare work-related exposure, lifestyle behaviors, and health or other related issues. The responses are summarized in Table 6.

Out of the 152 participants surveyed, 61 (40.1%; 95% CI: 32.3–48.3) believed that HBV infection could be acquired through healthcare-related activities. These included occupational exposure via patient care, contact with infected sharps and syringes, or accidental splashes of infected bodily fluids such as blood. This finding underscores the level of awareness among participants, particularly regarding nosocomial transmission risks. A slightly higher proportion of participants (48.7%; n = 74, 95% CI: 40.5–56.9) identified lifestyle-related behaviors as potential modes of HBV acquisition. This category included practices such as injecting drugs using shared needles, having multiple sexual partners, and undergoing tattooing. The fact that nearly half of the participants acknowledged these risks reflects a moderate awareness of behavioral transmission routes within the population. Similarly, 71 participants (46.7%; 95% CI: 38.6–55.0) recognized health-related and other general risk factors for HBV transmission. These included blood transfusions, mother-to-child transmission, and sharing personal items such as toothbrushes, blades, and towels. Although these routes are acknowledged in public health literature, the moderate level of participant recognition suggests a need for reinforced public education on both direct and indirect transmission pathways.

Table 6: Participants response on ways by which one could be infected with HBV

Risk factors	Observations		95% CI
	N=152	%	
Healthcare work-related	Yes (61)	40.1	32.3 - 48.3
	No (91)		
Lifestyle	Yes (74)	48.7	40.5 - 56.9
	No (78)		
Health and other related issues	Yes (71)	46.7	38.6 - 55.0
	No (81)		

Note: **Healthcare work-related**, Infection through patient care, infected sharps and syringes, Splashes of infected fluid (blood) on health worker(s); **Lifestyle**, Injectable drugs through sharing of syringes, multiple sexual partners and tattoo; **Health and other related issues**, Blood transfusion; mother-to-child transmission, sharing and contact with infected persons through sweat, toothbrushes, blades; **N**, Total number of observations; **CI**, Confidence interval

Perceived Sources of Hepatitis B Virus Infection

To understand the participants own perceptions of how they might have acquired hepatitis B virus (HBV) infection, participants were asked to indicate the most likely sources of their infection. Although this self-reported data may be subject to recall bias, it provides valuable insight into perceived transmission routes within the study population (Table 7).

Among the 152 HBV-positive participants, majority (94.08%; n = 143) reported that they did not know how they had acquired the infection. This substantial proportion reflects a general lack of awareness or difficulty in pinpointing the specific event or exposure that led to HBV acquisition, highlighting the need for improved public education regarding HBV transmission routes.

Only a small number of participants (n = 3, 1.97%) attributed their infection to sexual contact with a partner, while another 3 (1.97%) believed they had acquired the virus perinatally from birth, indicating mother-to-child transmission. Two participants (1.32%) suspected blood transfusion as the route of transmission, and one participant (0.66%) speculated that they may have contracted the virus through a salon visit, possibly due to contact with contaminated grooming instruments.

Table 7: Participants response on how possibly they could have been infected with HBV

Risk factors	Frequency (N=152)	%
Blood transfusion	2	1.32
Sexual partner	3	1.97
From birth	3	1.97
Suspects the salon	1	0.66
Unknown	143	94.08

Note: **N**-Total number of participants, **Unknown**, This group of participants did not know how they got infected with the hepatitis B virus.

Figure 2 represents the proportion of participants who are or have ever been involved with risk factors such as sharing items with infected HBV persons, having ever received blood transfusion, have or ever had multiple sexual partners, have tattoos on their bodies and have ever been pricked by needles or any other infected sharps. On the graph were also those who indicated none for

any of the risk factors and those who did not know if they had ever had blood transfusion and shared infected items or not. Though the participants gave their responses, there was the possibility of recall bias.

Other Health Conditions Among Participants with Hepatitis B Virus Infection

In addition to HBV infection, participants were asked to report any other known health conditions through a semi-structured questionnaire. For cases of HCC, diagnoses were verified from hospital records. The results are presented in Table 8.

Out of the 152 participants, approximately one-third (32.2%) reported having at least one other health condition aside from HBV infection, while the majority (67.8%) indicated no additional comorbidity. Among the comorbidities documented, hypertension was the most frequently reported condition, affecting 14 participants (9.21%). This aligns with broader epidemiological trends that show a rising burden of non-communicable diseases, such as cardiovascular disorders, among adult populations in sub-Saharan Africa.

Hepatocellular carcinoma (HCC), a serious complication commonly associated with chronic HBV infection, was documented in 9 participants (5.92%). This finding is clinically significant and supports the well-established link between chronic HBV infection and increased risk of liver cancer, underscoring the importance of routine screening and early intervention in HBV-positive individuals. Diabetes mellitus was the second most reported metabolic condition, affecting 8 participants (5.26%), followed by kidney diseases (n = 5; 3.29%) and stomach ulcers (n = 6; 3.9%). These comorbidities may potentially complicate the clinical management of chronic HBV and increase the risk of adverse health outcomes.

Other less common conditions included chest or joint pain (1.32%), neuro-disorders, enlarged prostate, *Helicobacter pylori* infection, cataracts, and glucose-6-phosphate dehydrogenase (G6PD) deficiency, each reported by a single participant (0.66%).

The presence of multiple comorbidities among HBV-infected individuals in this cohort highlights the need for integrated healthcare approaches. Effective management should not only focus on viral suppression but also encompass monitoring and treatment of concurrent non-communicable diseases. Such an approach will improve overall patient outcomes and reduce HBV-related morbidity and mortality.

Table 8: Other Health Conditions Among Participants with Hepatitis B Virus Infection

Comorbidities	Frequencies	%
G6PD full defect	1	0.66
Cataract	1	0.66
Helicobacter pylori	1	0.66
HCC	9	5.92
Neuro Disorder	1	0.66
Chest/joint pains	2	1.32
Diabetes	8	5.26
Enlarged prostate	1	0.66
Hypertension	14	9.21
Kidney diseases	5	3.29
Stomach ulcer	6	3.9
No other health issue	103	67.76

Note: **G6PD**, Glucose-6-phosphate dehydrogenase; **HCC**, hepatocellular carcinoma

Discussions

Co-infection of HBV and HDV poses a significant public health threat, and there is limited data on the prevalence of HDV in Ghana. The HDV infection is associated with an increased risk of cirrhosis and HCC compared to those who only have chronic HBV infection [14, 15]. Therefore, early diagnostic intervention in HDV-infected patients may be the right way to prevent end-stage liver disease and the development of cirrhosis. Considering that Ghana is endemic for HBV infection, an assessment of the prevalence of HDV co-infection or superinfection and its associated risk factors in Ghana is crucial.

This study sought to determine the prevalence of HDV and its associated risk factors among HBV infected patients in Ghana. The percentage of HBsAg among the participants was 94.7%, indicating a high prevalence of HBV infection, and this is corroborated by a study [16]. However, other studies [7, 17] contrast our study finding. Since HDV requires HBsAg protein for its replication and assembly, individuals who are HBsAg-positive are at risk of HBV/HDV superinfection. The prevalence of HBsAg observed in this study is high compared to previous reports from Africa, like South Africa with 7.6% [18], 1.1% in Mali [19], and 6.5% in southeastern Nigeria [20], 28.4% in southwestern Nigeria [21], and 21% in Cameroon [22]. In this hospital based cross-sectional study, a 1.4% prevalence of HDV among HBsAg positives was determined. This finding is closer to similar studies conducted which recorded 1.27% prevalence of HDV [23], 1.54% [24] and 1.8% reported by [13] but lower than a study which recorded 13.45% prevalence of HDV [25]. The study recorded lower prevalence when compared to a study done in Ghana [26] and Nigeria [5]. These results suggest that Ghana is a low endemic area for HDV infection. The low HDV prevalence in this study could be because of using only molecular techniques and not a combination of molecular and serology as employed in other studies. Nevertheless, further studies are needed to confirm these findings, especially since higher prevalence rates were reported in a cohort of children (9/44, 20.5%) [27], in a cohort of HIV patients in West Africa including Burkina Faso (10/67, 14.9%) [28] and in neighboring countries [26, 29]. These differences in HDV seroprevalence can result from a variety of factors, such as active preventive measures directed against sexually transmitted diseases, close family contact, education on proper use and disposal of needles and sharps, and other risk factors including improvement in economic conditions and better control practices for HBV infection. The detection of HDV exclusively in males aged 30–39 years, all of whom reported multiple sexual partnerships, though with the limited samples used, may suggest sexual transmission as an important route of HDV spread in this population. Multiple sexual partners have been identified in other studies as a significant risk factor for HDV acquisition [2, 30–32]. The significant association between age groups and both HBV ($p = 0.022$) and HDV ($p = 0.042$) infections further supports the need for targeted public health interventions aimed at sexually active adults.

Phylogenetic analysis revealed that the sequenced HDV isolate belonged to genotype 1 (HDV-1), clustering closely with strains from Nigeria [21], Cameroon [33], Gabon [34], and Pakistan [35]. The HDV-1 is the most widespread genotype globally and has been linked with severe liver disease progression, including cirrhosis and HCC [8, 36]. Although neither of the HDV-positive individuals in this study had HCC at the time of sampling, the established link between HDV infection and rapid progression to advanced liver disease underscores the importance of early detection and clinical monitoring [5, 8]. Though one of the HDV-positive patients had a higher HDV viral load than the other HDV patient, it was observed that the HDV viral loads of each patient was markedly higher compared to their respective HBV viral loads. This corroborates reports that HDV can suppress HBV replication [37]. While this interaction may modulate disease progression, HDV infection is associated with more severe liver injury and poorer outcomes than HBV mono-infection [38, 39]. Given the absence of routine HDV testing in Ghanaian healthcare facilities, these findings highlight the need to integrate HDV screening into standard HBV management protocols, particularly in endemic regions [11, 39].

The lack of association between HDV infection and other known HBV risk factors such as blood transfusion, tattoos, and sharing of contaminated items was consistent with findings from Nigerian and Ethiopian studies [24, 31]. However, this may reflect the small number of HDV-positive cases in the present study, limiting statistical power. Similarly, the absence of an association between education, economic status, or marital status and HBV/HDV infection aligns with earlier reports suggesting that viral hepatitis risk in endemic areas is less influenced by socioeconomic indicators than by behavioral and epidemiological factors [3, 40]. The comorbidity profile of participants, particularly the 5.9% prevalence of HCC and 9.2% prevalence of hypertension reflect the dual burden of infectious and non-communicable diseases in Ghana. The latter remains a major complication of chronic HBV infection, with higher risk documented among males [3, 41]. Although no HDV positive participants had HCC at the time of study, the established role of HDV in accelerating HBV related hepatocarcinogenesis warrants close clinical follow-up [8, 42].

The detection of HDV among HBV patients in Accra has important public health implications. The concentration of cases in sexually active males with multiple partners suggests a need for behavior change interventions and targeted screening. The presence of HDV-1, with its known association with severe outcomes, further reinforces the need for public awareness, routine HDV testing, and HBV vaccination for susceptible individuals [11, 43].

Limitations

A computation of the logistic regression could have been used to determine the direction of an association between the dependent and the independent variables. Also to understand the independent influence of each risk factor.

However, the analysis could not generate the expected results due to limitations with the dataset of this study. This is due to the small number (2) of HDV detected. Again, the numbers (results) are skewed to one side. Example, all two participants positive for HDV were males and not gender balanced, those 2 participants are in the same age-group and not different age-group and again they are from the same region of residency.

As a result, in doing the analysis, most of the variables, such as HBsAg, were omitted due to collinearity.

Also, the odds ratio and p-values for the logistic regression showed empty.

Additionally, as a hospital-based study, the findings may not be generalizable to the broader Ghanaian population, since there may be individuals who may be infected with HBV but have no access to healthcare.

Conclusions

This study provides molecular evidence of the presence of HDV among HBV-infected individuals from four hospitals in Accra, Ghana. This suggests the virus is in our communities, and the identified genotype HDV-1 is known to cause severe liver diseases such as HCC. Generally the prevalence of HBsAg was high and since HBsAg is the predominant serum marker for HBV infection, and HBV is endemic in Ghana, there is the likelihood that other individuals and even those other participants from the study who are HBsAg positive carriers are at risk of HDV infection. Additionally, the very high HDV viral loads compared to the HBV viral loads is an indication of HBV suppression by the HDV. Thus, low HBV DNA copies is not a surety of HBV clearing or progress in the health management of an HBV patient until it is proven by laboratory investigations that HDV infection is present or not. Finally, risk factors such as age and multiple sexual partners have association with HDV. Age group 30–39 years old was mostly at risk of HBV/HDV superinfection or coinfection.

These findings highlight the need for routine HDV testing in HBV-positive patients, public health education targeting high-risk groups, and strengthening of HBV vaccination programs. Since HDV testing is not done in hospitals in Ghana, further large scale, population-based studies are needed to better define the epidemiology and inform national hepatitis B and D elimination strategies.

Abbreviations

HBV

Hepatitis B Virus

HDV

Hepatitis D Virus

rRT

qPCR–Real time Reversed Transcription–Quantitative Polymerase Chain Reaction

cDNA

Complementary DNA

PCR

Polymerase Chain Reaction

G6PD

Glucose-6-phosphate Dehydrogenase

HCC

Hepatocellular carcinoma

HBsAg

Hepatitis B Surface Antigen

WHO

World Health Organization

GHS

Ghana Health Service

ERC

Ethical Review Committee

IRB

Institutional Review Board

RPM

Revolutions per minute

IU

International units

KBTH

Korle Bu Teaching Hospital

UGH

University of Ghana Hospital

GARH

Greater Accra Regional Hospital

MGH

Maamobi General Hospital

Declarations

Clinical trial number : not applicable

Human Ethics and Consent to participate declaration

Ethics approval and consent to participate

This study was ethically reviewed and approved by the Ghana Health Service's (GHS) Ethical Review Committee (ERC), and Korle Bu Scientific and Technical Committee and Institutional Review Board, with the following approval numbers: **GHS-ERC: 036/06/23, KBTH-IRB 000142/2023**, respectively. After explaining the study objectives and procedures to the participants, a written informed consent was obtained from each participant before data and sample collection. All other aspects of the study were conducted in accordance with the ethical guidelines of the Helsinki Declaration.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

The sequencing datasets generated and analyzed in this study have been deposited in the NCBI database. The HDV sequence generated was submitted via NCBI BankIt (submission ID BankIt3042924) and is available in GenBank under accession PX872187.

Competing interests

The authors declare no competing interests

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Figures



The phylogenetic tree shows different HDV genotypes. The first five sequences from the top of the tree are HDV-1 genotypes, where the sequence from the positive HDV sample from GARH clustered with. The seventh to ninth sequences in the middle of the tree are HDV-2 genotypes. Finally, the last four sequences on the tree are HDV-3 genotypes.

Figure 1

Phylogenetic tree of the HDV positive sample (with the red dot) sequenced

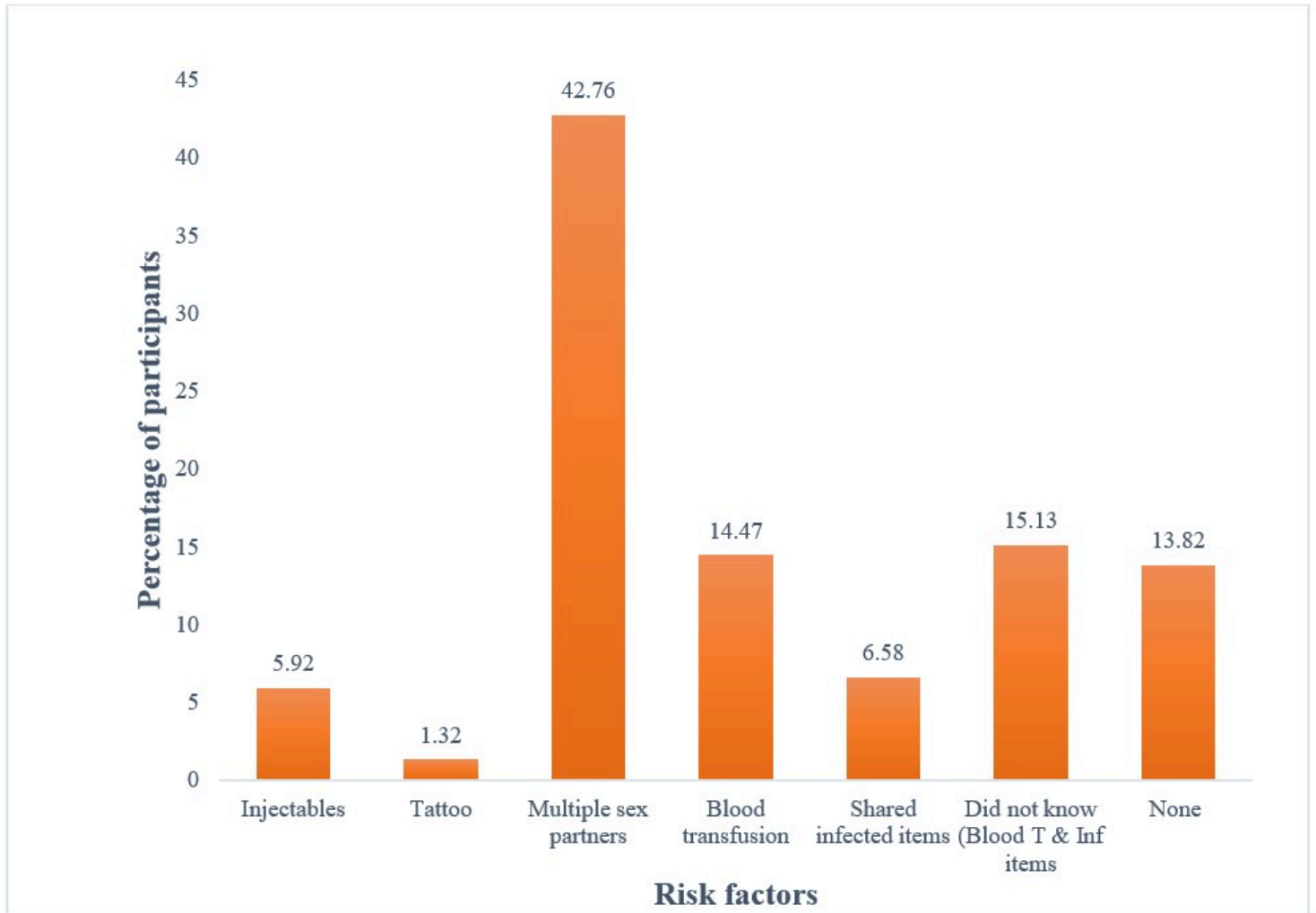


Figure 2

Representation of participants with different risk factors

Note: Blood T & Inf items, Blood transfusion and infected items

Supplementary Files

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