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Sero-epidemiology of Hepatitis D Virus among HBsAg-Positive Individuals in Port Harcourt, Nigeria

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Abstract

Hepatitis B virus (HBV) infection remains a major public health challenge in Nigeria, where coinfection with hepatitis D virus (HDV) can exacerbate liver disease and complicate management. This cross-sectional study evaluated the seroprevalence of HDV among 100 HBsAg-positive individuals in Port Harcourt, Nigeria, and examined demographic and clinical correlates of infection. Plasma samples were screened for HDV IgM using ELISA, and associations with age, gender, marital status, religion, education, occupation, and other clinical parameters were assessed using chi-square analysis. Overall, 36% of participants tested positive for HDV antibodies, with higher prevalence observed among females (40%), single individuals (41.1%), students (66.7%), and those aged 15–29 years (57.1%). Gender, marital status, age, and religion showed statistically significant associations with HDV seropositivity, while education and occupation did not. The findings indicate a substantial burden of HDV among HBV-infected individuals in this region, emphasising the need for routine HDV screening, targeted vaccination, and public health interventions aimed at younger and high-risk populations to mitigate disease progression and transmission.

Keywords: Hepatitis D Virus; Hepatitis B Virus; Coinfection; Seroprevalence; Nigeria; HDV IgM; Risk Factors

1. Introduction

Hepatitis B virus (HBV) infection remains a leading cause of chronic liver disease and a significant cofactor in viral-hepatitis coinfections across Africa. Nigeria is considered hyperendemic, with an HBV prevalence of 8–12% in the general population, among the highest on the continent (François-Souquière et al., 2015).

Transmission occurs mainly through sexual contact, exposure to contaminated blood, and perinatal routes, similar to HIV pathways. Approximately 10–15% of Nigerians living with HIV are also HBV-positive, increasing the risk of liver cirrhosis and complicating ART due to overlapping drug hepatotoxicities (François-Souquière et al., 2015). The hepatitis D virus (HDV), a defective single-stranded RNA virus, depends on HBV for replication and packaging (Rizzetto, 2015). Global estimates suggest that about 5% of chronic HBV carriers harbour HDV, but the prevalence is higher in high-risk and immunocompromised groups (Stockdale et al., 2020).

Coinfection or superinfection with HDV intensifies liver pathology, accelerating fibrosis and cirrhosis progression and markedly increasing hepatocellular carcinoma risk (Ifeorah et al., 2024). Despite these severe outcomes, HDV remains one of the most neglected viral infections in sub-Saharan Africa due to poor diagnostic coverage and limited molecular surveillance (Stockdale et al., 2020).

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In Nigeria, the overlapping epidemics of HBV and HDV represent a hidden syndemic that strains the healthcare system. Patients coinfected with HBV and HDV are prone to rapid clinical deterioration and may respond poorly to standard antiviral therapies, as effective HDV-specific treatments remain limited. Understanding the molecular characteristics of HBV/HDV coinfection is vital to improving patient management and preventing liver-related mortality. Port Harcourt in the Niger Delta provides a strategic setting for such studies, given its dense population, diverse demographics, and high burden of viral hepatitis (NACA, 2022). HBV/HDV coinfection in this region will provide essential data for epidemiological surveillance, inform antiviral strategies, and support Nigeria's public-health response to viral-hepatitis control.

2. Materials and methods

2.1. Study Area

This investigation was centred on Rivers State, located in the Niger Delta region of Southern Nigeria. Specifically, the study was conducted at the University of Port Harcourt in Port Harcourt.

2.2. Study Design

This research utilised a cross-sectional approach, encompassing the collection and examination of samples using HDV IgM ELISA kits.

2.3. Sample Size Estimation

The sample size for this study was determined using the established formula (MacFarlane, 1997; Niang et al., 2006; Awando et al., 2013): $N = [Z^2(pq)] / d^2$. The total sample size was calculated as 73.

2.4. Population for the Study

Around 100 confirmed HBV patient samples, labelled 1-100, were systematically collected and appropriately stored. These samples were obtained from the University of Port Harcourt (UPTH), situated in the state of Rivers, Nigeria. Trained medical laboratory professionals conducted the collection. Subsequently, the samples were transported to the Virus and Genomics Research Unit, Department of Microbiology at the University of Port Harcourt (UNIPORT) for the necessary laboratory analyses. Alongside the testing, pertinent clinical history, behavioural patterns, and demographic information were also documented.

2.5. Sample and Sampling Techniques

A stratified random sampling technique was employed to ensure proportional representation across different demographic strata. The study enrolled a total of 100 participants. Each participant's sample was assigned a unique identification number. Data on demographic and clinical characteristics were obtained using a structured questionnaire and hospital records. This design ensured both representativeness and traceability of samples.

2.6. Inclusion Criteria

The following criteria were included in the inclusion criteria: males and females, all ages, must have lived in Rivers State for at least five years and must be HBV positive.

2.7. Exclusion Criteria

Any individuals who did not fulfil any of the aforementioned eligibility criteria were not considered eligible to participate in this study.

2.8. Sample Collection

Venipuncture, the procedure of drawing blood from a vein, was employed to collect five millilitres (5 ml) of venous blood from each participant. The collected blood was then placed in EDTA bottles and subjected to centrifugation at 3000 rpm for five minutes. This centrifugation process aimed to separate the plasma, which is necessary for detecting Hepatitis D Virus antibodies. The obtained plasma was carefully stored at -20°C until it underwent laboratory testing. In the laboratory, the testing process utilised the ELISA technique. Specifically, kits designed for capturing HDV IgM antibodies. This technique is a widely used and effective method for detecting specific antibodies in biological samples.

The data consisted of both quantitative and qualitative information from study participants. Quantitative data collection methods were employed for gathering the data.

2.9. Serological Analysis

All plasma samples were screened for hepatitis D Immunoglobulin M (HDV IgM) using a commercially available ELISA kit (Fortress Diagnostics, UK), following the manufacturer's protocol. Optical density (OD) readings were measured using an ELx800 ELISA reader (BioTek Instruments, USA) at 450 nm and 630 nm wavelengths. Interpretation of results followed the manufacturer's guidelines, with each test plate including positive, negative, and blank controls. Samples with OD values equal to or greater than the cut-off were considered positive.

2.10. Data Analysis

Sociodemographic and clinical data, including age, gender, marital status, occupation, and educational level, were coded and entered into WPS Spreadsheet version 12.2 for analysis. Descriptive statistics (frequency and percentage) were used to summarise demographic data, while the Chi-square test was applied to determine associations between categorical variables such as demographic factors and seroprevalence of HBV and HDV. A significance level of $p < 0.05$ was considered statistically significant. The statistical analysis employed the chi-square method to investigate potential relationships between the identified characteristics and the seroprevalence rates among patients. Additionally, further statistical analyses were conducted to pinpoint specific categories within each risk factor characteristic that exhibited heightened vulnerability. This involved a detailed examination of their contribution factors to the observed seroprevalence rates.

2.11. Ethical Approval

This study received ethical approval from the University of Port Harcourt Research Ethics Committee, ensuring compliance with ethical guidelines governing medical research involving identifiable human subjects and their data or materials.

3. Results

3.1. Socio-demographical Characteristics of Study Participants

The categorical data detailing the socio-demographical characteristics of the study participants have been systematically organised and are presented in Table 1.

3.2. Overall HDV IgM Assay Results

Out of the 100 HBV-positive individuals screened, 36 (36.0%) tested positive for anti-HDV antibodies, indicating previous or ongoing exposure to hepatitis D virus (HDV), as indicated in Table 1.

3.3. HDV Ab/HDV IgM Seroprevalence Concerning Socio-demographic Characteristics

In terms of gender distribution, females exhibited a higher seroprevalence rate (40.0%) compared to males (28.6%) as indicated in Table 1. Age distribution further highlighted that participants aged 15–29 years had the highest HDV seroprevalence (57.1%), followed by those aged 45–59 years (33.3%) and 30–44 years (32.4%) (Table 1). Marital status also influenced seropositivity, with single participants showing the highest prevalence (41.1%), followed by married individuals (27.9%), while the only divorced participant was positive (100.0%), as indicated in Table 1. Regarding religion, the highest seroprevalence was recorded among individuals practising Traditional religion (66.7%), followed by those of Islamic faith (50.0%) and other religions (50.0%), while Christians accounted for a lower but substantial rate of 34.1% (Table 1). Educational attainment also showed varying prevalence rates. Participants with tertiary education had a seroprevalence of 40.0%, those with primary education 38.5%, and those with no formal education 35.7%, while those with secondary education recorded the lowest rate of 32.5% (Table 1).

In terms of occupation, students showed the highest seroprevalence (66.7%), followed by unemployed individuals (32.0%), employed persons (31.0%), and self-employed individuals (29.0%), as shown in Table 1.

3.4. Statistical Relationship

Gender showed a statistically significant relationship with HDV infection ($p = 0.003$). Marital status was also strongly associated with HDV seroprevalence ($p < 0.001$). Religion demonstrated a highly significant association with HDV

infection ($p < 0.001$). Age also showed a statistically significant relationship with HDV infection ($p < 0.001$). In contrast, education level ($p = 0.246$) and occupation ($p = 0.970$) did not show statistically significant associations with HDV seropositivity (Table 1).

Table 1 HDV IgM Serological Prevalence and Statistical Analysis Results

Characteristic	Category	No. Tested (%)	No. Positive (%)	p-value
Gender	Female	65 (65.0)	26 (40.0)	0.003
	Male	35 (35.0)	10 (28.6)	
Age Range	15–29	14 (14.0)	8 (57.1)	< .001
	30–44	68 (68.0)	22 (32.4)	
	45–59	18 (18.0)	6 (33.3)	
Marital Status	Single	56 (56.0)	23 (41.1)	< .001
	Married	43 (43.0)	12 (27.9)	
	Divorced	1 (1.0)	1 (100.0)	
Religion	Christianity	91 (91.0)	31 (34.1)	< .001
	Islamic	4 (4.0)	2 (50.0)	
	Traditional	3 (3.0)	2 (66.7)	
	Others	2 (2.0)	1 (50.0)	
Education Level	Secondary	40 (40.0)	13 (32.5)	0.246
	Primary	26 (26.0)	10 (38.5)	
	Tertiary	20 (20.0)	8 (40.0)	
	None	14 (14.0)	5 (35.7)	
Occupation	Self-employed	31 (31.0)	9 (29.0)	0.970
	Employed	29 (29.0)	9 (31.0)	
	Unemployed	25 (25.0)	8 (32.0)	
	Student	15 (15.0)	10 (66.7)	
Total		100 (100.0)	36 (36.0)	

4. Discussion

The study revealed a 36% anti-HDV positivity rate among HBsAg-positive participants, indicating a significant incidence of HDV exposure relative to other endemic regions, thereby underscoring the necessity for frequent HDV screening in HBV carriers.

A notable gender disparity was identified, with females exhibiting higher anti-HDV positivity (40.0%) compared to males (28.6%) ($p = 0.003$). This pattern diverged from studies in Thailand and Iran, where male dominance was attributed to occupational exposure and increased participation in hazardous behaviours, including intravenous drug use (Ananchuensook et al., 2023; Joshaghani et al., 2007). The heightened female frequency in this cohort may have indicated disparities in healthcare consumption, as women frequently received screenings during antenatal care. Moreover, marital status demonstrated a significant correlation ($p < 0.001$), with single individuals displaying the highest seropositivity (41.1%), corroborating prior research that linked unmarried status to an elevated risk of horizontal transmission via unprotected sexual intercourse (Sobajo et al., 2023).

Religion appeared to influence the findings, as individuals practising traditional and Islamic faiths exhibited elevated anti-HDV positivity rates (50–67%). This trend might have been associated with cultural behaviours such as the sharing

of sharp objects or scarification, both of which promote blood-borne virus transmission (Akande and Fowotade, 2020). Despite the lack of statistical significance in education level ($p = 0.246$), anti-HDV positivity was more prevalent among individuals with lower educational attainment, reflecting global findings that associate low education and inadequate awareness of HBV/HDV transmission with higher infection rates (Sanou et al., 2018; Shen et al., 2021).

Students exhibited the greatest anti-HDV positivity (66.7%) among occupational groups, perhaps indicative of communal exposure and inadequate vaccination coverage in younger demographics. The age range of 15–29 years demonstrated the highest seropositivity (57.1%, $p < 0.001$), consistent with research from Nigeria and Korea that suggested increased HDV exposure among younger individuals due to lifestyle choices and waning vaccine-induced immunity (Ibrahim et al., 2021; Kim et al., 2011). These findings underscored the susceptibility of younger, sexually active individuals in endemic areas.

Globally, HDV co-infection rates among HBV-positive persons exhibited significant variability, ranging from 1–2% in East Asia (Chang et al., 2022) to 15–38% in West Africa (Sobajo et al., 2023; Ibrahim et al., 2021). The 36% positivity identified in this study aligned with findings from sub-Saharan Africa, where inadequate HBV immunisation, insufficient infection control, and customary practices facilitated ongoing HDV transmission. Comparative data from Burkina Faso indicated a comparable co-infection rate, underscoring regional endemicity (Sanou et al., 2018). Conversely, multicenter studies in China revealed an HDV prevalence of less than 5% among blood donors, indicating the effectiveness of immunisation and blood safety initiatives (Chang et al., 2022).

From a clinical standpoint, anti-HDV positivity signified either a resolved or active infection, with such individuals frequently exhibiting more severe hepatic pathology compared to those with HBV monoinfection (Akande and Fowotade, 2020; Ananchuensook et al., 2023). Individuals with co-infection had elevated risks of cirrhosis, hepatic decompensation, and hepatocellular carcinoma (Huang et al., 2025). This highlighted the essential importance of regular anti-HDV testing in HBsAg-positive patients, especially in environments where HDV screening was infrequent. Recent evidence indicated that HDV co-infection might have expedited HBV replication and hindered immune clearance, thereby exacerbating disease progression (Sobajo et al., 2023).

The analysis concluded that anti-HDV seropositivity in HBV-infected individuals was significantly affected by demographic and behavioural characteristics, including age, gender, and marital status. The 36% positivity rate underscored the pressing necessity for comprehensive HBV/HDV testing initiatives and focused vaccination measures, particularly for youth and high-risk groups. Enhancing awareness campaigns and facilitating access to HBV vaccination were essential for mitigating HDV transmission in endemic areas.

5. Conclusion

In conclusion, the study demonstrated a 36% anti-HDV positivity rate among HBsAg-positive individuals, indicating a considerable burden of HDV exposure within the study population. The findings highlighted significant associations between HDV seropositivity and gender, marital status, and age, with females, single individuals, and participants aged 15–29 years showing the highest positivity rates. Although factors such as religion and education level were not statistically significant, they reflected behavioral and cultural influences on infection risk. Overall, the results underscored the need for integrated HBV and HDV screening programs, particularly targeting younger and high-risk groups, alongside strengthened public health education and vaccination initiatives to reduce HDV transmission and its clinical complications among HBV carriers.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have declared that no competing interests exist.

Statement of ethical approval

All authors declare that all experiments have been examined and approved by the University of Port Harcourt Research Ethics Committee. Therefore, the study is performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

Statement of informed consent

All authors declare that informed consent was obtained from all individual participants included in the study.

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