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Prevalence of Hepatitis D Virus Co-Infection among HBsAg-Positive Patients in Abakaliki Metropolis, Ebonyi State, Nigeria

Okosigha Saviour Azibanyam[1], Imarenezor Edobor Peter Kenneth[2], Anyiam Ifeoma Vivian[3]

[1]Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

[2,3]Federal University Otuoke, Bayelsa State, Nigeria

imarenezorep@fuotuoke.edu.ng

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Abstract

Hepatitis delta virus (HDV) is a defective RNA virus that requires co-infection with *Hepatitis B virus* (HBV) for replication and expression. This study aimed to determine the prevalence of HDV co-infection among HBsAg-positive patients in Abakaliki Metropolis, Ebonyi State, Nigeria. A cross-sectional analytical design was employed, involving 1,000 patients who presented at the Federal Teaching Hospital Abakaliki (FETHA) and Mile 4 Hospital Ishieke during the study period. Screening for HBV infection was conducted using the Skytec one-step rapid diagnostic kit, identifying 89 (8.9%) HBsAg-positive individuals. Subsequently, anti-HDV IgM serology testing was performed using ELISA on serum samples from HBsAg-positive patients to detect HDV co-infection. HDV IgM antibodies were detected in 5 (5.6%) of the HBsAg-positive patients—four pregnant women and one blood donor. Age-specific HBsAg prevalence was highest in the 24–28-year age group (13.5%), followed by 19–23 years (9.4%) and 29–33 years (4.0%). Married individuals exhibited a higher prevalence (16.6%) than singles (4.8%).

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Educational level was also associated with prevalence, with tertiary education holders showing the highest rate (20.0%) compared to those with primary education (4.8%). Based on occupation, the highest HBsAg prevalence was recorded among housewives (25.0%), followed by traders (14.7%), students (9.4%), and civil servants (4.3%). The presence of HDV co-infection highlights its potential to exacerbate the clinical course of HBV infection. The study recommends routine HDV screening among HBsAg-positive patients for early detection and clinical management. Additionally, public health interventions should include RNA-based diagnostic tools, increased awareness of HBV/HDV risks in high-risk populations, and integrated care strategies tailored to region-specific epidemiological patterns in Nigeria.

Keywords: Abakaliki; *Hepatitis B Virus* (HBV); *Hepatitis Delta Virus* (HDV); Co-Infection; Prevalence; Nigeria

INTRODUCTION

The virus responsible for the hepatitis B condition is the hepatitis B virus (HBV), whose primary target is the hepatocytes. Hepatitis B virus (HBV) can illicit both short and long-term contaminations (Imarenezor *et al.,* 2016). Hepatitis B virus (HBV) infection is a major cause of liver disease in the world. WHO has estimated that more than 350 million people, that have acute infection have turned to chronic infection. It is also estimated that more than 500,000 deaths occur annually mainly due to cirrhosis and hepatocellular carcinoma caused by chronic HBV infection (Alavian *et al*., 2007). Although many infected individuals present no clinical sign during onset of illness. Some patients do present certain fast clinical signs and symptoms ranging from vomiting, yellowish skin, tiredness, dark urine and abdominal pain. Onset of illness rarely causes death, although symptoms could persist for some days (Hunt and Richard, 2007).

Incubation period may range from thirty to one hundred and eighty days for clinical signs to commence. However, reports have it that about ninety percent of persons infected at birth could stand the chance of getting infected with a long-term HBV as opposed to the 10 % of those who could become infected above the age of five with acute cases. Those with chronic infection, without any obvious clinical manifestation could come down with several hepatocelular-carcinomas. In about 15-25 % of those with long-term infection, death could occur (Imarenezor *et al.,* 2023).

Various routes of viral transmission include, close contact with contaminated body fluids. Infection via childbirth as well as infected body fluids remains the most effective means of HBV propagation in endemic areas. But in places with low HBV occurrence, disease progression is usually via intravenous drug use and sexual relationships (Hunt and Richard, 2007).

Some of the predisposing factors or occupation to acquiring this disease include taking a job in the hospital were several blood transfusions are made on regular basis, dialysis, living with an infected persons, travelling to countries where the infection level is very high (Görlach *et al.,* 2008). Drawing of tattoo including vein puncture led to relevant count of observations in the 1980s; however, with improved sterility, this has become less common (Imarenezor *et al*., 2023). Spread of the virus cannot occur via hand shake, combined use of sharp objects, kissing, body contact, coughing, sneezing, or breast-milk (Görlach *et al.,* 2008). The root cause of this infection can be ascertained within 30 - 60 days upon exposure, and this is usually via a blood examination to detect either viral particles or immunoglobullins against the virus. HBV is amongst the five known types of liver destroying viruses: A, B, C, D, and E. However, reports indicated that the disease can be prevented via vaccination (Hunt and Richard, 2007). Vaccine administration at day one of child birth has been recommended by World Health Organization, 2015, after which 2 or 3 more vaccine doses are expected to be administered later in life to achieve complete vaccine effect. According to WHO, 2015 HBV vaccines are about 95% efficient and active (Imarenezor and Benjamin 2024).

Although HBV vaccination under the children immunization schedule has been available in Nigeria since 2004, HBV infection still remains a serious public health issue in this country with a prevalence of 10–15 % in the population (Opaleye *et al*., 2016). Response to vaccination programs has also been effective over the years with about one hundred and eighty countries participating in the exercise as at 2006 (Görlach *et al.,* 2008).

HBV is the most common and significant chronic viral infection worldwide. The prevalence of chronic HBV infection varies from ≥8 % in Africa, Asia, and Western Pacific to <2 % in Western Europe, North America and Australia (Weiss *et al*., 2005). In Nigeria where HBV infection is considered endemic, different prevalence rates have been reported. Several reports have established the endemic nature of HBV by the presence of HBsAg in different population groups from different parts of the country.

Recently, cases of adequate screening of blood before transfusion and prompt use of sex tools have also been known to help avert HBV infection. At first infection, treatment is basically hinged on certain clinical presentations from the patients. For patients with long-term infection with HBV, certain antiviral drugs like tenofovir or interferon alpha2A could be useful though these drugs are said to be very expensive. Liver transplant, is sometimes used to treat cirrhosis patients (Hunt and Richard, 2007).

Overtime, HBV has been reported to have a global spread touching many lives, with an over-lapping long-term infection rate ranging from about 240 million to 350 million (Drosten *et al*., 2013).

As at 2013, about 129 novel cases of infection were identified out of which an estimated number of about 750,000 patients die of HBV annually (Hunt and Richard, 2007). Out of the total death toll, 300,000 cases were attributed to liver-related uncontrolled cell growth. The disease is common in East Asia and sub-Saharan Africa, where between 5 and 10% of adults are chronically infected. Rates in Europe and North America are less than 1%. It was originally known as serum hepatitis. The hepatitis Delta virus or HDV is RNA defective virus composed of a core presenting the delta-specific antigen, encapsulated by HBsAg that requires the helper function of HBV to support its replication. Infection by HDV occurs in the presence of acute or chronic HBV infection. When acute delta and acute HBV simultaneously occur, the illness becomes severe and clinical and biochemical features may be indistinguishable from those of HBV infection alone.

In contrast, a patient with chronic HBV infection can support HDV replication indefinitely, usually with a less severe illness appearing as a clinical exacerbation. The determination of HDV specific serological markers (HDV Ag, HDV IgM AND IgG) represents in these cases an important tool to the clinician for the classification of the etiological agent, for the follow up of infected patients and their treatment (Imarenezor *et al.,* 2016). The detection of HDV IgM and IgG antibodies allows the classification of the illness and the monitoring of the sero-conversion event (Görlach *et al.,* 2008). This study is to determine the prevalence of Hepatitis D Virus (HDV) co-infection among HBsAg-positive patients in Abakaliki Metropolis, Ebonyi State, Nigeria. Specifically, the study seeks to evaluate the seroprevalence of HDV among HBsAg-positive patients and identify potential risk factors for HDV co-infection.

MATERIAL AND METHODS

Study Area

The study was carried out in Ebonyi State, also known as the "Salt of the Nation" because of its large salt deposits, and it is one of the 36 states in Nigeria. It was created in 1996, making it one of the youngest states in Nigeria. The State capital and largest town is Abakaliki which is the focus point of this study. The inhabitants are primarily members of Igbo Nations with farming as their pre-dominant occupation. The study area has a geographical population of about 134,102 with Geographic coordinates: (*decimal degrees*) Latitude: 6.32° N Longitude: 8.12° Elevation: 117 m.

Study Population

A prospective cross-sectional study was carried out among 1000 patients who visited the FETHA-11 Hospital and the Mile Four Maternity Hospital, Ebonyi State, Nigeria and were screened for the HBsAg. Further studies were then carried out to clearly ascertain cases of ongoing co-infection between HBsAg/ HDV-IgM. The age of subjects was 19-23, 24-28, 29-33, 34-39 and 40-45. The age group, 19–23 years constituted the largest population making up 35 % (n=350) while 40-45 years were the least making up 1 % (n=10). Gender, females constituted the largest population of 62 % (n=620) while the males were 38 % (n=380) of the total population. The singles were 65 % (n=650) whereas married made up 35 % (n=350). FETHA-II had 35.4 % (n=354), Mile 4 had 64.6 % (n=646).

Questionnaire

A brief closed-ended questionnaire was designed to collect demographic data from patients. The information needed were age, sex, marital status, patient's status, occupation, educational status and location of each subject.

Informed Consent Form

An informed consent form was first given to all who consented to enroll in the study through the patient's physician. All the patients gave their consent.

Ethical Considerations

Ethical approval was obtained from the management of FETHA-11 and Mile 4 Hospital all in Abakaliki before we proceeded with the study.

Sampling Technique for HBsAg

Blood samples were collected from all subjects for serological test first for HBsAg antibody. Five millitres (5mL) of venous blood was obtained from each participant under aseptic procedure into a properly labeled serial number-tagged clean EDTA. Sera extracted were then placed into a plain bottle and stored until the time of use for analysis.

Laboratory Investigations for HBsAg

Hepatitis B surface antigen (HBsAg) was detected using Skytec Rapid Diagnostics Test Kit which was made in USA Stored and Sealed between 2-30ºC (OR 36-86 F).

Test Procedure for HBsAg Detection

The sealed pouch was removed by tearing along the notch and the test strip was removed from the pouch, the test strip was then immersed into the container with the arrow end pointing towards the container. It was ensured that the test strip was not immersed past the maximum line, the strip was removed after 8-10 seconds and laid on a clean sterile dry glass tile, result was then read 10-20 minutes afterwards. Results after 30 minutes were not read.

Serological Analysis for the HBsAg

All 89 samples that tested positive for the HBsAg were then analyzed using ELISA assay technique to detect the presence of HDV-IgM antibodies. The kit used for the study was DIA.PRO Diagnostic Bioprobes Srl Via G. Carducci nº 27 20099 Sesto San Giovanni (Milano) – Italy. All tests were performed according to manufacturer's specifications as described briefly. Each kit contains the components described below and sufficient reagents to carry out 89 tests.

Microplate for ELISA Analysis

The microplate upon storage was first allowed to reach room temperature before opening the seal and after use, the remaining strips were then put in the bag and then resealed with desiccant and kept for future use at 4ºC. The microplate contained 12 strips of 8 break-wells coated with purified anti human IgM specific mouse monoclonal antibody.

Negative Control for ELISA Analysis

The negative control was contained in a 1× 2.0 ml/vial with the following components; ready to use, human IgM antibodies positive to HDV, 3 % skimmed milk,

0.2M Tris buffer pH 6.0 +/- 0.1, 0.2 % Tween 20, 0.09 % Na azide and 0.1 % Kathon GC as preservatives. The negative control is pale yellow in colour. We ensured that the ready to use negative controls are thoroughly mixed on vortex before use.

Positive Control for ELISA Analysis

Positive control was in a 1× 2.0 ml/vial with the following components; ready to use, human IgM antibodies positive to HDV, 3 % skimmed milk, 0.2M Tris buffer PH 6.0 +/- 0.1, 0.2 % Tween 20, 0.09 % Na azide and 0.1 % Kathon GC as preservatives. The positive control is green yellow colour coded. Calibrator lyophilized reagent was then dissolved with EIA grade water as reported in the label and it contains fetal bovine serum human IgM antibodies to HDV, 0.2mg/ml gentamicine sulphate and 0.1 % kathon GC as preservatives.

Wash Buffer Concentrate for ELISA Analysis: WASHBUF

The wash buffer concentrate was in 1×60ml/bottle and it contained; 20× concentrated solution, 10mM phosphate buffer pH 7.0+/-0.2,0.05% Tween 20 and 0.1% kathon GC. The whole content of the concentrated solution was diluted with distilled water up to 1200ml and mixed gently end – over – end before it was used. We also avoided foaming during preparation as this could impact on the efficiency of the washing cycles

Interpretation of Results for HBsAg

Negative: Result is negative when only one colour band appears on the control region. This indicates that there is no detectable HBsAg.

Positive: Results are positive when distinct colour bands appear on both the control and test region, this is an indication that the specimen contains detectable amount of HBsAg.

Invalid Results: Occurs when no visible band occurs at all or when only one colour band appears on the test region, this could be due to possible error in performing the test and such tests were repeated using a new device

Statistical Analysis for HBsAg/HDV-IgM

Data generated for HBsAg/HDV-IgM co-infection in this study was then analyzed using SPSS (statistical package for social sciences) software package, version 13.0 (USA).

RESULTS

Detection of HBsAg Infection

From the 1000 blood samples analyzed for HBsAg, a prevalence rate of 8.9 % (n=89) was found within the study population.

Prevalence of HBsAg among Patients in FETHA-11 and Mile Four Hospital with Respect to Age

The highest prevalence rate of 20 % (n=2) was observed in the age group of 40-45 years while the lowest prevalence rate of 2.5 % (n=2) was observed in the age group of 34-39 years. However, the age groups of 19-23 years, 24-28 years and 29-33 years had prevalence rate of 9.4 %(n=33), 13.5 % (n=42) and 4 % (n=10) respectively as shown in Table 1 below.

Prevalence of HBsAg among Patients with Respect to Gender and Marital Status

The prevalence rate of 9.7 % (n=37) and 8.38 % (n=52) was found for the males and females. The total of 89 samples which tested seropositive for HBsAg infection was stratified into two groups; the singles and married based on their marital status. However, it was found that the married group had a higher prevalence rate of 16.6 % (n=58) wherein 4.8 % (n=31) was the prevalence rate of the single group as shown in Table 2 below.

Prevalence of HBsAg/HBV-IgM Co-infection in relation to Location

Mile 4 had the highest HBsAg/HBV-IgM co-infection prevalence rate of 7.5 % as opposed to FETHA-II which had a much lower HBsAg/HBV-IgM co-infection prevalence rate of 2.8 % in relation to location (Table 3). There is no significant difference between the various locations studied at ($p > 0.33$).

Prevalence of HBsAg/HBV-IgM Co-infection in relation to Educational Status

Participants with the primary level of Education showed the lowest prevalence rate of 0 % HBsAg/HBV-IgM co-infection which is closely followed by their secondary school counterparts having a prevalence rate of 4.5 %. The highest prevalence rate in this category is shown by participants in the tertiary level of education having a prevalence rate of 13.6 % (Table 3). There is no significant difference on the basis of education at ($p > 0.13$).

Prevalence of HBsAg/HBV-IgM Co-infection in relation to Occupational Status

Participants in the study who were traders and housewife had showed the lowest prevalence rate of 0 % while the highest HBsAg/HDV-IgM co-infection prevalence rate for this category is shown by the student participant having a rate of 8.9 % (Table 3). There is no significant difference with respect to occupation at ($p>0.44$).

Table 1: Prevalence of HBsAg among Patients with respect to Age

Age Bracket	No. Tested %	No. Positive (%)	No. Negative %
19-23	350 (35.0)	33 (9.4)	317 (31.7)
24-28	310 (31.0)	42 (13.5)	268 (26.2)
29-33	250 (25.0)	10 (4.0)	240 (24.0)
34 – 39	80 (8.0)	2 (2.5)	78 (7.8)
40 – 45	10 (1.0)	2 (20.0)	8 (0.8)
Total	1000	89	911

Table 2: Prevalence of HBsAg among Patients with Respect to Gender and Marital Status

Socio-Demographic Factor	Gender/ Marital status	No. Tested %	No. Positive (%)	No. Negative %
Gender	Male	380 (38.0)	37 (9.7)	343 (34.3)
	Female	620 (62.0)	52 (8.38)	568 (56.8)
Marital Status	Single	650 (65.0)	31 (4.8)	619 (61.9)
	Married	350 (35.0)	58 (16.6)	292 (29.2)

Table 3: The Socio-demographic Characteristics of the Study Population for HBsAg /HDV-IgM Co-infection

Socio-Demographic Characteristics		No. Tested	No. Positive	No. Negative	Percentages
Gender (n=89)	Male	37	1	36	41.6
	Female	52	4	48	58.2
Marital Status (n=89)	Single	58	1	57	65.2
	Married	31	4	27	34.8
Age Group (n=89)	19-23	33	1	32	37.1
	24-28	42	3	39	47.2
	29-33	10	1	9	11.2

Socio-Demographic Characteristics		No. Tested	No. Positive	No. Negative	Percentages
	34 – 39	2	0	2	2.2
	40 – 45	2	0	2	2.2
Location (n=89)	FETHA – II	36	1	35	40.4
	Mile 4	53	4	49	59.6
Educational Status (n=89)	Primary	14	0	14	15.7
	Secondary	44	2	42	49.4
	Tertiary	22	3	19	24.7
Occupational Status (n=89)	Student	45	4	41	50.6
	House Wife	10	0	10	11.2
	Civil Servant	15	1	14	16.9
	Trader	19	0	19	21.3
Patient Status (n=89)	Pregnant women	42	4	38	47.2
	Blood Donor	47	1	46	52.8

DISCUSSION

Prevalence of Hepatitis B Surface Antigen (HBsAg) Infection

This study establishes the prevalence of HBsAg infection among 1000 patients within Abakaliki metropolis, Ebonyi State, Nigeria. The prevalence rate of 8.9 % obtained in this study was higher when compared to a similar study by Eze *et al.,* 2015 among adolescents in Enugu, Nigeria which was 3.1 %. Studies conducted in Abidjan, Ivory Coast Africa show 8.5 % prevalence of HBV Imarenezor *et al.* (2023).

Terwase *et al.* (2015) reported a prevalence rate of 7.3 % from a study conducted among Residents of Julius Berger Staff Quarters, Kubwa, Abuja which is almost similar to values obtained in this study. However, the prevalence rate of 8.9 % obtained in this study was also in sharp disagreement with previous studies done among the medical students of Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria having a prevalence rate of 15.5 % (Alo *et al.*, 2015) while a prevalence rate of 25.0 % was reported among HIV infected individuals in Jos, Nigeria (Uneke *et al.*, 2005). An HBsAg seroprevalence rate of 14.3 % was reported in Jos, Nigeria (Uneke *et al.*, 2005).

The presence of HBsAg in serum or plasma is an indication of active Hepatitis B infection, either acute or chronic. A 4.3 % prevalence rate was reported in pregnant women in Port Harcourt, Nigeria (Imarenezor *et al.*, 2016). Among blood donors in Benin City, a prevalence rate of 5.4 % was reported by Umolu *et al.* (2005).

Also, a 5.6 % HBV prevalence was reported in Sudan by Elsheikh *et al.* (2007), all of which were lower than the results obtained in this stud**y**.

Age-wise, the highest incidence of HBsAg in this study occurred among those within the age bracket of 19-23 with a prevalence of 35 %, which was higher in this study as opposed to the 11.36 % recorded by Esan *et al.* (2014) and lower among the 40-45 age bracket which is in disagreement with Esan *et al.* (2014) with a 36.36 % prevalence for HBV from previous work done in Federal Medical Centre, Ido-Ekiti Okitipupa South-Western Nigeria. The prevalence rate of 9.7 % (n=37) and 8.38 % (n=52) which was found for the males and females were higher than the 3.2 % and 2.7 % reported by Esan *et al.* (2014). On marital status, it was found that the married group had a higher prevalence rate of 16.6 %(n=58) which was higher compared to the 7.2 % prevalence rate obtained among the married as reported by Onwuakor *et al.* (2014) when they worked on the Sero-prevalence of Hepatitis B surface antigen (HBsAg) amongst pregnant women attending antenatal clinic at the Federal Medical Centre Umuahia, Abia State, Nigeria, whereas the 4.8 % (n=31) prevalence rate of the single group obtained in our study did not tally with the 6.7 % recorded by Onwuakor *et al.* (2014). This could be attributed to marital infidelity on the part of the married couples.

Results of the analysis also showed a higher prevalence rate of 20 % (n=22) in the study population who had attained tertiary level of education, contradicts the 16.7 % value observed by Onwuakor *et al.* (2014). Primary level of education showed the lowest prevalence rate of 4.8 % (n=14).

This value disagrees with the 25.0 % prevalence rate recorded by Onwuakor *et al.* (2014) in Abia State. This could be as a result of the high sexual drive and other predisposing practices (such as intravenous drug use, alcoholism) among those in tertiary schools than in those who have attained primary level of education.

In relation to occupational status, this study showed that HBsAg infection was higher among house wife 25.0 % (n=10) which was higher compare to the 14.6 % rate value recorded by Onwuakor *et al.* (2014) among housewives, traders in this study had 14.7

% (n=19) as opposed to the 5.3 % prevalence value reported by Onwuakor *et al.* (2014), students had 9.4% (n=45) which is in agreement with the 9.4 % prevalence reported by Onwuakor *et al.* (2014) while civil servants in our study had the lowest rate of 4.3 % (n=15) similar study by Onwuakor *et al.* (2014) reported a higher prevalence rate of 8.0 % among civil servants. This could occur due to lack of knowledge about some of the predisposing factors that could possibly elicit transmission. On the bases of location blood donors from FETHA- II showed the highest prevalence rate of 10 % (n=36) compared to the 8 % (n=53) observed among pregnant women attending the Mile Four Maternity Hospital. No comparative data has yet been observed.

Prevalence of HBsAg/HDV Coinfection

Although HBV is endemic in Nigeria, data on HDV seroprevalence are limited. However, this study obtained a prevalence rate of 5.6 % which is lower than 9 % obtained in a Nigerian study population that tested positive to HBsAg and the HDV (Opaleye *et al.*, 2016).

Another study showed that HDV antigen was detectable in 6.5 % of patients with chronic hepatitis B in Southwest Nigeria (Ojo *et al.*, 1998). In addition, another study reported an anti-HDV prevalence of 12.5 % in 96 HBsAg positive patients which is higher than that recorded in this study. Moreover, a recent study (Andernach *et al.*, 2014) showed that HDV1 prevails with 53.3 % in Southwestern Nigeria followed by the HDV5 (33.3 %) and HDV6 (13.3 %) which were higher than the 5.6 % observed in this study. In association with HBV, HDV produces significantly more severe illness than HBV alone (Imarenezor *et al.*, 2016).

Similar results were obtained by Ghadir *et al.* (2012), in Qom Province, Center of Iran, who detected HDV (2 %) in their study which was lower than the 5.6 % value obtained in this study. In Egypt, Gomma *et al.* (2013) also found HDV antibodies in 8 (4.7 %), from 170 HBsAg positive healthy individuals.

This prevalence rate was slightly similar to the 5.6 % obtained in this current study. In Tehran, Iran, WHO reported 37(7.7 %), of HDV from their patients (Tahaei *et al.,* 2014), this value was similar but slightly higher than the 5.6 % recorded in this study. There was significant difference among pregnant women (<0.029 at $p<0.05$). However, there was no significant difference between gender, age, education, occupation and location at ($p>0.05$). The variation may be due to difference in sample size; technique used for analysis

or even difference in the demographic characteristics of the study population or difference in hepatitis epidemiology in these countries. Conclusively, there is urgent need for larger studies on a national scale to accurately evaluate the public health significance of this infection. HDV affects individuals of all ages and various ethnic groups. Our data also support revising screening guidelines to advocate for all patients with HBV to be screened for HDV in order to both give the individual patient important information related to the possible need for treatment and to support the public health goal of reducing transmission by educating HDV-negative patients about the need for protection against super-infection and HDV-infected patients about the need to protect against transmission to others.

Proper management of maternal hepatitis during the prenatal phase ensures better outcomes in the infant, therefore screening of pregnant women for hepatitis B and D virus are highly necessary in order to identify those at risk of transmission, to whom preventive intervention can be instituted irrespective of maternal hepatitis B and virus carriage status; this may be the most effective approach to hepatitis B and D virus prevention and control. It is therefore recommendations that;

- Routine HDV Screening: Implement routine HDV screening for HBsAg-positive patients to enable early detection and management of co-infection.

- RNA-Based Testing: Utilize RNA-based testing to detect active HDV infections and differentiate between resolved and active infections.

- Public Health Education: Enhance public health education and awareness about the risks of HBV/HDV co-infection, particularly in high-risk populations.

- Integrated Care Models: Develop integrated care models that address the complex clinical management of HDV/HBV co-infected patients, including those with concurrent HIV infection.

- Regionalized Public Health Strategies: Prioritize region-specific public health strategies, considering the varying prevalence rates and risk factors across different regions in Nigeria.

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