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Hepatitis B and D Virus Co-Infection: A Study of Seroprevalence and IgM Response among Patients in Abakaliki Metropolis, Ebonyi State, Nigeria

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Abstract

Hepatitis, an inflammation of the liver commonly caused by viral infections, exists in five primary forms: Hepatitis A, B, C, D, and E. Among these, *Hepatitis D virus* (HDV) is a defective virus that requires co-infection with *Hepatitis B virus* (HBV) for replication. Hepatitis may present as acute or chronic, and early diagnosis and treatment are essential to prevent complications. This study investigated the seroprevalence of HBV and HDV co-infection among patients in Abakaliki Metropolis, Ebonyi State, Nigeria. A cross-sectional analytical design was employed, involving 1,000 patients who attended the Federal Teaching Hospital Abakaliki (FETHA) and Mile 4 Hospital Ishieke. Serological testing was conducted using ELISA kits in accordance with the manufacturer's protocols and standard laboratory procedures. Of the 1,000 participants, 89 (8.9%) tested positive for HBsAg, indicating HBV infection, while 5 (5.6%) of these were also positive for HDV-IgM, confirming co-infection. The prevalence of HBV/HDV co-infection was

higher among females (7.7%) compared to males (2.7%) and more pronounced among married individuals (6.9%) than singles (3.2%). These findings indicate a notable burden of HBV and HDV co-infection in the study area. The results underscore the importance of routine HDV screening for all HBsAg-positive patients and the implementation of targeted public health interventions to prevent HDV transmission and reduce the severity of HBV-related disease.

Keywords: Abakaliki; Co-Infection; *Hepatitis B Virus*; *Hepatitis D Virus*; IgM; Seroprevalence; Public Health Intervention

INTRODUCTION

Hepatitis D (hepatitis delta) is a disease caused by the hepatitis D virus (HDV), a small spherical enveloped virusoid. This is one of five known hepatitis viruses: A, B, C, D, and E. HDV is considered to be a subviral satellite because it can propagate only in the presence of the hepatitis B virus (HBV) (Makino *et al.,* 1987; Imarenezor *et al*., 2016). Transmission of HDV can occur either via simultaneous infection with HBV (coinfection) or superimposed on chronic hepatitis B or hepatitis B carrier state (superinfection). Both superinfection and coinfection with HDV results in more severe complications compared to infection with HBV alone.

These complications include a greater likelihood of experiencing liver failure in acute infections and a rapid progression to liver cirrhosis, with an increased risk of developing liver cancer in chronic infections (Fattovich *et al.,* 2000). In combination with hepatitis B virus, hepatitis D has the highest fatality rate of all the hepatitis infections, at 20 %. The HDV is a small, spherical virus with a 36 nm diameter. It has an outer coat containing three kinds of HBV envelope protein - large, medium, and small hepatitis B surface antigens - and host lipids surrounding an inner nucleocapsid. The nucleocapsid contains single-stranded, circular RNA of 1679 nucleotides and about 200 molecules of hepatitis D antigen (HDAg) for each genome. The central region of HDAg has been shown to bind RNA (Poisson *et al.,* 1993). Like Hepatitis B, HDV gains entry into liver cells via the NTCP bile transporter. HDV recognizes its receptor via the N-terminal domain of the large hepatitis B surface antigen, HBsAg (Engelke *et al.,* 2006). Mapping by mutagenesis of this domain has shown that amino acid residues 9–15 make up the receptor binding site. After entering the hepatocyte, the virus is uncoated and the nucleocapsid

translocated to the nucleus due to a signal in HAD-Ag (Schulze *et al.,* 2010). Since the nucleocapsid does not contain an RNA polymerase to replicate the virus' genome, the virus makes use of the cellular RNA polymerases. Initially just RNA pol II, (Lehmann and Cramer, 2007) now RNA polymerases I and III have also been shown to be involved in HDV replication (Greco- Stewart *et al.*, 2011).

Normally RNA polymerase II utilizes DNA as a template and produces mRNA. Consequently, if HDV indeed utilizes RNA polymerase II during replication, it would be the only known animal pathogen capable of using a DNA-dependent polymerase as an RNA-dependent polymerase. The RNA polymerases treat the RNA genome as double stranded DNA due to the folded rod-like structure it is in. Three forms of RNA are made; circular genomic RNA, circular complementary antigenomic RNA, and a linear polyadenylated antigenomic RNA, which is the mRNA containing the open reading frame for the HDAg.

Synthesis of antigenomic RNA occurs in the nucleolus, mediated by RNA Pol I, whereas synthesis of genomic RNA takes place in the nucleoplasm, mediated by RNA Pol II. HDV RNA is synthesized first as linear RNA that contains many copies of the genome. The genomic and antigenomic RNA contain a sequence of 85 nucleotides, the Hepatitis delta virus ribozyme, that acts as a ribozyme, which self-cleaves the linear RNA into monomers. These monomers are then ligated to form circular RNA (Branch *et al.,* 1989). There are eight reported genotypes of HDV with unexplained variations in their geographical distribution and pathogenicity.

A significant difference between viroids and HDV is that, while viroids produce no proteins, HDV is known to produce one protein, namely HDAg. It comes in two forms; a 27kDa large-HDAg, and a small-HDAg of 24kDa. The N-terminals of the two forms are identical; they differ by 19 more amino acids in the C-terminal of the large HDAg.

Both isoforms are produced from the same reading frame which contains an UAG stop codon at codon 196, which normally produces only the small-HDAg. However, editing by cellular enzyme adenosine deaminase-1 changes the stop codon to UGG, allowing the large-HDAg to be produced (Weiner *et al.,* 1988). Despite having 90% identical sequences, these two proteins play diverging roles during the course of an infection.

HDAg-S is produced in the early stages of an infection and enters the nucleus and supports viral replication. HDAg-L, in contrast, is produced during the later stages of an infection, acts as an inhibitor of viral replication, and is required for assembly of viral particles. Thus, RNA editing by the cellular enzymes is critical to the virus' life cycle because it regulates the balance between viral replication and virion assembly (Weiner *et al.,* 1988).

The routes of transmission of hepatitis D are similar to those for hepatitis B. Infection is largely restricted to persons at high risk of hepatitis B infection, particularly injecting drug users and persons receiving clotting factor concentrates (Imarenezor *et al.,* 2016) Worldwide more than 15 million people are co-infected. HDV is rare in most developed countries, and is mostly associated with intravenous drug use. However, HDV is much more common in the immediate Mediterranean region, sub-Saharan Africa, the Middle East, and the northern part of South America (Weiner *et al.,* 1988). In all, about 20 million people may be infected with HDV (Taylor, 2006).

The HDV is a small, spherical virus with a 36 nm diameter. It has an outer coat containing three kinds of HBV envelope protein - large, medium, and small hepatitis B surface antigens - and host lipids surrounding an inner nucleocapsid. The nucleocapsid contains single-stranded, circular RNA of 1679 nucleotides and about 200 molecules of hepatitis D antigen (HDAg) for each genome. The central region of HDAg has been shown to bind RNA. Several interactions are also mediated by a coiled-coil region at the N terminus of HDAg (Mason *et al.,* 2008).

The hepatitis D circular genome is unique to animal viruses because of its high GC nucleotide content. The HDV genome exists as an enveloped negative sense, single-stranded, closed circular RNA. Its nucleotide sequence is 70 % self-complementary, allowing the genome to form a partially double-stranded, rod-like RNA structure. With a genome of approximately 1700 nucleotides, HDV is the smallest "virus" known to infect animals. It has been proposed that HDV may have originated from a class of plant pathogens called viroids, which are much smaller than viruses (François, 2005).

Hepatitis D virus was first reported in the mid-1977 as a nuclear antigen in patients infected with HBV who had severe liver disease (Rizzetto *et al.,* 1981). This nuclear antigen was then thought to be a hepatitis B antigen and was called the delta antigen. Subsequent experiments in chimpanzees showed that the hepatitis delta antigen (HDAg) was a

structural part of a pathogen that required HBV infection to replicate (Wang *et al.,* 1976). The entire genome was cloned and sequenced in 1986. It was subsequently placed in its own genus: *Deltavirus* (Wang *et al.,* 1976).

Deltaviridae, has HDV as the only virus belonging to this genus. HDV is not classified into a viral family because it is a unique virus dependent on HBV. HDV is a co-infection of HBV. The envelope of HDV particles contains the Hepatitis B surface antigen (HBsAg). The production and transmission of HDV is entirely dependent on HBV to provide HBsAg. Thus, HDV is considered a satellite virus of HBV. Unlike a classical satellite virus, however, HDV does not share sequence similarity with HBV, and it can replicate independently of HBV.

There are at least three HDV genotypes: I, II, and III. HDV isolates of Genotype I have been reported in every part of the world, and the pathogenesis of Genotype I infections varies from fulminant hepatitis to asymptomatic chronic liver disease. The milder HDV II genotype is found primarily in Asia, including Japan, Taiwan, and Russia.

Some sequences from Taiwan and the Okinawa islands have been assigned to a subtype of Genotype II, called Genotype IIB. HDV genotype III has been isolated only in northern South America (Peru, Venezuela, and Columbia) and is associated with severe acute hepatitis. Furthermore, HDV genotype I is the only genotype found in some locations, including Europe and North America.

The mechanism in which HDV causes more severe hepatitis and a faster progression of fibrosis than HBV alone remains unclear (WHO, 2017). Chronic HBV carriers are at risk for infection with HDV. People who are not immune to HBV (either by natural disease or immunization with the hepatitis B vaccine) are at risk of infection with HBV which puts them at risk of HDV infection (WHO, 2017). HDV infection is diagnosed by high titres of Immunoglobulin G (IgG) and Immunoglobulin M (IgM) anti-HDV, and confirmed by detection of HDV RNA in serum.

However, HDV diagnostics are not widely available and there is no standardization for HDV RNA assays, which are used for monitoring response to antiviral therapy. Type D hepatitis should be considered in individuals who are HBsAg positive or who have evidence of recent HBV infection. The diagnosis for Hepatitis D infection is made following serologic tests for the virus. Total anti-HDV antibodies are detected by radioimmunoassay (RIA) or enzyme immunoassay (EIA) kits. To monitor ongoing HDV

infection, reverse transcriptase-polymerase chain reaction (RT-PCR) should be used. RT-PCR can detect 10 to 100 copies of the HDV genome in infected blood serum. Each of the markers of HDV infection, including IgM and IgG antibodies, disappears within months after recovery.

In chronic Hepatitis D infection, on the other hand, HDV RNA, HDAg, IgM anti-HD antibodies, and IgG anti-HD antibodies persist (WHO, 2017).

Co-infection occurs when both HDV and HBV are contracted simultaneously. This results in acute HDV and HBV infection. Depending on the relative amounts of HBV and HDV, one or two episodes of hepatitis occurs. Co-infections of HDV and HBV are usually acute and self-limiting infections. HBV/HDV co-infections cause chronic HDV infections in less than 5 % of co-infected patients. Although clinical symptoms disappear, fatigue and lethargy may persist for weeks or months (WHO, 2017). Super-infection occurs when chronic HBV carriers are infected with HDV. This leads to severe acute hepatitis and chronic Hepatitis D infection in 80 % of the cases. Super-infection is associated with the fulminant form of viral hepatitis. Fulminant viral hepatitis, the most severe form of acute disease, is about ten times more common in HDV infections than in the other types. It is characterized by hepatic encephalopathy that is manifested by changes in personality, disturbances in sleep, confusion, difficulty concentrating, and sometimes abnormal behaviour and coma. The mortality rate of fulminant hepatitis is about 80 %.

When poorly sensitive molecular hybridization assays were used to monitor HDV RNA in serum, it was observed that patients were HDV RNA–negative in serum despite the rare occurrence of HDAg in the hepatocyte nuclei—as detected by immune-histochemistry—well before HBV recurrence. This was interpreted as consistent with the infrequent evasion of neutralizing antibodies by some HDV particles, thus capable of infecting hepatocytes. Without the concomitant infection of HBV, a productive cycle of HDV could not be completed. This view was corroborated by the notion that the helper virus is necessary for particle formation but not for viral replication (Kuo *et al.,* 1989). In these patients, circulating HDV RNA was only detected—by molecular hybridization assays—several months after transplantation, for instance, when residual HBV had evaded neutralization and co-infected hepatocytes harboring replicating HDV, thus allowing for HDV rescue and cell-to-cell spread (Ottobrelli *et al.,* 1991). This pattern of infection,

however, has been revisited with the use of more sensitive RT-PCR–based techniques for detecting HDV RNA.

In the Western world, where most natural history studies detailed in the previous paragraph have been conducted, HDV genotype 1 is prevailing (Imarenezor and Benjamin, 2024). In Taiwan, where the predominant genotype is 2, acute HDV infection evolves less frequently toward acute liver failure, and even chronic hepatitis D seems less rapidly progressive (Wu *et al.,* 1995).

HDV only replicates in the liver, and therefore pathologic changes are limited to this organ. Liver damage in HDV infection is thought to be mostly immune mediated, although initial data from experimentally infected chimpanzees had suggested a direct cytopathic effect of HDV on hepatocytes, particularly during the primary infection (Kamimura *et al.,* 1983; Canese *et al.,* 1984; Govindarajan *et al.,* 1986). It was observed that in acute hepatitis D, infected hepatocytes were undergoing degenerative changes characterized by shrunken eosinophilic cytoplasm and pyknotic nuclei, with minimal inflammatory cells in the liver parenchyma, consistent with a cytopathic hepatocellular damage.

Variation in immune-mediated responses during acute and chronic HDV infection has been noticed (Casey *et al.,* 2006), which may explain the variability of the clinical course of HDV infection. Cytotoxic T lymphocytes are mainly responsible for clearing the virus by destroying HDV-infected cells. Delayed and insufficient immune response with the ability to recognize only limited viral epitopes has been implicated in failure to clear the infection coupled with establishment of chronic infection. An exaggerated immune response, particularly a cell-mediated one, is proposed to be involved in causing massive hepatocyte necrosis and liver damage in acute liver failure (Imarenezor *et al.,* 2016). Thus, a vigorous immune response involving HDAg-specific T-cell response and cytotoxic killing of HDV-infected cells leads to both viral clearance and increased liver damage.

Response to HDV involves the activation of antigen-specific helper T cells secreting a variety of cytokines, including interleukin (IL)-2, IL-2 receptor, IL-10, and interferon (Imarenezor *et al.,* 2019). IL-2, in turn, stimulates both additional HDV-specific helper T cells and $CD8^+$ cytotoxic T cells that target infected hepatocytes. HDV-specific Th1 and cytotoxic T cells produce large amounts of IFN-γ, which, in addition to its known immunological effects (among others, the induction of class I and class II MHC proteins at

the surface of hepatocytes) (Franco *et al.,* 1988), may also inhibit viral replication (Magrin *et al.,* 1989). HDAg-specific T-cell responses in peripheral blood of HDV-infected individuals is associated with reduced HDV replication levels (Nisini *et al.,* 1997).

Hepatitis Delta Virus infections are found worldwide, but the prevalence varies in different geographical areas. Anti-HDV antibodies are found in 20-40 % of HBsAg carriers in Africa, the Middle East, and Southern Italy. HDV infection in the United States is relatively uncommon, except in drug addicts and haemophiliacs, who exhibit prevalence rates of 1-10 %. Homosexual men and health care workers are at high risk for contracting HBV, but are surprisingly at low risk for HDV infection for unclear reasons.

Additionally, HDV infection is uncommon in the large population of HBsAg carriers in Southeast Asia and China. Additional high-risk groups for contracting HDV include haemodialysis patients, sex contacts of infected individuals, and infants born to infected mothers (rare). Worldwide, over 10 million people are infected with HDV.

Three genotypes (I–III) were originally described. Genotype I has been isolated in Europe, North America, Africa and some Asia. Genotype II has been found in Japan, Taiwan, and Yakutia (Russia). Genotype III has been found exclusively in South America (Peru, Colombia, and Venezuela). Some genomes from Taiwan and the Okinawa islands have been difficult to type but have been placed in genotype 2. However, it is now known that there are at least 8 genotypes of this virus (HDV-1 to HDV-8). Phylogenetic studies suggest an African origin for this pathogen (Fields *et al*., 2001).

An analysis of 36 strains of genotype 3 estimated that the most recent common ancestor of these strains originated around 1930. This genotype spread exponentially from early 1950s to the 1970s in South America. The substitution rate was estimated to be 1.07×10^{-3} substitutions per site per year. Genotype 8 has also been isolated from South America. This genotype is usually only found in Africa and may have been imported into South America during the slave trade (Fields *et al*., 2001). This research study is to determine the seroprevalence of HBV and HDV co-infection and investigate the IgM response among patients in Abakaliki Metropolis, Ebonyi State, Nigeria.

MATERIALS 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).

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).

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.

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

Enzyme Conjugate for ELISA Analysis

The enzyme conjugate was contained in a 1×0.8ml/vial. Concentrated solution contains a peroxidase labelled polyclonal antibody to HDV. The reagent is dissolved into a buffer solution 10mM Tris buffer pH 6.8+/-0.1, 5 % BSA, 0.1 % Kathon GC and 0.2 % gentamicine sulphate as preservatives.

HDV Antigen for ELISA Analysis

The HDV antigen was in a 1×6 vials. Lyophilized reagents were dissolved with 1.9ml proper diluents and it contained non infective recombinant HDV Ag, 25mM Tris buffer pH 7.8+/-0.1 and 5 % human serum proteins.

HDV Antigen Diluent for ELISA Analysis

HDV diluents were in 1×16 ml/vial. The buffered solution for the dissolution of the lyophilized HDV antigen contained 0.2M Tris buffer ph 6.0+/-0.1 % kathon GC and 0.2 % Triton × 100. The component is red colour coded.

Specimen Diluent for ELISA Analysis (DILSPE)

Specimen diluent was in a 2 × 60.0 ml/vial. It's a buffered solution for the dilution of the samples; it contained 0.2M Tris buffer pH 6.0+/-0.1, 0.2 % Tween 20, 3 % skimmed milk, 0.1% kathon GC and 0.09 % sodium azide as preservatives. The component is blue colour coded.

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

Prevalence of HBsAg/HDV-IgM Co-infection among Study Population

A total of 89 samples that tested positive for HBsAg were investigated to ascertain the occurrence of HBsAg/HDV-IgM co-infection. A prevalence rate of 5.6 % (n=5) was recorded in this study.

Prevalence of HBsAg/HDV-IgM Co-infection According to Gender

The prevalence rate for the male HBsAg/HDV-IgM co-infection was found to be 2.7 % which is lower when compared to the prevalence rate of HBsAg/HDV-IgM co-infection among the females which was 7.7 %. However, there was no significant difference between gender at ($p > 0.31$) (Table 1).

Prevalence of HBsAg/HDV-IgM Co-infection According to Marital Status

With regards to marital status, HBsAg/HDV-IgM co-infection was higher among the married, having a prevalence rate of 6.9 % which is much lower when compared to their single counterparts which have a prevalence rate of 3.2 % (Table 1). There was a significant difference for marital status at ($p < 0.02$).

Prevalence of HBsAg/HDV-IgM Co-infection According to Age

The age group of 29-33 years is shown to have the highest HBsAg/HBV-IgM co-infection prevalence rate of 10 %, a rate that is closely followed by the age group 24-28 years with 7.1 % prevalence rate. However, the age groups of 34-39 years and 40-45 years showed the lowest HBsAg/HDV-IgM co-infection prevalence rate of HBsAg/HDV-IgM co-infection which is 0 % (Table 1). There is no significant difference between the various age groups at ($p > 0.88$).

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 1). 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 1). 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 1). There is no significant difference with respect to occupation at ($p > 0.44$).

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

Participants who were pregnant women showed the highest HBsAg/HDV-IgM co-infection prevalence rate of 9.5 % while participants who were blood donors had the lowest prevalence rate of 2.1 % (Table 1). There is no significant difference with respect to patient type at ($p > 0.13$).

Table 1: 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
	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

Socio-Demographic Characteristics		No. Tested	No. Positive	No. Negative	Percentages
	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

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 Imarenezor *et al.* (2016) 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 Imarenezor *et al.* (2016). 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 Imarenezor *et al.* (2023), 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.

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 (Imarenezor *et al.*, 2024) 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 (Gupta *et al.*, 2005).

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, the study reveals a significant

prevalence of HBV and HDV co-infection among patients in Abakaliki Metropolis, Ebonyi State, Nigeria. The findings highlight the need for routine screening of HDV among HBsAg-positive patients and targeted public health interventions to prevent HDV co-infection and manage HBV infection severity. Effective preventive measures should be put in place to mitigate the severity of HBV infection contributed by HDV co-infection.

REFERENCES

Branch, A. D., Benenfeld, B. J., Baroudy, B. M., Wells, F. V., Gerin, J. L. and Robertson, H. D. (1989). An Ultraviolet-sensitive RNA Structural Element in a Viroid-like Domain of the Hepatitis Delta Virus. *Science,* 243 (4891): 649–652.

Casey, J. L., Brown, T. L., Colan, E. J., Wignall, F. S. and Gerin, J. L. (1993). A Genotype of Hepatitis D Virus that occurs in Northern South America. *Procreate of the National Academy of Science,* 90: 9016–9020.

Casey, J. L., Tennant, B. C. and Gerin, J. L. (2006). Genetic Changes in Hepatitis Delta Virus from Acutely and Chronically Infected Woodchucks. *Journal of Virology* 80: 6469–6477.

Engelke, M., Mills, K., Seitz, S., Simon, P., Gripon, P., Schnölzer, M., and Urban, S. (2006): Characterization of a Hepatitis B and Hepatitis Delta Virus Receptor Binding Site *Hepatology*. 43 (4): 750–760.

Fattovich, G., Bortolotti, F., and Donato, F. (2008): Natural History of Chronic Hepatitis B: Special Emphasis on Disease Progression and Prognostic Factors. *Journal of Hepatology*. 48: 335–352.

Fattovich, G., Giustina, G., and Christensen, E. (2000): Influence of Hepatitis Delta Virus Infection on Morbidity and Mortality in Compensated Cirrhosis Type B. Journal of General Virology. 46 (3): 420–426.

Fattovich, G., Giustina, G., Christensen, E., Pantalena, M., Zagni, I., Realdi, G., and Schalm, S. W. (2000): Influence of Hepatitis Delta Virus Infection on Morbidity and Mortality in Compensated Cirrhosis type B. *The European Concerted Action on Viral Hepatitis*. 46: 420–426.

Francois, M., and Roggendorf, M. (2005): Immunology of HDV Infection. *Current Top Microbiology and Immunology*. 307: 187–209.

Govindarajan, S., De Cock, K. M., and Redeker, A. G. (1986): Natural Course of Delta Super Infection in Chronic Hepatitis B virus-infected Patients: Histopathologic Study with Multiple Liver Biopsies. *Hepatology* 6: 640–644.

Govindarajan, S., Fields, H. A., Humphrey, C. D., and Margolis, H. S. (1986): Pathologic and Ultrastructural changes of Acute and Chronic Delta Hepatitis in an Experimentally infected Chimpanzee. *American Journal of Pathology*. 122: 315–322.

Greco-Stewart, V. S., Schissel, E., and Pelchat, M. (2009): The Hepatitis Delta Virus RNA Genome Interacts with the Human RNA Polymerases I And III. Journal of Virology. 386 (1): 12–15.

Imarenezor, E.P.K., N. P. Ofiri, O. E. Yakubu, S. T. C. Brown, F. U. Ebuara and S. Sani (2019). CD4, CD8 T-Lymphocytes and WBC profiles during pregnancy among HIV-seronegative women in Wukari, North East, Nigeria. FUW Trends in Science & Technology Journal, 4 (3): 930 – 933

Imarenezor EPK, Brown STC, Yakubu OE, Soken DC, 2016. Survey of Hepatitis B and C among students of Federal University Wukari, Taraba State, Nigeria. Int Res J Med Med Sci, 4(3): 31-37.

Imarenezor Edobor Peter Kenneth, Anyiam Ifeoma Vivian, Abhadionmhen Abel Onolunosen, Ndubuisi Miracle Nneoma, Ekeh Amarachi Promise (2023). Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) Co-Infections among Patients attending General Hospital, Wukari, Taraba State, North East, Nigeria. *International Journal of Medical and All Body Health Research*, 4 (2):36-42

Imarenezor Edobor Peter Kenneth and Benjamin Nanisi Daniel (2024). Unveiling Co-Infections: Hepatitis C Virus and Malaria Sero Prevalence among Outpatients Attending General Hospital Wukari in Taraba State, Nigeria. *Int. J. Adv. Biol. Biomed. Res.*, 12(2), 192-205

Imarenezor Edobor Peter Kenneth, Anyiam Ifeoma Vivian, Abhadionmhen Abel Onolunosen, Iduku Husseni (2023). The immunological evaluate of Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) co-infection with plasmodium in Wukari, Taraba State, North East, Nigeria. *International Journal of Medical and All Body Health Research,* 4(2): 43-47

Imarenezor Edobor Peter Kenneth, Anyiam Vivian Ifeoma, Ofiri Pascal Ngozi, Abhadionmhen Onolunosen Abel (2024). Pervasiveness of Human Immuno-Deficiency Virus (HIV), among Individuals in Wukari, Taraba State, North-East Nigeria. International Journal of Advanced Biological and Biomedical Research, 12(3), 237-247.

Kamimura, T., Ponzetto, A., Bonino, F., Feinstone, S. M., Gerin, J. L., and Purcell, R. H .(1983). Cytoplasmic tubular structures in Liver of HBsAg carrier Chimpanzees infected with Delta agent and comparison with cytoplasmic structures in non-A, non-B hepatitis. *Hepatology,* 3: 631–637.

Kuo, M. Y., Chao, M., and Taylor, J .(1989). Initiation of Replication of the Human Hepatitis Delta virus Genome from cloned DNA: Role of Delta antigen. *Journal of Virology,* 63: 1945–1950.

Lehmann, E., Brueckner, F., and Cramer, P .(2007). Molecular basis of RNA-dependent RNA Polymerase II Activity. *Nature,* (7168): 445–449.

Nisini, R., Paroli, M., Accapezzato, D., Bonino, F., Rosina, F., Santantonio, T., Sallusto, F., Amoroso, A., Houghton, M., and Barnaba, V .(1997). Human $CD4^+$ T-cell response to hepatitis Delta virus: Identification of multiple epitopes and characterization of T-helper cytokine profiles. *Journal of Virology,* 71: 2241–2251.

Onwuakor, C. E., Eze, V. C., Nwankwo, I. U. and Iwu, J.O. (2014). Sero-prevalence of Hepatitis B Surface Antigen (HBsAg) amongst Pregnant Women Attending Antenatal Clinic at the Federal Medical Centre Umuahia, Abia State, Nigeria. *American Journal of Public Health Research,* 6;255-259

Opaleye, O. O., Tijani, B. A., Zakariyahu, T. O and Taiwo, S. S (2016). Prevalence of HBsAg and HIV among blood donors in Osogbo, Osun State, Nigeria. International Resource Journal of Medical Science, (1):68-71.

Ottobrelli, A., Marzano, A., Smedile, A., Recchia, S., Salizzoni, M., Cornu, C., Lamy, M. E., Otte, J. B., De Hemptinne, B., and Geubel, A .(1991). Patterns of Hepatitis Delta virus Reinfection and Disease in Liver Transplantation. *Gastroenterology,* 101: 1649–1655.

Poisson, F., Roingeard, P., Baillou, A., Dubois, F., Bonelli, F., Calogero, R.A. and Goudeau, A. (1993): Characterization of RNA-binding Domains of Hepatitis Delta Antigen. Journal of General Virology, 74 (11): 2473–2478.

Rizzetto, M., Canese, M. G., Purcell, R. H., London, W. T., Sly, L. D., and Gerin, J. L. (1981). Experimental HBV and delta infections of chimpanzees: occurrence and significance of intrahepatic immune complexes of HBcAg and delta antigen. Hepatology. 1 (6): 567–574.

Schulze, A., Schieck, A., Ni, Y., Mier, W., and Urban, S .(2010). Fine Mapping of Pre-S Sequence Requirements for Hepatitis B Virus Large Envelope Protein-Mediated Receptor Interaction. Journal of Virology. 84 (4): 1989–2000.

Schulze, A., Schieck, A., Ni, Y., Mier, W., and Urban, S. (2010): Fine Mapping of Pre-S Sequence Requirements for Hepatitis B Virus Large Envelope Protein-Mediated Receptor Interaction. Journal of Virology, 84 (4): 1989–2000.

Tahaei, S. M. E., Mohebbi, S. R., Azimzadeh, P., Behelgardi, A., Sanati, A., and Mohammadi, P (2014): Prevalence of Hepatitis D Virus in Hepatitis B Virus Infected Patients Referred to Taleghani Hospital Tehran, Iran. *Journal of Gastroenterology and Hepatology*, 7 (3):144-150.

Taylor, J. M. (2006): Hepatitis Delta Virus. Virology. 344 (1): 71–76.

Wang, K (1986): "Capture" Enzyme Immuno-Assay (ELISA) for the Determination of IgM antibodies to Hepatitis Delta Virus in Human Plasma and Sera. *Nature,* (323):508-514.

Wang, K. S., Choo, Q. L., Weiner, A. J., Ou, J. H., Najarian, R. C., Thayer, R. M., Mullenbach, G. T., Denniston, K. J., Gerin, J. L., and Houghton, M .(1986). Structure, sequence and expression of the hepatitis delta (delta) viral genome. Nature, 323 (6088): 508–514.

Wu, H. N., Lin, Y. J., Lin, F. P., Makino, S., Chang, M. F., and Lai, M. M .(1989). Human hepatitis delta Virus RNA Subfragments contain an Autocleavage Activity. *Proceedings of the National Academy of Sciences of the United States of America.* 86 (6): 1831–1835.