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Prevalence of Hepatitis D virus infection among Hepatitis B virus surface antigen positive children attending two selected Hospitals in Jos, Nigeria

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Abstract

Hepatitis B virus is a member of *Hepadnaviridae* family that cause liver infections in humans. These viral infections are characteristically linked with cirrhosis, chronic hepatitis, and hepatocellular carcinoma that are the leading cause of morbidity and mortality in the population of developing countries including Nigeria. Young children and newborns are more likely to have liver damage early in life and become chronic HBV carriers. This study identified the frequency of hepatitis B and D co-infection in children visiting the emergency pediatric units of Plateau State Specialist Hospital and Bingham University Teaching Hospital in Jos, Plateau State, Nigeria. The virus was screened using immunochromatographic Skytec® HBsAg test strips and Alere chase buffer for the qualitative detection of HBsAg in serum. HBsAg positive samples were confirmed using HBsAg Monolisa ELISA kit. Using an Italian HDV ELISA kit, samples that tested positive for HBsAg were further examined to evaluate the presence of HDV co-infection. The finding of this study revealed that 4.4% of children had HBsAg markers on their bodies. None of the children with HBV infection were co-infected with HDV. The HBV prevalence was found to be highest in children aged 6 to 10 years. Females had higher levels of HBV seropositivity (5.5%). Risk factors in children included birthplace (native households), failure to check children's HBV status after immunization, and history of jaundice. To properly manage and prevent this infection, it is advised that children should be promptly immunized at birth, their HBV status should be confirmed following immunization and they should be revaccinated if necessary.

Keywords: Hepatitis B Virus (HBV); Hepatitis Delta Virus (HDV); Hepatocellular carcinoma (HCC); Chronic hepatitis B (CHB); Hepatitis B surface antigen (HBsAg)

1. Introduction

Hepatitis B is a partly double-stranded DNA virus, that is a member of the *Orthohepadna* virus genus and of the *Hepadnaviridae* family [1]. Hepatitis B infection caused by HBV can result in both acute and chronic hepatitis. The majority of those who are acutely or chronically infected are asymptomatic, which is why it is called a "quiescent epidemic". Hepatitis B infection that is acute lasts for less than six months. Within a few months, recovery will be complete and the body will be free of acute hepatitis B. The incubation period for chronic hepatitis B is six months or more. It persists as

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a result of the immune system's inability to eradicate the infection [2]. A lifetime of chronic hepatitis B infection may result in life-threatening conditions like cirrhosis and liver cancer [3].

A faulty RNA virus that resembles some plant viroids[1], the hepatitis delta virus (HDV) depends on HBV as a helper virus to spread [4]. About 15 to 20 million of the 240 million chronic HBV carriers estimated globally [5] also have HDV infection [6,7]. Of the estimated 65 million chronic HBV carriers in Africa, roughly one-fourth of HBsAg-positive people have dual HDV infection [6]. Over 360 million cases of chronic hepatitis and 620,000 fatalities each year are attributable to HBV, which is a major cause of liver disease morbidity and mortality globally [8]. In Sub-Saharan Africa (SSA), it is hyperendemic (i.e., > 8% of the population is infected), and it is a significant contributor to chronic liver disease [9,10,11]. Perz and his coworkers reported that HBV is responsible for 44% of cases of cirrhotic liver disease and 47% of cases of hepatocellular carcinoma in SSA [11]. There are thought to be 10-15 million HDV carriers worldwide as a result of the projected co-infection of 5% of HBsAg carriers with HDV [12]. Several investigations have demonstrated that HDV infection worsens the course of HBV co-infection, hastening the development of cirrhosis and causing early liver function decompensation in comparison to HBV mono-infection [13,14,15]. According to epidemiological research, HDV is widespread and that its local incidence depends on the prevalence of HBV [15]. Any population with a high proportion of HBV carriers may also be at risk for HDV infection outbreaks, which, when co-infected with HBV, may worsen the clinical course and outcome of hepatitis [16,17]. These risks are in addition to the risks associated with HBV infection.

Due to the most hazardous methods of HBV spread, children are more vulnerable and exposed to it. The first generation is passed vertically from mother to children, while the second occurs when they are exposed to the virus at a young age through horizontal transmission. According to a World Health Organization (WHO) report from 2009, other primary sources of infection include transfusion of blood from unscreened donors, sharing of household items like toothbrushes, exposure to contaminated or unsterilized surgical equipment for medical and dental procedures, body and ear piercing, tattooing, and the use of unsterilized objects for traditional rituals, circumcision, and face marking, among others [18]. Additionally, it is risky to presume that all children who have received the vaccine are immune to HBV because the majority of vaccines may not be effective. After a brief interval, every child who received vaccination should be retested to determine whether they are immune to the virus or susceptible to it. If they are susceptible, they should get revaccinated because chronic carriers are more likely to have first contracted the virus when they were younger and hence have a 15-40% chance of developing complications from chronic hepatitis B [19]. Getting to the bottom of HBV in Nigeria is crucial, but doing so will hamper public health. Lack of understanding about the virus's route of transmission, its effects on children, and immunization may be the main factors contributing to the virus' spread among Nigerian children.

The findings from this study have identify infant-related HBV infections that frequently progress to chronic cases, which typically cause liver cirrhosis and hepatocellular cancer, highlighting the urgent need to prevent mother-to-child transmission [20,21]. Additionally, it's critical to identify children who have hepatitis B and D co-infection because this opens the door for additional research on the condition. Co-infection with B and D tends to be more severe than when the child has 'B' alone. To the best of the researcher's knowledge, published data on HDV infection in Nigeria are scarce compared to information on the frequency of HBV infection in children in Nigeria. Prior research on HDV infection in Nigeria has only been done on a small number of people, such as adults [23], patients on highly active antiretroviral drugs [22]. However, anti-hepatitis B virus core antibody (anti-HBc), which persists during infection resolution and thus represents both past and present infection, and HBsAg are the most reliable markers for assessing the burden of HBV infection [24]. In order to ascertain the seropositivity rate of the HBsAg marker among children visiting the Plateau State Specialist Hospital and Bingham University Teaching Hospital, Jos, Nigeria, this study was conducted. We examined all the children who tested positive for HBsAg for the presence of HDV because it has been established that only people who have HBV can become infected by HDV.

2. Material and methods

2.1. Study Site

The study was carried out at Plateau State Specialist Hospital (PSSH) and Bingham University Teaching Hospital (BHUTH) both in Jos, Plateau State, Nigeria.

2.2. Study Design

A cross sectional study design was used for conducting this research among children 1-16years attending the Emergency Pediatrics Unit (EPU) BHUTH and the EPU of PSSH in Jos, Plateau State.

2.3. Study Population

One hundred and eighty children were recruited for this study, aged 1-16years attending EPU of BHUTH and PSSH Jos.

2.4. Inclusion and Exclusion Criteria

Children attending EPU of BHUTH and PSSH whose parents/guardians gave consent, children 1-16years attending EPU of BHUTH and PSSH, and children between 1year to 16years were included in this study, whereas children 1-16years attending EPU of BHUTH and PSSH whose parents/guardians did not give consent, children 1-16years not attending EPU of BHUTH and PSSH were excluded.

2.5. Sample Size

This was determined using the formula described by [25] as thus:

$$N = \frac{Z^2 pq}{d^2}$$

P = Local prevalence of 11.5% (0.115) was used from a study on Seroprevalence and Associated Risk Factors of Hepatitis B Virus Infection Among Children in Enugu Metropolis. [27]

To accommodate poor response rate, 10% (156.3) increase on the sample was allowed.

Approximately minimum sample size =172. However, 180 samples were collected.

2.6. Sample Collection and Preparation

Following the participant interviews, 5 ml of venous blood was drawn from kids attending the EPU of BHUTH and PSSH in Jos using a vacutainer needle and tube while applying a tourniquet. At the Department of Serology at the Aids Prevention Initiative in Nigeria (APIN), JUTH (Jos University Teaching Hospital), blood samples were centrifuged at 5000 rpm for 10 minutes to produce the serum, which was then stored at -20^{o}C in cryovial tubes till the time of analysis and several freeze-thaw cycles were avoided. Samples were examined at JUTH, APIN, and Jos.

2.7. Laboratory Evaluation

Between November 2020 and May 2021, specimen testing was carried out in the Department of Serology at the Aids Prevention Initiative in Nigeria (APIN), JUTH (Jos University Teaching Hospital).

2.8. Testing for HBsAg And HDV Antibodies

For HBV serology, serum samples were tested for HBsAg using immunochromatographic Skytec® HBsAg test strips (produced by Skytec Diagnostics USA) and Alere chase buffer (produced by Alere LTD, UK). Samples positive for HBsAg were confirmed using ELISA kits by MonolisaTM HBs Ag (US) ULTRA assay, and the confirmed HBsAg positive samples were screened for antibodies of HDV using DAB.Italian company PRO Diagnostic Bioprobes Srl is located in Via G. Carducci 27, 20099 Sesto San Giovanni (Milan).

2.9. Statistics Analysis

Chi-square was used to test for significant association between sociodemographic characteristics and HBV/HDV co-infection in children at the 95% confidence level, using statistical Package for social sciences (SPSS) version 22.0, results were presented using contingency tables.

3. Results

The prevalence of HBsAg was determined among children attending Emergency Pediatric Units of PSSH and BUTH in Jos Plateau State. Seroprevalence of HDV infection among a group of known HBsAg-positive subjects was also measured. A total of 180 children age 1-16 years participated in this study, 107 were males while 73 were females with a Male to Female sex ratio of 1.5:1. Of the 180 children screened, 8 were positive for HBsAg giving a prevalence of 4.4%. HBsAg positivity remained the same (4.4%) after confirmatory test. There was no HBV/HDV coinfection observed in study participants (Table 1.

Table 2 shows the Socio-demographic characteristics of children with respect to age and sex There was no statistically significant association observed with respect to sex (p=0.856), and age (p=0.403).

Table 1 Overall Prevalence of Hepatitis B Surface Antigen Among Children

Infection For	Number Screened	Number Infected	HBV Prevalence (%)	HDV prevalence (%)
Hepatitis B	180	8	4.4	0.0

Table 2 Age and sex distribution of serologic markers of HBV and HDV infection in Jos, Nigeria

Variables	Response	No. examined	HBsAg (%)	HBsAg ELISA (%)
Age (years)	1-5	66	2(3.0)	2(3.0)
	6-10	52	4(7.7)	4(7.7)
	11-16	62	2(3.2)	2(3.2)
	Total	180	8(4.4)	8(4.4)
Sex	Male	107	4(3.7)	4(3.7)
	Female	73	4(5.5)	4(5.5)
	Total	180	8(4.4)	8(4.4)

Table 3 Prevalence of Hepatitis B virus infection in Relation to Medical history of the children

Medical history	Responses (Yes)	HBsAg Positivity	Responses (No)	HBsAg Positivity	p-value
Place of birth					0.001
Hospital	135	4(3.0)			
Native homes	10	3(30.0)			
At home	14	1(7.1)			
Others	21	1(4.8)			
Assumed HBV status					0.044
Positive	0	0			
Negative	119	3(2.5)			
Unknown	55	5(9.5)			
History of vaccination	108	5(4.6)	72	3(4.2)	0.536
Blood transfusion	13	0	167	8(4.7)	0.573
Circumcision	55	0	125	8(6.4)	0.180
History of jaundice	18	3(16.7)	161	5(3.1)	0.008

Result is significant were $p<0.05$

Table 3, shows the Prevalence of Hepatitis B virus infection in Relation to Medical history of the children. Place of birth of the children which include; (hospital, native home, at home and others), native home had a higher prevalence of (30.0%), p=0.01. HBV status based on post HBV vaccination, none of the children was observed to be positive, of the 119 children that accepted to be HBV negative, 3 children turned out to be positive after screening giving a prevalence of 2.6% while 55 children were not sure of their status (unknown), out of which, 5 children turned out to be positive

giving a prevalence of 9.5%. HBV status of the children after vaccination has a statistical association with HBV infection p=0.04. Among the following medical history which include, History of immunization of the children, blood transfusion, circumcision, and history of jaundice. History of jaundice was observed to be the only risk factor that was statistically significant (p=0.008.)

Table 4: Prevalence of Hepatitis B Virus Infection of Children in Relation to medical history of the mother which include; history of immunization of the mother, and treatment of HBV positive pregnant mothers. History of immunization of mothers and treatment of HBV positive pregnant mothers were statistically significant (p=0.03) and (p=0.001) respectively.

Table 4 Prevalence of Hepatitis B Virus Infection of Children in Relation to HBV Status of the mothers

Medical history	**Responses Yes**	**HBsAg Positivity**	**Responses (No)**	**HBsAg Positivity**	**p-value**
History of vaccination	26	1(3.8)	119	7(5.8)	0.029
Assumed HBV status of mothers when pregnant:					0.290
Positive	9	2(22.2)			
Negative	98	4(4.1)			
Unknown	70	2(2.8)			
Treatment of HBV mother including during pregnancy	5	2(3.6)	172	6(3.5)	0.001

Result is significant where p<0.05

4. Discussion

Hepatitis Delta Virus (HDV) continues to be a serious global health issue, impacting 15–20 million people worldwide. HDV co-infection or super infection means that the host liver cells are previously been infected by hepatitis B virus.[36] There are several reports on prevalence of Hepatitis B Surface Antigen (HBsAg) amongst children in Nigeria, however, there is paucity of information on the prevalence of HDV or its co-infection with HBV. The Center for Disease Control estimated the prevalence of chronic hepatitis B (CHB) infection in Nigeria to be at least 8%. [35] The prevalence of HBV among children has been reported to be 29.0% in Riyom, Plateau state Nigeria [26] and 9.75% in Central Nigeria [27] which are relatively higher compared to our study that showed (4.4%) within the same region. Conversely, studies conducted in Enugu [28] and Calabar [29] had shown a lower prevalence of 3.1% and 1.2%, respectively. Another study in Enugu, Nigeria, reported a HBsAg prevalence of (4.3%), which is in agreement with the findings of this study [30].

Of the 4.4% HBsAg positive children, none of the children tested positive for HDV, indicating a zero prevalence of HDV co-infection or superinfection among the study participants. This study is in agreement with a study in Bangladesh that reported zero prevalence of HDV in children under the age of 15 years [24]. Unlike this study, other similar studies have reported HDV superinfection. HDV superinfection of 1.76 % was reported among children in Turkey [37], another study has reported 10.3% co-infection among children in Taiwan [36]. The cause of these discrepancies is largely unknown; however, it may be due to the higher number of HBV chronic carriers among study participants than in the current study. Also, HDV cannot stand on its own without a helper virus which is HBV, thus higher prevalence of HBV in those studies could underscore the superinfection, as children not infected with HBV may likely not contact HDV. The zero prevalence of HDV superinfection among participants in this study could be multifactorial. The fact that Jos, the study's location, is home to multiple primary and secondary healthcare centers, in addition to two tertiary health institutions may account for the zero prevalence. This can be as a result of greater rate of childhood vaccinations in the city against HBV, accompanied by robust health education on preventive measures, early healthcare seeking behaviors, and an efficient use of these medical services by the respondents. Also, the children selected for this study were born after the hepatitis B vaccine was included into the Nigerian National Program on Immunization (NPI) in 2004 giving at birth, which predates their birth. This could be the cause of the zero HDV superinfection or co-infection found in this study.

Age and sex related HBsAg seropositivity revealed that respondents who were 1-5 years showed the lowest prevalence (3.0%), while the highest was in respondents who were 6-10 years (7.7%). This finding is consistent with a related study [28,] elsewhere, the prevalence of HBsAg was found to be lowest in children aged 1-5 years (2.0%) and highest in those aged 6-10 years (6.5%). Lower prevalence among children 1-5 years is expected since the prevalence of HBV

among pregnant women in Jos remained low as the major source of infection among these age group is via vertical transmission. Higher prevalence rate among children 6-10 years may serve as an additional evidence of the Jos HBV vaccine's efficacy. The high prevalence in this age group may be due to the fact that most children are quite active and exploring the world at this age, it also highlights the high rate of horizontal transmission of HBV infection in our environment. The difference in prevalence per age group may be due to an increase vulnerability of older children to other risky behaviors such as adult unprotected sexual habits, sharing of sharp objects, unsafe circumcision and blood transfusion. Female children exhibited a higher prevalence of HBsAg (5.5%) than their male counterparts (3.7%). This is consistent with a studies elsewhere[38] who also reported female children to have higher prevalence of HBV than male subjects.[36,37] While the exact cause of this female predilection remains largely unknown, one possibility could be the female sex ratio in the study, this could also be due to the number of female children born to infected mothers which predispose their unborn children to the virus. HBV-infected mothers will develop chronic HBV infection without postexposure immune prophylaxis, and some may eventually pass away from chronic liver disease or transmit to their fetus.[33,39]

5. Conclusion

The results of this study show a zero-prevalence rate of HDV-HBV co-infection in Jos. Females were more infected with HBV than Males. Factors that favored this outcome was the high level of child HBV vaccination status, low HBV prevalence among pregnant women and place of child birth in Jos. Nigeria is an endemic country for HBV infection which predispose to HDV infection, a study covering larger number of children and location in other part of the country is recommended to ascertain the distribution of HDV co-infection. Therefore, the general public, health authorities, practitioners and healthcare managers should be made aware of the risk factors associated with dual infection of HBV and HDV. Especially place of birth, blood transfusion, circumcision e.t.c. Proper vaccination against HBV at birth and awareness programs should be started to prevent this HBV/HDV dual coinfection.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors hereby state that they have no competing interests.

Statement of ethical approval

The Health Research Ethics Committee of BHUTH, Jos, and the Health Research Ethics Committee of PSSH, Jos, both granted me their approval. Informed consent, both verbal and written, was obtained from parents, caregivers, and the children while permission to conduct the study was received from several Heads of EPU. The necessity and advantages of the study were properly explained to them.

Statement of informed consent

Informed consent was received from the subject and/or the patients in both written and verbal form. They were, however, well informed about the significance and advantages of the study.

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