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Hepatitis Delta Virus in Patients Referred for Malaria Parasite Test in Ile-Ife, Nigeria

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Abstract
Hepatitis delta virus (HDV) infection is considered the most severe form of human viral hepatitis. The infection only occurs in the presence of a concomitant hepatitis B virus (HBV) and leads to severe liver disease that includes fulminant liver failure and rapid progression to cirrhosis, as well as increased risk of hepatocellular carcinoma (HCC). Diverse data exist on HBsAg carrier rates, but not much is known about the rates of HDV co-infection in cases of HBV infections in Nigeria. More so, HDV has not been detected in people with unknown HBV status or people who had no clinical evidence of liver disease in Nigeria. We therefore determined the prevalence of HBV and HBV/HDV co-infection in patients without clinical evidence of liver disease referred for malaria parasites test from two health institutions in Ile-Ife, Nigeria, using one step HBsAg diagnostic kit (Nova Diagnostic®, USA) and HDV IgM and IgG ELISA kit (Dia.Pro, Italy). Of the 275 patients tested, 38 (13.8 %) were HBsAg positive and 12 (31.6%) of them had antibody to HDV, hence HBV/HDV prevalence in the study population was 4.4 % (12/275). The result of this study shows a significant HBV/HDV prevalence in the study population, and the need for public enlightenment to further improve management. There is also the need to consider HDV screening in patients with no clinical evidence of liver disease.
Keywords: HDV, Hepatitis B, co-infection,, ELISA, Nigeria

1. Introduction

Hepatitis delta virus (HDV) is still a neglected pathogen (Elazar and Glenn 2017), although it causes the most severe form of human viral hepatitis (Alves *et al*., 2013; Carvalho *et al*., 2016; Lempp and Urban, 2017) and is associated with a higher risk of cirrhosis, liver decompensation and liver cancer (Siederdissen and Cornberg, 2016; Elazar and Glenn 2017; Lempp and Urban, 2017). To disseminate in its host, HDV, a unique RNA virus, depends on a helper virus, the human Hepatitis B virus (HBV), which provides the envelope proteins required for HDV assembly (Lempp and Urban, 2017; Nguyen *et al*, 2017). Hence, HDV requires the presence of HBV to infect the hepatocytes (Rizzetto *et al*., 1980). Accordingly, HDV infection either establishes as a superinfection of an HBV-carrier or by simultaneous contact with HBV and HDV. Both co-infection and superinfection with HDV result in more severe complications compared to infection with HBV alone. (Pascarella and Negro, 2011; Jamjoom *et al*., 2017; Lempp and Urban, 2017).

HDV affects an estimated 15-20 million out of the 240 million chronic HBV-carriers globally (Baatarkhuu *et al*., 2017; Lempp and Urban, 2017). Higher HDV prevalence is found in Africa and other continents including the Middle East, Mediterranean, Amazonas, and in Asian countries. (Mumtaz *et al*., 2005; Wedemeyer and Manns, 2010; Hughes *et al*., 2011; Andernach *et al.,* 2014). In West Africa, little is known about the epidemiology of HDV infection (Coffie *et al*., 2017). Though, HDV is not routinely diagnosed, it has been estimated that approximately 5% of HBV carriers are co-infected with HDV worldwide (Hughes *et al*., 2011; Rizzetto and Ciancio 2012) and between 3% and 25% in West Africa (Diop-Ndiaye *et al*., 2008; Mansour *et al*., 2012; De Paschale *et al*., 2014; Honge *et al*., 2014). Patients with HBV/HDV co-infection have a significantly increased risk for HCC compared with patients with HBV mono-infection and the general population (Ji *et al*., 2012).

Many hospitals do not routinely check for anti-HDV status of HBV patients, therefore a major problem of HDV diagnosis remains the low testing rates in HBV-infected individuals in different countries. Screening for both viruses should be performed to allow a better management. This study therefore aimed at estimating the prevalence of HBV and HBV/HDV co-infection in patients referred for malaria parasite tests.

2. Materials and Methods

2.1 Sample Collection and Preparation

Blood samples were obtained from patients with fever, hence referred for malaria parasite test at the Haematology Laboratories of the Obafemi Awolowo University Teaching Hospital and the Obafemi Awolowo University Health Centre between July and October 2009. A total of 275 patients (Male = 176, Female = 99; Age range= 1-80 years) were enrolled for the study. Five millilitres of blood was collected from each of them into labelled sterile plain bottles free of anticoagulants or preservative. Each blood specimen was separated by low-speed centrifugation at 500 g for 5 minutes, the sera were transferred into labelled cryovials, and then kept frozen at -20°C until analyzed.

2.2 Methodology

Hepatitis B surface antigen (HBsAg) was tested using one step HBsAg diagnostic kit (Nova Diagnostic®, USA). The Kit is based on the principle of sandwich immunoassay for detection of HBsAg in serum. Sensitivity and accuracy is at 2ng/ml and 99% respectively. HBsAg positive serum samples were then tested for total anti-HDV antibodies (IgG and IgM) using Diapro HDV Ab Diagnostic Bioprobes (Milano, Italy). All analyses were performed according to the manufacturers' instructions.

3. Results

3.1 HBV, HDV and HBV/HDV

Of the 275 patients screened, 38 (13.8 %) had detectable HBsAg. Twelve (31.6%; 12/38) of them had detectable HDV antibodies in addition to the HBsAg. Thus, the prevalence of HBV/HDV co-infection in the study population was 4.4% (12/275) (Table 1).

3.2 Prevalence of HBV by age and location

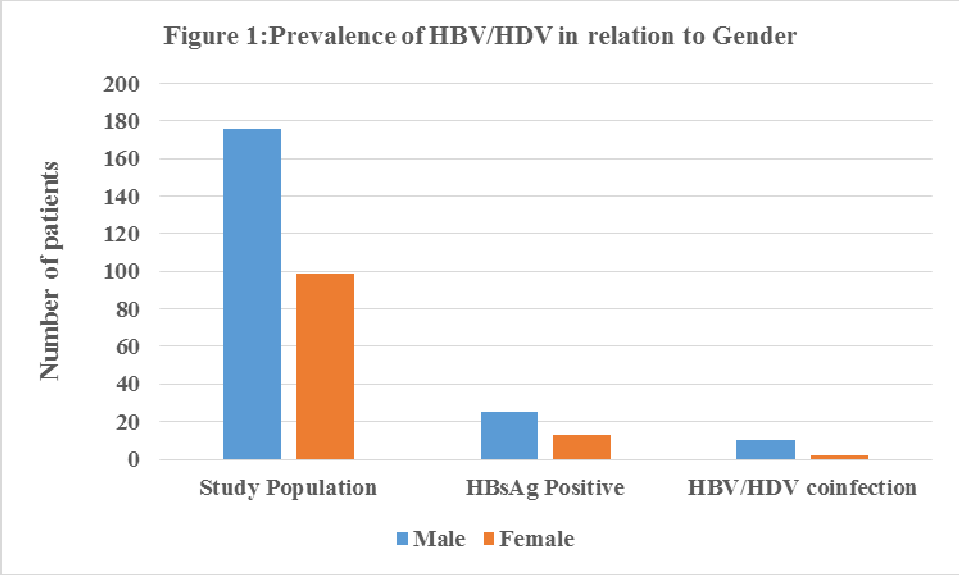
Highest HBV prevalence rate (20.0%) was recorded among patients in age groups 1-10 and 51-60 years while the highest HDV prevalence was found in age group 1-10 (6.7%) and in age group 21-30 (6.4%). Majority (more than 50%) of the HBV and HBV/HDV infection occurred between ages 1-40 years (81.6% and 91.7% respectively). No HBV/HDV co-infection was detected in patients above 50 years of age (Table 1).

3.3 HBV/HDV co-infection by Gender

Figure 2 shows the distribution of HBV/HDV co-infection according to gender. Of the 176 male included in the study, 25(14.2%) were seropositive for HBsAg while 13(13.1%) of the 99 females were seropositive. HBV/HDV co-infection among male and female were 10 (83.3%) and 2(16.7%) respectively.

Table 1: ***Age distribution of the study population and HBV/HDV co-infection***

Age Category (Years)	Study population	HBsAg Positive (%)	HBV/HDV co-infection (%)
1-10	15	3 (20)	1 (6.7)
11-20	28	4 (14.3)	0 (0)
21-30	125	17 (13.6)	8 (6.4)
31-40	57	7 (8.8)	2 (3.5)
41-50	28	4 (14.3)	1 (3.6)
51-60	10	2 (20)	0 (0)
61-70	6	0(0)	0 (0)
71-80	6	1 (16.7)	0 (0)
Total	275	38 (13.8)	12 (4.4)



4. Discussion

This is the first report of HDV antibody detection in patients not previously diagnosed for HBV and with no

clinical evidence of liver disease in Nigeria.

HDV is still a neglected pathogen, despite the fact that it causes chronic Hepatitis D infection which presents as the most severe form of viral hepatitis, leading to accelerated progression of liver dysfunction including cirrhosis and hepatocellular carcinoma and a high mortality rate (Lemp and Urban, 2017). Even though HDV is spread worldwide and is endemic in some regions, screening and treatment has been often neglected (Elazar *et al*., 2017). Test and treatment programs have been reported to be highly efficient in reducing HBV and HDV prevalence in the population (Goyal and Murray, 2017). The disease is not routinely diagnosed in Nigeria and there are so far no recommended standard therapy and diagnosis in place for HDV in the country. HDV remains a problem in any region where HBV is endemic (Nguyen *et al*., 2017) hence, a problem in Nigeria.

The study reports the prevalence of HBV and HBV/HDV co-infection in a population with unknown HBV status, presenting with fever and referred for malaria parasite test. The observed prevalence rates of HBV (13.8%) infection and HBV/HDV (4.4%) co-infection were relatively high, much more that the study population is not being managed for known liver disease. HDV prevalence rates of 31.6% (12/38) in the HBsAg positive population is also high. This results suggests that HDV is contributing to significant morbidity and mortality rates as previously observed (Nwokediuko and Ijeoma, 2009) in HBV-related liver diseases in Nigeria

A few studies have been carried out in Nigeria to detect the presence of HDV antibody, antigen or DNA among HBsAg positive individuals and the HDV prevalence rates ranges from of 0% to 12.5% (Amazigo and Chime, 1988; Ojo *et al*., 1995; Ojo *et al*., 1998; Nwokediuko and Ijeoma, 2009; Olal *et al*., 2012; Onyekwere *et al*., 2012; Andernach *et al*., 2014; Opaleye *et al*., 2016). HDV was not detected in the first Nigerian study on HDV (Ojo *et al.,*1998), in people who had no clinical evidence of liver disease (blood donors and University fresh men) and the study concluded that HDV prevalence is low in our community. Contrary to the study, this study detects the presence of HDV among people with no clinical evidence of liver disease, showing a significant HDV prevalence (4.4%) in the community, suggesting that HDV prevalence is increasing in Nigeria even among people with no clinical evidence of liver disease. However, HDV prevalence among HBsAg in our study is within the range (0-12.5%) detected by previous studies in Nigeria.

It has been suggested that the distribution of HDV prevalence in HBsAg positive individuals varies across different geographical regions and even within a country from region to region (Wedemeyer and, Manns, 2010; Hughes *et al*., 2011). Similarly, our study tends to conform to this observation. For instance, the prevalence of 4.4% in this study carried out from the South-western part of Nigeria is similar to that from other studies within the south-western Nigeria with a prevalence of 4.0% and 4.9% (Ojo *et al*., 1995 and Opaleye *et al*., 2016) compared to the study from the Eastern part of Nigeria where a prevalence as high as 12.5% was reported (Nwokediuko and Ijeoma, 2009). The differences might be due to region-related burden of the disease, partly explained by socio-demographics, cultural and risk behaviours. As more research on HDV are carried out, this could be established or found to be just a coincidence.

The presence of HDV in apparently healthy people have been reported from other Sub-saharan African countries (Andernach *et al*., 2014). This is also confirmed by our study. HDV has been described as a culprit for more severe hepatitis than HBV monoinfection in which it can accelerate the progression of chronic hepatitis B to cirrhosis and HCC (Rizzetto *et al*., 1997; Uzunalimoglu *et al*., 2001; Siederdissen and Cornberg, 2016; Elazar and Glenn 2017; Lempp and Urban, 2017). However, there is the need for further studies with larger samples among apparently healthy, asymptomatic HBsAg positive individuals as well as those with liver disease, to clarify the role of HDV in HBV pathogenesis and prognosis.

A limitation of our study is the inability to further confirm and define the circulating HDV genotypes using molecular assays. However, the results were broadly in line with other studies from the region, which suggests that there were no significant biases.

5. Conclusion

To conclude, this study reports the presence of HDV among individuals with no clinical evidence of liver disease. It also reports a significant HBV/HDV co-infection among the study population. HBV/HDV co-infection was found to be age related. Analysis of gender relatedness to HDV infection in our study shows that male gender is predominantly affected with HDV (83.3%; 10/12) compared to female (16.7%; 2/12). We therefore recommend routine screening for HDV in HBV positives; every HBsAg-positive individual should be tested for the presence of anti-HDV antibody. Public awareness programs could also help to reduce HBV/HDV transmission.

LIMITATIONS OF THE STUDY

There are limitations of this study. Because of insufficient funds (research works are self-funded in Nigeria), no molecular tests were done either to detect HDV RNA or carry out genotyping from blood samples. HDV infection status was detected by serological assay for anti HDV antibody by ELISA method.

CONFLICT 0F INTEREST
The authors declare that no conflict of interests exist.

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