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Hepatitis D Virus in Chronic Liver Disease Patients with Hepatitis B Surface Antigen in University of Calabar Teaching Hospital, Calabar, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author DCO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors DCO, MKA, ZAO, JOA, ENA and VUN managed the literature searches, analyses of the study, performed the spectroscopy analysis and managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aims: It is well known that patients with hepatitis B virus (HBV) could be co-infected with hepatitis D virus (HDV), thus worsening and complicating their condition. The prevalence of HBV and HDV co-infection in University of Calabar Teaching Hospital has not been ascertained. This study was therefore carried out to determine the frequency of hepatitis D virus among chronic liver disease

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(CLD) patients with hepatitis B surface antigen (HBsAg) in University of Calabar Teaching Hospital, Calabar, Nigeria.
Place and Duration of Study: This study was carried out in University of Calabar Teaching Hospital, Cross River State, Nigeria, from September 2012 to March 2013.
Methodology: A total of 91 subjects were recruited for this study, 76 were CLD patients, while 15 were apparently healthy subjects. Sera samples were subjected to HBsAg using enzyme linked immunosorbent assay (ELISA) technique and finally the HBsAg positive and negative samples were screened using ELISA technique for hepatitis D virus.
Results: Out of the 91 subjects recruited for this study, 76 (83.5%) tested positive for HBsAg, and were CLD patients. Out of the 76 CLD patients who tested positive for HBsAg, 46 (60.5%) had a co-infection with HDV, while 30(39.47%) showed no co-infection. HBV/HDV co-infection was higher in males 27(58.7%) than females 19 (41.3%).
Conclusion: This study shows a high rate of HDV/HBV co-infection which was higher in males than females with chronic liver disease in UCTH, Calabar, Nigeria.

Keywords: Chronic liver disease; hepatitis B; hepatitis D; liver.

1. INTRODUCTION

The hepatitis D virus (HDV) also known as hepatitis delta virus, is a single stranded defective RNA virus consisting of 1679 nucleotides [1,2]. For its penetration into hepatocytes and assembly of virion, it needs the help of hepatitis B virus (HBV) that provides the viral coat with the surface antigen [2–4]. Hepatitis D virus can cause both rapid progression of already existing HBV Hepatitis and fulminant hepatitis [5].

Hepatitis B is one of the most common infectious diseases of the world and has infected 2 billion people worldwide; including an estimated 400 million chronically infected cases [6]. It is hyperendemic in Sub-Saharan Africa and Asia [7,8]. It is thought to be the main etiological factor in over 75% of chronic liver disease [8]. Hepatitis B virus infection is the most common in Nigeria, with 25% of the population being infected with the virus [9].

Hepatitis B virus and HDV have similar routes of transmission, namely; through blood and blood products, intravenous drug abuse, unsafe injections and sexual activity [10,11].

In Nigeria, about 12.5% of the population is infected by hepatitis D virus [11]. HDV infection can cause either co-infection in individuals with HBV or super-infection in chronic HBV carriers [3,12,13]. Individuals who had HBV-HDV co-infection may have more severe acute disease and higher risk of fulminant hepatitis compared to mono infection with HBV [14,15]. Persons suffering from HDV/HBV super-infection have more risks of progressing rapidly to cirrhosis than the individuals suffering from HBV mono-infection [3,12,14,15]. It is observed that most individuals infected with HDV develop the chronic form of the disease and in approximately 80% of these individuals, chronic Hepatitis D progresses to cirrhosis within 5 – 10 years [1]. Another important feature of chronic hepatitis D is that it can be complicated by hepatocellular carcinoma in the infected individuals [16].

An important trend in Pakistan HDV infection is that a decline in the prevalence of hepatitis D infection has been observed. This is true for both acute and chronic forms of the disease [13]. However, Mumtaz et al. [17] noted a prevalence of 16.6% in HBsAg positive patients from different areas of Pakistan. The aim of this study was to determine the frequency of hepatitis D in hepatitis B surface antigen (HBsAg) positive patients visiting the Medicine out-patient Department of University of Calabar Teaching Hospital.

2. MATERIALS AND METHODS

2.1 Subjects

This study was carried out at the University of Calabar Teaching Hospital, Nigeria. A total of ninety-one (91) subjects (76 CLD patients and 15 apparently healthy subjects) were enrolled for this study from September 2012 to February 2013. The consent from each of the subjects was taken with the aid of a questionnaire and biodata obtained for this study in order to fulfill the ethical guidelines of research conducted on humans, as stipulated in the guidelines of the ethics committee of the University of Calabar Teaching

Hospital. The inclusion criteria for the selection of the 76 CLD patients for this study were jaundice, ascites, hepatomegaly, edema while the laboratory investigations were Prothrombin time test and deranged Liver function test (alanine amino transferase, ALT). The control subjects were also subjected to the clinical examination and laboratory investigations and their (control subjects) results shows normal, i.e not having CLD.

All the 91 subjects were screened for HBsAg using ELISA technique and the 76 CLD patients were all positive for HBsAg while the 15 apparently healthy subjects were negative. Furthermore, the 76 patients who were positive for HBsAg using ELISA technique were then screened using ELISA method for hepatitis D for qualitative determination of anti-HDV in human serum. Both ELISA test is a solid-phase microtiter plate coated with monoclonal antibodies to human IgM which is based on "sandwich principle". ELISA for hepatitis B, HBsAg test kit (Catalog number KAPG4SGE3, DIA source Immuno Assays, Belgium) was used. For hepatitis D, 'EIA-Anti HDV-M' (Ref GWB-HDV IgM, GenWay Biotech, Inc, San Diego, USA) was used.

2.2 Statistical Analysis

PRIMER version 17 was used for analysis of data in this study. The X^2 (Chi-square) test was performed for quantitative variables to check for relationship of HBV and HDV infection. Percentages were calculated directly for HBV and HDV. P = 0.05 was used as the accepted significance level were necessary.

3. RESULTS

3.1 Subjects

A total of ninety one (91) participants attending Medicine out-patient Department (MOPD) of the University of Calabar Teaching Hospital (UCTH) were recruited for this study. Seventy-six of the subjects were chronic liver disease (CLD) patients, 46 were males while 30 were female and were positive for HBsAg, while 15 (9 males and 6 females) were apparently healthy subjects who tested negative for HBsAg.

3.2 Demographic Profile of the Subjects

3.2.1 Age

The age range of the subjects was 19 – 75years, with a mean of 37.1±1.28 years. It was observed that majority of the subjects were aged 26 – 35 years, followed by the 36 – 45 years age group (Table 1).

Table 1. Age range of subjects deployed for this study

Age range	Frequency (%)
19-25	11(12.1)
26-35	38(41.8)
36-45	23(25.3)
46-55	12(13.2)
56-65	3(3.3)
66-75	4(4.4)
Total	91(100)

3.2.2 Sex

Fifty-five patients were males (60.4%), while thirty-six were females (39.6%), giving a male to female ratio of 1.5:1. Among the 76 chronic liver disease (CLD) patients who tested positive for HBV, 46(60.5%) were males and 30(39.5%) were females.

3.2.3 Frequency of the co-infection of HBV and HDV

Out of the 76 CLD patients tested positive for HBsAg, 46 (60.5%) had a co-infection with HDV, significantly ($P<0.05$) higher, compared with 30 (39.49%) which showed no co-infection. Of the 46 CLD patients who had HBV/HDV co-infection, it was observed that 27 (58.7%) were males and 19 (41.3%) were females. In the control group (15 subjects), 9 were males and 6 were females which had no co-infection with HDV (Figs. 1 and 2).

4. DISCUSSION

Worldwide, the pattern of hepatitis D infection is different from that of hepatitis B infection but have similar modes of transmission and it has been estimated that 15 million people with hepatitis B (HBsAg) are infected with hepatitis D [18]. People with chronic hepatitis B who are infected with hepatitis D (co-infection) usually develop chronic (long-term) hepatitis D infection. Long-term studies of patients who had HDV super-infection show that between 70% and 80% will ultimately develop cirrhosis compared to 15% to 30% of patients who had only chronic hepatitis B [19].

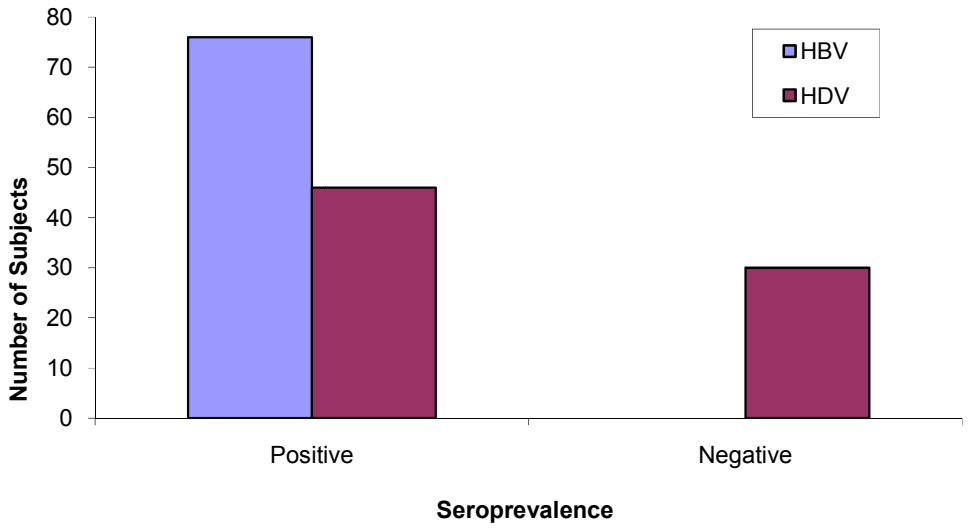

Fig. 1. Frequency of 76 sero-positive HBV subjects that had co-infection with HDV

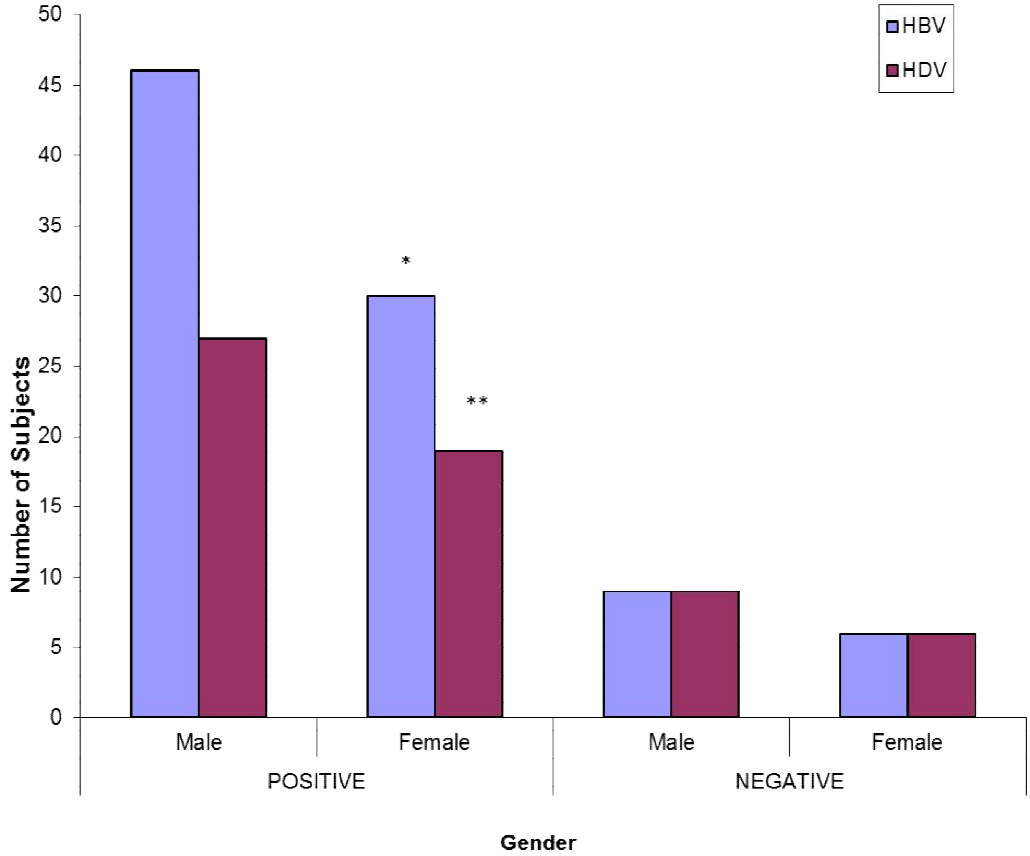

Fig. 2. Frequency of positive and negative HDV with co-infection with HBV among male and female subjects, N = 91

This study had demonstrated a high frequency of HDV co-infection among HBV positive chronic liver disease patients in Calabar, Nigeria. However, the rate of HBV infection was observed to be higher (83.5%) in the 91 enrolled subjects enrolled, while no HBV infection was found among the control group. The reason for the high rate of HBV in this study is that the enrolled patients were diagnosed to have chronic liver disease, of which HBV is the major cause. The reported rate for HBV infection in this study is very high as compared to a previous study in Ibadan – Nigeria, which reported that 57.1% of patients with primary liver cell carcinoma were positive for HBsAg. In Jos and Gombe, prevalence rate showed 25.9% and 26.5% respectively among patients with human immune deficiency syndrome (HIV) [20–22]. This could be because HIV and HBV share similar mode of transmission and risk factors. Nwokediuko and Ijeoma [11] reported that in Nigeria patients with acute hepatitis and asymptomatic infection has prevalence of 4.3% while in patients with chronic hepatitis, liver cirrhosis and primary liver cell carcinoma has prevalence of 15%. HDV still contributes to significant morbidity and mortality in HBV-related liver disease in Nigeria. In Pakistan, Bukhtiari et al. [23], reported that a high percentage of patients of CLD in their study had evidence of HBV. Hepatitis B virus prevalence rate was higher in males (60.4%) than in females (39.6%), with the majority of the subjects in the age range of 26 – 45 years old. This could be due to the fact that this age group is mostly exposed to multiple risk factors associated with HBV. The mean ages of both test and control group was similar, and that ruled out age effect in this study. Bukhatiari et al. [23], reported 10% prevalence of HBV in second decade of life. He also reported 20% prevalence of HBV in third and fourth decade of life. Twenty-five percent was reported to have HBV among patients on antiretroviral drug therapy (ART), and gender showed that males (9.1%) were more infected than females (5.4%) in a recent study in Calabar by Inyang-Etoh and Philip-Ephraim [24].

The interesting finding of this study is the observation of a high seropositivity rate of anti-HDV ELISA that is 50.5% in HBV positive CLD patients. This positivity rate is very high as compared to previously reported rates from Nigeria, though the sample size of our study was not as large as that of earlier research reported.

In this study, we observed that all HDV infected patients were positive for HBV. This finding may be attributed to the fact that HBV and HDV have a common mode of transmission. This positivity rate is very low as compared with other studies from Pakistan [25,26], where it was reported that HDV prevalence rate was 58.6% and 88.8% respectively in HBV positive patients. Although, Mumtaz et al. [17], reported low prevalence rate of 16.6%, our study found a much higher prevalence than the previous study. On the other hand, overall decline in the worldwide HDV infection has been observed globally [27].

Currently, HBV vaccine is available and its administration can prevent infection of HBV, thus, reducing the possibility of co-infection with HDV. It is rather unfortunate that in Calabar - Nigeria, the decline of HDV infection as a result of HBV vaccination programs has not been achieved. This could be attributable to lack of awareness and unavailability of the HBV vaccine to the people. Importantly, the higher prevalence rate of HDV co-infection with HBV was observed in young males compared to females. Our observation is similar with the previous findings from Iran by Mohammed et al. [28] and from Pakistan [17,25,26]. These researchers observed higher HDV prevalence rate in young males compared to females. The reason young males are infected more than their female counterparts could be because there may be an influence of estrogen in the protection and defense of hepatic cells against the development of chronic liver disease in females; hence their low susceptibility to hepatitis [29,30]. It could also be because of the different sexual behavior and practices between males and females due to traditional and moral culture.

5. CONCLUSION

The results from this study have demonstrated a high prevalence of HDV-HBV co-infection in University of Calabar Teaching Hospital, Nigeria. Nigeria is an endemic country for Hepatitis B infection. Males are more infected than the females. We therefore encourage care givers to pay attention to the likelihood of patients' co-infection with HDV-HBV, as this knowledge will help early detection and rapid provision of adequate care to cob the situation.

CONSENT

Consent was sort and obtained from all patients involved in this study.

ETHICAL APPROVAL

Approval of study was sort and obtained from the Ethics Committee of the University of Calabar Teaching Hospital, Cross River State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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