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Frequency of Hepatitis B and C Co-Infection in Chronic Liver Disease Patients in Calabar, Cross River State, Nigeria

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Summary: Hepatitis B (HBsAg) and C (HCV) virus are becoming a significant causative factors in the aetiology of chronic liver disease (CLD) worldwide. However, the information on the frequency of HBsAg and HCV virus co-infection in CLD is sparsely reported in Nigeria. In this study, we assessed the frequency of HBsAg and HCV co-infection in CLD. One hundred and eleven subjects aged 19 - 76 years, comprising of 76 CLD patients and 35 apparently healthy subjects without CLD were tested for both HBsAg and HCV virus antibodies using ELISA test kits. Out of the 111 subjects recruited for this study, 76 (68.5%) were CLD patients tested positive for HBsAg and 35 (31.5%) tested negative for HBsAg and served as control. Out of the 76 CLD patients that tested positive for HBsAg, 34 (44.7%) of them also tested positive for HCV, thus, having co-infection with HBV. Incidence of co-infection was highest in those aged 36 - 45 years, and greater in males than females. Among the control group, 4 (11.4%) of the subjects (3 males and 1 female) tested positive for HCV, while 31 (88.6%) subjects (20 males and 11 females) tested negative. This work has shown that the co-infection with HBV and HCV among chronic liver disease patients and the incidence of HCV is high in our locality. Also, some of the supposed apparently healthy subjects in this study tested positive for HCV, hence the need for improving the awareness of this virus. It is therefore necessary to give immunization and test for HBsAg and HCV in both rural and urban areas.

Keywords*:* Chronic liver disease, Co-infection, Hepatitis B, Hepatitis C, Calabar

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INTRODUCTION

Viral hepatitis is an infection of the liver caused by a group of viruses having affinity for the liver and causing an overlapping pattern disease. These viruses include hepatitis A, B, C, D, E, G (Crawford, 1999). Hepatitis B virus, a major public health problem worldwide is more prevalent in the developing countries (WHO, 2000).

Hepatitis B virus, which causes serious liver damage is one of the World Health Organization's (WHO's) target for global eradication by 2020 (Dusheiko *et al*, 1999). The virus can be passed on through blood transfusion or sexual contact, and has an incubation period ranging from 3 weeks to several years before any symptoms appear (Peter and Tokyo, 2000). It is a resilient virus that can exist on almost any surface for about 1 month. More than 2 billion people are infected with HBsAg worldwide, while some 280 million are chronic carriers harbouring the virus in their liver (Clement *et al*, 1990). Sometimes, one may be co-infected with HDV/HBV. Okpokam *et al*, (2015), reported high rate of HDV/HBV coinfection in Calabar, Nigeria, which was higher in males than females.

Hepatitis C is an infected disease caused by HCV. The infection is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis, which is generally apparent after many years. In some cases, those with cirrhosis will go on to develop liver failure, liver cancer, or life threatening esophageal and gastric varies (Ryan and Ray, 2004). Hepatitis C differs from HBsAg because it tends to stay longer in the body not causing any problem (Otegbayo *et al.*, 2012). The primary route of transmission in the developed world is intravenous drug use (IDU), while in the developing world, the main routes are blood transfusion and unsafe health procedures (Maheshwari *et al*, 2008). Hepatitis C virus has become a leading cause of CLD worldwide (Edemariam, 2004). An estimated 130 - 170 million people worldwide are infected with hepatitis C. In Nigeria, about 6 - 20% of the population are infected with Hepatitis C virus (Abiodun and Agumadu, 2012). This study therefore seeks to determine the incidence of HCV in CLD patients tested positive for HBsAg in the University of Calabar Teaching hospital, Calabar,

Cross River State, Nigeria.

MATERIALS AND METHODS

Study place and duration

This study was done in the Medicine out-patient and Haematology Department, University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State, from September 2012 to March 2013.

Subjects

A total of one hundred and eleven (111) subjects were used for this study. Out of the 111 subjects, 76 were CLD patients visiting the University of Calabar Teaching Hospital (UCTH) and 35 were apparently healthy (control) subjects. Biodata and consent from each of the subjects was taken for this study in order to fulfil the ethical guidelines of research conducted on humans. The inclusion criteria for the selection of the 76 CLD patients for this study were presence of jaundice, ascites, hepatomegaly, edema while the laboratory investigations were prothrombin time test and deranged liver function test (alanine amino transferase). The control subjects were also subjected to clinical examination and laboratory investigations and their results showed absence of CLD. Out of the 111 subjects, 69 were males and 42 were females. Moreover, out of the selected 76 CLD patients that were positive for HBsAg using ELISA technique, 46 were males and 30 were females, while out of 35 apparently healthy subjects that were negative for HBsAg, 23 were males and 12 were females. These positive (76 patients) and negative (35 apparently healthy) subjects were then screened using ELISA method for hepatitis C. 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, DIAsource ImmunoAssays, Belgium) was used. For hepatitis C, Anti-HCV ELISA 4.0 test kit (Catalog number KAPG4NAE3, DIAsource ImmunoAssays, Belgium) was used.

Statistical analysis

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

RESULTS

Subject distributions

One hundred and eleven (111) subjects were recruited in this study. Seventy-six (76) were selected patients attending Medicine out-patient Department (MOPD) of the University of Calabar Teaching Hospital (UCTH). These 76 patients were CLD patients and all tested positive for Hepatitis B surface antigen (HBsAg) while thirty-five (35) were apparently healthy control subjects and all tested negative for HBsAg (Figure 1).

Age distribution of subjects

HBsAg and HCV seropositivity were not statistically significant (P > 0.05) when associated with age group as shown in table 1 above. In this study, the seropositivity of 111 subjects was higher in those aged 26 - 35 years than those aged 36 - 45 years. This was similar to HBsAg seropositive subjects. However, seropositivity of HCV subjects was higher in those aged 36 - 45 years than those aged 26 - 35 years (Table 1).

Gender distribution of subjects

According to gender, the seropositivity of 111 subjects in this study was highest in males (62.2%) than in the females (37.8%). The same applied to those in HBsAg and HCV. There was no statistically significant difference (P > 0.05) between gender and seropositivity rate in this study (Table 2).

Co-infection of HBV and HCV

All 76 CLD patients tested positive (100%) for HBsAg and 34 (44.7%) tested positive for HCV, while 42 (55.3%) tested negative for HCV (Figure 2). Out of the 76 CLD patients positive for HBsAg, 46 (60.5%) were males and 30 (39.5%) were females, while in those CLD patients positive for HCV, 22 (64.7%) of the patients were males and 12 (35.3%) were females. However, in those tested negative, 24 (57.1%) were males and 18 (42.9%) were females (Figure 3). Among the 35 apparently healthy subjects, we observed that 4

Table 1: Seropositivity of HCV and HBsAg according to age

Age Group	Subjects Tested (%)	Subjects Tested Positive (%) for HCV	Subjects Tested Positive (%) for HBsAg
19-25	19(17.1)	5(13.2)	16(17.8)
26-35	44(39.6)	12(31.6)	36(40.0)
36-45	28(25.2)	14(36.8)	21(23.3)
46-55	13(11.7)	4(10.5)	10(11.1)
56-65	3(2.7)	1(2.6)	3(3.3)
>76	4(3.6)	2(5.3)	4(4.4)
Total	111 (100)	38(100)	90(100)

Table 2: Seropositivity of HCV and HBsAg according to gender

Sex	Subjects Tested (%)	Subjects tested Positive (%) for HCV	Subjects Tested Positive (%) for HBsAg
Male	69(62.2)	25(65.8)	55(61.1)
Female	42(37.8)	13(34.2)	35(38.9)
Total	111(100)	38(100)	90(100)

P>0.05

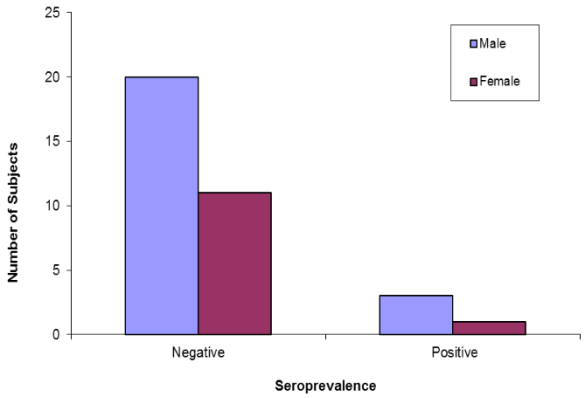

Figure 1: Subject Distribution

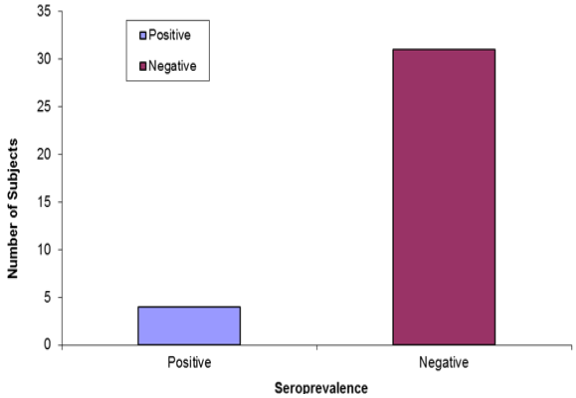

Figure 2: Seropositivity and seronegativity of HBsAg and HCV among CLD patients

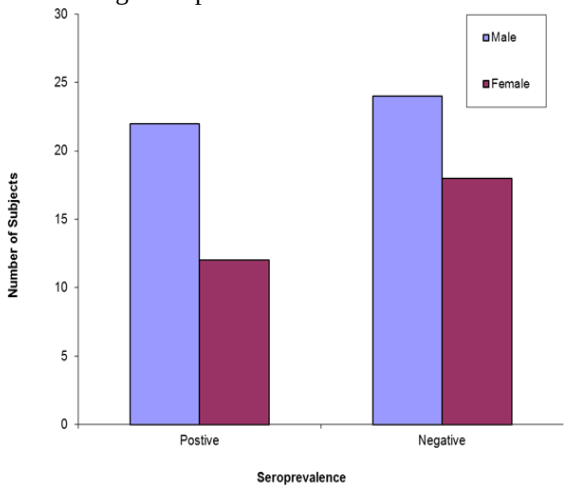

Figure 3: Seropositivity and seronegativity of HCV among CLD patients based on sex

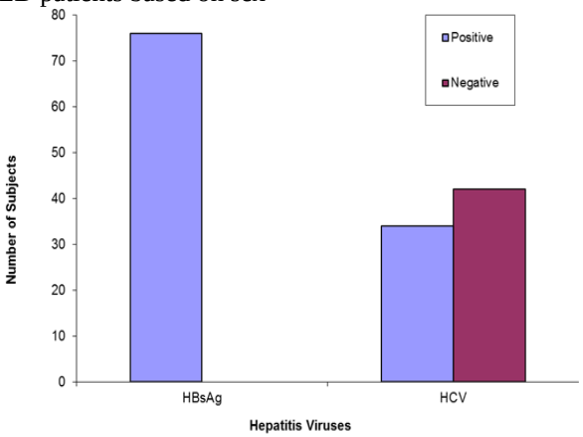

Figure 4: Frequency of HCV in 35 apparently healthy subjects (control)

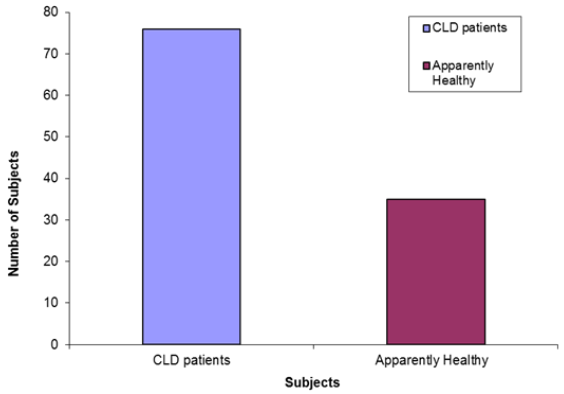

Figure 5: Frequency of seropositive and seronegative HCV among 35 apparently healthy subjects (control) based on sex

(11.4%) tested positive for HCV (3 males and 1 female), while 31 (88.6%) tested negative for HCV (20 males and 11 females) (Figures 4 and 5).

DISCUSSION

Hepatitis B virus and HCV share common transmission pathways. Therefore, coinfection can be anticipated. The percentages of 111 subjects at various age groups were similar with a mean of 36.2 ± 1. 17 and showed no significant difference (P > 0.05). However, we observed that the majority of 111 subjects were in the age group 26 - 35 years which are in the third decade of life. From the results obtained from this study, those in age group 26 - 35 years had the highest number of positive HBsAg (81.8%) while those with HCV (50.0%) had the highest number of positive in age group 36 - 45 years. Inyang-Etoh *et al*, (2014), also reported recently in their study in Calabar that those in age group 31 - 40 years had the highest prevalence rate with HCV infection (7.3%). The percentages of HBsAg and HCV infection according to gender showed that in both infections (HBV and HCV), males (61.1% and 65.8%) were more infected than females (38.9% and 34.2%) respectively, but there was no statistically significant difference (P > 0.05) based on gender in this study. In this study, the results of co-infection with HBsAg and HCV among 76 CLD patients showed that all were positive for HBsAg using ELISA test and 34 (44.7%) of the CLD patients were also positive for HCV, thus, showing co-infection with HBV. Out of the 34 (44.7%) CLD patients that were positive for HCV, 64.7% were males while 35.3% were females. The reason for the co-infection with HBV and HCV could be attributed to the fact that both virus infection presupposes from same source and also have same transmission pathway. Although, the number of samples used in the study is few, it still indicates the interactions between the two viruses which occur in chronic infections like in our study. This study conforms with the findings by Omuemu *et al*, (2012), which assessed 115 subjects with CLD. In their study, the prevalence of HBsAg in the CLD patients was 40.9%, of which 85.1% were

males and 14.9% were females, while prevalence of HCV among CLD patients was 1.7% of which all (100%) were males. In Pakistan, 52 patients with positive HBsAg and anti-HCV antibodies were included in their study, 32 (61.5%) were males and 20 (38.5%) were females. The percentage of HBV infection was 38.85% while percentage of HCV infection was 24.0%. Their mean age in years was 40 ± 10.125 SD (Mazhar *et al*, 2014). Also, in this study, the CLD patients were not classified into their various subtypes (i.e. chronic hepatitis, liver cirrhosis and HCC) so as to know the stage and duration when the infection occurs. A study by Ola *et al*, (2004) in Ibadan, South-Western Nigeria found HCV infection in 20% of their patients with liver cirrhosis and 14% of their patients with HCC. In another study, in Ibadan, Olubuyide *et al* (1997), found HCV infection in 18.7% of their patients with HCC. Furthermore, Shehu, (2002), in his study in Jos, North-Central Nigeria found that 11.8% of their patients with CLD had evidence of HCV infection, which is lower than the percentage (44.7%), observed in our study. It was also observed that 11.4% of our apparently healthy subjects tested positive for HCV, of which 3 were males while 1 was female. They were found not tested positive for HBsAg. The reason for this could also be linked to lack of awareness and unavailability of vaccines for HCV.

In our study and locality, HCV has been observed to be more in males than in females. According to recent work done by Inyang-Etoh *et al*, (2014) in Calabar, it was discovered that males were more infected with HCV (5.6%) than females (2.2%), but there was no statistically significant difference ($P > 0.05$) in the infections. The World Health Organisation (WHO) at the sixty-third World Health Assembly in 2010 had recognised HCV as a growing public health threat (WHO, 2011). The reason for more males in this study than females could be due to high risk behaviour of men having this viruses for example; having multiple sexual partners, scarification, living in a crowded environment, being uneducated, etc. Bwogi *et al*, (2009), reported that rural residence could also be risk factors for HBV and HCV infection. Also, socioeconomic conditions, especially in the rural areas, may contribute to HBV and HCV exposure.

This study has shown that there appear to be a high percentage of CLD co-infection with HBV and HCV in our locality than in other studies mentioned. Therefore, we recommend nonstop public awareness of both infections especially to the grass root level, sticking to one sex partner, using of protective devices like condom, better environmental sanitation,
drinking safe water, complete immunization against HBV and introducing vaccines for HCV. We also advocate that those infected should take adequate nutritionally balanced meals and develop a better attitude towards taking their drugs. Larger sample size using advanced parameters like the molecular biology techniques [polymerase chain reaction (PCR)] should be carried out in our locality to determine dominant HBV and HCV infection.

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