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Occurrence of hepatitis 'B' and 'C' amongst patients on antiretroviral drug therapy (ART) in a treatment centre in Calabar, Nigeria

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The occurrence of hepatitis 'B' and 'C' virus amongst patients on antiretroviral drug therapy (ART) was studied. Two hundred (200) human immunodeficiency virus (HIV) positive subjects on ART and 100 apparently healthy HIV negative subjects (control) were recruited for the study. The subjects aged 1 to 75 years were screened for hepatitis B and C viral antibodies using hepatitis B and C test strips manufactured by ACON Laboratories. Questionnaire were also administered. CD4 counts of the subjects were determined using CyFlow Counter manufactured by GEM Laboratories, Germany. Fourteen (7%) of the subjects were positive for hepatitis B virus (HBV) infection, 6 (3%) for hepatitis C virus (HCV) and 2 (1%) for mixed infections. In the control group, a prevalence of 6 (6%) was recorded for HBV, 4 (4%) for HCV and none for mixed infections. Among the test group, subjects in age group 51 to 60 years had the highest prevalence rate for HBV (25%), 31 to 40 years for both HCV (7.3%) and mixed infection (3.6%). There was no statistically significant difference in infection according to age P=0.475. Males had a higher prevalence rate (9.1%) than the females (5.4%) for HBV, but there was no statistically significant difference in HBV infections according to gender P=0.404. In HCV infection, males had a higher prevalence rate (5.7%) than females (0.9%), but there was no statistically significant difference in HCV infection according to gender P=0.089. Subjects with CD4 counts in the range of 1401 to 1600 had the highest infection rate (50%) for HBV and 201 to 400 for HCV (7.7%) and mixed infection (5.1%). This work has shown that HBV and HCV are common among patients on ART and the need for routine screening of this category of patients in order to aid in the effective management of co-infections.

Key words: Hepatitis B, Hepatitis C, antiretroviral therapy, HIV, Calabar.

INTRODUCTION

In Nigeria and other developing countries, human immunodeficiency virus/acquired immunodeficiency virus (HIV/AIDS) disease is a major public health problem, and a serious threat to development. Since the introduction of potent antiretroviral drug therapy (ART), HIV/AIDS has been successfully converted from a uniformly fatal illness

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to a manageable chronic infection. Correspondingly, during the past years, the opportunistic infections that complicate profound immunosuppression have been replaced with newer forms of morbidity and even mortality. Chief among these has been the development of progressive liver disease due to hepatitis C virus (HCV) and hepatitis B virus (HBV). Due to their shared routes of transmission, HCV and HBV are frequently found in the HIV-infected host, while HCV co-infection has deservedly gained considerable attention as a major cause of mortality in the post-highly active antiretroviral therapy era (Bonacini et al., 2001). Complications of HBV-related liver disease are being increasingly recognized especially as drug-resistant forms of HBV have become nearly universal (Saravanan et al., 2007).

HBV and HCV co-infection in HIV positive individuals is of utmost importance due to the underlying conesquences such as the hepatological problems associated with these viruses, which have been shown to decrease the life expectancy in the HIV-infected patients (Koziel and Peters, 2007; Major, 2009). Moreover, among the HIV-infected patients, 2 to 4 million are estimated to have chronic HBV co-infection, while 4 to 5 million are co-infected with HCV (Soriano et al., 2009). In Nigeria, the average carrier rate of hepatitis B in the general population is estimated to be 4% (Taylor et al., 2006). This study was an attempt to investigate the current prevalence of HBV and HCV among patients on ART in the study centre.

MATERIALS AND METHODS

Study location

The study centre was ART Laboratory, General Hospital, Calabar located in Calabar Municipality in Cross River State. Cross River is a coastal state in South Eastern Nigeria, bordering Cameroon to the east and it is located in Nigeria's Delta region.

Patients' recruitment

The study subjects were 200 consecutive patients aged 1 to 75 years on ART, while 100 HIV negative apparently healthy control subjects who were randomly selected from members of the general hospital community whose HIV status were negative at the time of study. The study subjects and the controls were age and sex matched. The study was conducted between November 2011 and June 2012. Questionnaires were administered to obtain the demographic data of the subjects.

Ethical clearance was sought and obtained from the State Ministry of Health. Prior to specimen collection, verbal consent from each of the subjects and/or their guardians were sought and obtained. Those who declined participation were excluded from the study.

CD4 T lymphocytes count

Partec CyFlow Counter was the machine used for analysis of CD4 count with serial No. 090746022 manufactured by GEM Laboratories, a biotechnological company in Germany (Pantec GmbH Am flugplatz 13 D-02828 Glorilitz Germany).

The Partec CyFlow Counter which is a fully equipped portable/mobile flow cytometry system (FCM) was used for the identification and the enumeration of the CD4 T lymphocytes which is the first point of attack by the HIV virus.

HBV screening test

Sample from each subject was screened serologically for hepatitis B surface antigen. The test was done using ACON hepatitis B surface antigen rapid test strip manufactured by ACON Laboratory Inc 4108 Serrente Valley Boulevard, San Diego, CA 92121 in United States of America.

The HBsAg one step hepatitis B surface antigen test strip (serum/plasma) is a qualitative, lateral flow immunoassay for the detection of HBsAg in serum or plasma. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the strip. During testing, the serum or plasma specimen reacts with the particle coated with anti-HBsAg antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colour line. The presence of this coloured line in the test region indicates a positive result, while its absence indicates a negative result. This test strip has been compared with a leading commercial HBsAg EIA test and the correlation between this two is over 90%. The relative sensitivity, specificity and accuracy are 99, 97 and 98.5%, respectively (Blumberg et al., 1971).

HCV screening test

Hepatitis C virus antibodies was screened using ACON one strip hepatitis C virus test strip manufactured by ACON Laboratory Inc 4108 Serrente Valley Boulevard, San Diego, CA 92121 in United States of America. This HCV one step hepatitis C test strip (serum/plasma) is a qualitative, membrane based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is coated with recombinant HCV antigen on the test line region of the strip. During testing, the serum or plasma specimen reacts with the protein A coated particles. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a coloured line. The presence of this coloured line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear at the controlled line region indicating that proper volume of specimen has been added and membrane wicking has occurred. This test strip has been compared with a leading commercial HCV EIA test. The relative sensitivity, specificity and accuracy are >99.0, 98.6 and 99.3%, respectively (van der Poel et al., 1991; Wilber, 1993).

HIV screening test

HIV screening was done using the serial algorithm of screening with determined and confirmed result with Unigold (WHO, 1993).

Alere determined HIV1/2 is an immunochromatographical test for the qualitative detection of antibodies to HIV-1 and HIV-2.

HIV confirmatory test using uni-gold

All the test samples that were positive were confirmed with a second test using uni-gold. For testing, two drops of whole blood from the pricked finger were allowed to fall into the sample port, followed by two drops of wash buffer and allowed to react.

Table 1. Prevalence of HBV and HCV amongst subjects examined according to age.

Age group	Test subject				Control subject			
	No. examined	No. (%) with HBV infection	No. (%) with HCV infection	No. (%) with both infections	No. examined	No. (%) with HBV infection	No. (%) with HCV infection	No. (%) with both infections
1-10	6	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)
11-20	7	0 (0)	0 (0)	0 (0)	7	0 (0)	0 (0)	0 (0)
21-30	58	2 (3.45)	0 (0)	0 (0)	37	4 (10.8)	1 (2)	0 (0)
31-40	55	5 (9.1)	4 (7.3)	2 (3.6)	32	1 (3.1)	1 (3.1)	0 (0)
41-50	57	4 (7.0)	2 (3.5)	0 (0)	16	0 (0)	1 (6.25)	0 (0)
51-60	8	2 (25)	0 (0)	0 (0)	8	1 (12.5)	1 (12.5)	0 (0)
61-70	8	1 (12.5)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)
71 above	1	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)
Total	200	14 (7)	6 (3)	2 (1)	100	6 (6)	4 (4)	0 (0)

Table 2. Prevalence of infection among subject examined according to gender.

Gender	Test subject				Control subject			
	No. examined	No. (%) with HBV infection	No. (%) with HCV infection	No. (%) with both infections	No. examined	No. (%) with HBV infection	No. (%) with HCV infection	No. (%) with both infections
Female	112	6 (5.4)	1 (0.9)	1 (0.9)	46	4 (8.7)	1 (2.2)	0 (0)
Male	88	8 (9.1)	5 (5.7)	1 (1.1)	54	2 (3.7)	3 (5.6)	0 (0)
Total	200	14 (7)	6 (3)	2 (1)	100	6 (6)	4 (4)	0 (0)

Antibodies of HIV-1 or HIV-2 proteins were bound to the colloidal gold linked antigens. The antibody protein colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device. A positive reaction is visualised by a pink band in the test region of the device and in the control line. A negative reaction occurs in the absence of human immunoglobin antibodies to HIV in the analysed specimen. Consequently, no visually detectable band develops in the test region of the device.

Data analysis

Variables were analysed using Statistical Package for Social Sciences (SPSS) software.

RESULTS

Two hundred (200) HIV positive subjects on ART and 100 apparently healthy HIV negative subjects (control) were recruited into the study.

Table 1 shows the prevalence of HBV and HCV amongst subjects examined according to age. In the test subjects group, those in the age group 51 to 60 years had the highest prevalence rate of infection with HBV (25%), but there was no statistically significant difference in the prevalence of HBV infection by age (χ^2 = 6.9, df (7), P > 0.05). Those in age group 31 to 40 years had the highest HCV rate infection (7.3%). But there was no statistically significant difference in HCV infection by age (χ^2 = 6.2, df (7), P >0.05).

Amongst those with mixed infection, subjects in age group 31 to 40 years had the highest prevalence of infection (3.6%), but there was no statistically significant difference in mixed infection (χ^2 = 5.3260, df (7), P >0.05).

Among the control subjects, age group of 51 to 60 years had the highest infection with HBV and HCV (12.5 and 12.5%), respectively. There was no statistically significant difference in infection according to age (χ^2 = 0.1199, df (7), P >0.05) and (χ^2 = 1.813, df (7), P >0.05), respectively. There was no mixed infection in the control subjects.

The prevalence of HBV and HCV infection according to gender is as shown in Table 2. In HBV infection, males (9.1%) were more infected than the females (5.4%), but there was no statistically significant difference between infections in males and females (χ^2 = 1.055, df (1), P = 0.404). Males were also more infected (5.7%) than females (0.9%), and there was also no statistical significant difference in HCV infection (χ^2 = 3.884, df (1), P > 0.05). Among those with mixed infection, males (1.1%) were more infected than females (0.9%), but there was also no statistically significant difference in mixed infection according gender (χ^2 = 0.030, df (1), P = 0.864).

In the control subjects, females were more infected

Table 3. Distribution of infection according to CD4 count of subject examined.

CD4 count range	Test subject				Control subject			
	No. examined	No. (%) with HBV infection	No. (%) with HCV infection	No. (%) with both infections	No. examined	No. (%) with HBV infection	No. (%) with HCV infection	No. (%) with both infections
0-200	21	2 (9.5)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)
201-400	39	4 (10.2)	3 (7.7)	2 (5.1)	0	0 (0)	0 (0)	0 (0)
401-600	49	3 (6.4)	1 (2.0)	0 (0)	22	1 (4.5)	2 (9.1)	0 (0)
601-800	32	3 (9.4)	0 (0)	0 (0)	24	3 (12.5)	0 (0)	0 (0)
801-1000	19	1 (5.3)	1 (5.3)	0 (0)	26	2 (7.7)	2 (7.7)	0 (0)
1001-1200	14	0 (0)	1 (7.1)	0 (0)	15	0 (0)	0 (0)	0 (0)
1201-1400	6	0 (0)	0 (0)	0 (0)	5	0 (0)	0 (0)	0 (0)
1401-1600	2	1 (50)	0 (0)	0 (0)	4	0 (0)	0 (0)	0 (0)
1601-1800	18	0 (0)	0 (0)	0 (0)	4	0 (0)	0 (0)	0 (0)
Total	200	14(7)	6(3)	2(1)	100	6(6)	4(4)	0(0)

(8.7%) with HBV than males (3.7%), but there was no statistically significant difference in infection according to gender (χ^2 = 1.098, df (1), P = 1.410), while males were more infected (5.6%) with HCV than females (2.2%), but there was also no significant difference in the infection (χ^2 = 0.740, df (1), P > 0.05).

Table 3 shows the distribution of infection according to CD4 count. Subjects with a CD4 count of 1401 to 1600, had the highest infection rate (50%) with HBV. In HCV infection, the CD4 count of 201 to 400 had the highest prevalence rate of infection (7.7%). In the control group, subjects with CD4 count of 601 to 800 had the highest infection rate (12.5%) with HBV.

Among those with HCV infection, subjects with CD4 count of 401 to 600 had the highest infection rate (9.1%). No mixed infection was recorded in the control group.

DISCUSSION

From the results obtained from the test subjects, those in age group 51 to 60 years had the highest prevalence rate of infection for HBV (25%). This can be compared to the work done by Denue et al. (2011) at medical wards of University of Maiduguri Teaching Hospital, Nigeria on the survey of hepatitis B and C virus prevalence in HIV positive patients, who had a prevalence rate of 12.3% for HBV and 0.5% HCV infection with no mixed infection obtained. Similarly, this work can be compared to the work done by Adewole et al. (2009) at the Department of Medicine, Obafemi Awolowo University, Ile-Ife, Nigeria, on hepatitis B and C virus co-infection in Nigeria patients with HIV infection. Adewole et al. (2009) had 11.5% prevalence rate for HBV, 2.3% prevalence rate for HCV and 1.5% for mixed infections. This result can also be compared with the work carried out by Soriano et al. (2009) on hepatitis B and C in HIV/AIDS, Hong Kong, who had 23% for HBV, 16% for HCV and 5 to 10% mixed infections. Those in age group 31 to 40 years had the highest prevalence rate with HCV infection (7.3%). Among those with mixed infection, subjects in age group 31 to 40 years had the highest prevalence of infection (3.6%), but there was no statistically significant difference in mixed infection (P > 0.05). Among the control subjects, age group of 51 to 60 years had the highest infection with HBV and HCV (12.5 and 12.5%), respectively. There was no mixed infection in the control subjects.

The prevalence of HBV and HCV infection according to gender showed that in HBV infection males (9.1%) were more infected than females (5.4%), but there was no statistically significant differences between infections in males and females (P > 0.05). Among those with mixed infection, females were more infected (8.9%) than males (5.7%) and there was a statistically significant difference in mixed infection according to gender P < 0.05. In the control subjects, female were more infected with HBV (8.7%) than males (3.7%), but there was no statistically significant difference in infection according to gender P > 0.05, while males were more infected with HCV (5.6%) than females (2.2%), but there was no statistically significant difference in the infections P > 0.05. This can be compared to the work done by Denue et al. (2011), where blood donors were used as their controls, with a percentage prevalence of 5.2% for HBV and 1.4% for HCV. The distribution of CD4 count showed that CD4 count group of 1401 to 1600 had the highest infection rate (50%) with HBV. In HCV infection, the CD4 count group of 201 to 400 has the highest prevalence rate of infection (7.7%). In the control group, those with CD4 count of 601 to 800 CD4 group had the highest infection with HBV (12.5%). Among those with HCV infection subjects in CD4 count group of 401 to 600 had the highest infection rate (9.1%). No mixed infection was recorded in the control group. This can also be compared with the work done by Denue et al. (2011) at medical wards of University of Maiduguri, Nigeria on the survey of

hepatitis B and C virus prevalence in HIV positive patients. The mean CD4 count of the control group was significantly higher (181 cell/µl) than the test subjects (117 cell/µl).

According to WHO estimates, the global burden of HIV, HBV and HCV is 33.2, 400 and 170 million, respectively. Knowledge of the prevalence and distribution of blood borne viruses and sexually transmitted disease (STDs) in different part of the world, and particularly in Africa it is important for the planning of prevention measures and the development of vaccination programmes. More females than males were presented for care during the study period, but majority of males in the control subjects were blood donors. The gender inequality in presentation for therapy is consistent with the sex distribution documented in majority of treatment centres, particularly in the first decade of ART. The reason for more females at the study centre is that women present for care after positive HIV test on their sick children, death of their husbands or perhaps they are more sensitive to changes in their health and may be socially conditioned to seek and receive assistance for their sickness. This however does not mean that more women are infected with HIV in study centre, as study in Nigeria actually found that more men were afflicted with HIV/AIDS (Ola et al., 2005).

HIV has been shown conclusively to be an independent risk factor for more rapid CD4 decline, although it has been associated with increased occurrence of HBV, but HCV has not been known to decline CD4 count. The limitation of this study has been the availability funds for serotyping of these viruses, that is, confirmation of HBV and HCV, respectively. This work has shown that HBV and HCV are common amongst patients on ART. It is therefore advisable to screen for these viruses in all the HIV infected individuals and their sexual partners as a routine management and check up in order to aid in the proper management of the disease.

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