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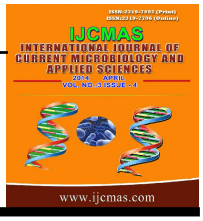

Original Research Article

Patterns of CD4+ cells and Liver Enzymes and their correlation with prevalence of HBV and HCV among HIV Positive Individuals at Orlu Hospital Counseling and Testing Unit, Imo state, South-East Nigeria

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A B S T R A C T

Co-infection with viral hepatitis caused by HBV and HCV is associated with severe liver damage and immunological complications among HIV positive patients. The aim of the present study was to evaluate the patterns of CD4 cells and liver enzymes and their correlation with prevalence of HBV and HCV among HIV infected individuals naïve to HAART. A Cross-sectional study was carried out within seven (7) months period (March-September, 2011) at Orlu Hospital counseling and testing (HCT) unit, Imo state, south-East Nigeria. HBsAg and anti-HCV serological tests and liver enzymes (AST and ALT) as well as levels of CD4 T cell count were determined using standard procedures. Socio-economic data were collected by using structured questionnaire. Using statistical software (SPSS version 15.0) data were entered and analyzed. Statistical significant was considered at $P < 0.05$. The study population comprises 846 HIV positive and 34 HIV negative control patients. Among 846 HIV positive subjects evaluated, the overall prevalence of HIV-viral hepatitis co-infection was 126(14.89%). The prevalence of HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infections were 83(9.8%), 34(4.0%) and 9(1.1%) respectively. Study participants who had HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infection have relatively high mean liver enzyme levels (ALT and AST) than HIV mono-infected ones. Individuals with HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infection also had a lower mean CD4 levels than HIV mono-infected individuals examined. The mean CD4 value in females was higher than males.The prevalence of HBV and HCV was higher than reports from general population of the country. Raised levels of liver enzymes and lowered mean CD4 counts were seen in HIV-HBV, HIV-HCV and HIV-HBV-HCV co-infections. These findings underscore the importance of screening all HIV positive individuals before initiating antiretroviral treatment.

Keywords

HBsAg, HCV, . HIV, CD4+ T cells, Liver enzymes

Introduction

Infection with HIV is still a global challenge. The complications associated with this all important infection has remained a pertinent concern. Hepatitis B

Virus (HBV) and Hepatitis C Virus (HCV) are the most common cause of chronic liver diseases especially in HIV patients (WHO, 2002). The unsatisfactory treatment and lack of vaccine make HIV a uniquely troubling infection. Worldwide HIV prevalence is estimated at 40 million, chronic Hepatitis B virus (HBV) infection accounts for an estimated 370 million, while Hepatitis C virus (HCV) infection is estimated at 130 million (Alter, 2005). It is well known that infections with HBV and/or HCV have a severe and invasive impact on the health of millions of people around the world and the infection is often asymptomatic (Wondimeneh, *et al*., 2013). There is body of evidence that HBV and HCV among other opportunistic infections manifest in the course of HIV progression with attendant depletion below normal values of CD4+ count (Alberti *et al.*, 2005; Chen and Morgan, 2006; Fenton, 2007; Volf *et al*., 2008). In HIV infected persons, about 2-4 million have chronic HBV co-infection, whereas 4-5 million have HCV co-infection (Alter, 2005). HIV, HBV and HCV share common routes of transmission but they differ in their prevalence depending on geographic region, cultural practices, nutrition, age, sex etc. (Soriano, *et al*., 2006; Wondimeneh, *et al*., 2013). In spite of significant decline in morbidity and mortality rates since introduction of highly active antiretroviral therapy (HAART), liver diseases due to chronic HBV and HCV infections is among the leading cause of death in HIV positive patients (Alter, 2005; Wondimeneh, *et al*., 2013). In HIV-HBV co-infections, HIV infection causes increased rates of persistent HBV infection, increased cirrhosis and liver-related mortality and increased risk of hepatocellular carcinoma at lower CD4+ counts (Thio, 2009). Similarly in HIV-HCV co-infections, there is a more rapid progress to cirrhosis, end-stage liver disease and hepatocellular carcinoma (Romeo, *et al.,* 2000). In addition, the impact of HBV and HCV in HIV positive patients could not be limited to causing hepatocellular toxicity but also results in failure in immunological recovery. For instance, Christian *et al*., (2010) reported slow rate of immunologic recovery after initiation of HAART and higher risk of hepatotoxicity among HIV/HBV and HIV/HCV co-infected patients. Therefore, the management of HBV and HCV in HIV infection is complicated. Consequently, HIV, HBV and HCV become the major public health concerns globally (Modi and Feld, 2007; Leeratanapetch and Suseangrut, 2008). In most advanced countries, screening of HIV-infected individuals for HBV and HCV is highly recommended before initiation of antiviral treatment (Chung, 2006) but this scheme is not readily available in our center.

Evaluation of CD4+ level is a vital tool in the control and prophylactic strategy in HIV infection (CDC, 1992a). In West Africa, there is still paucity of information as to what point could CD4+ count depletion in HIV patients may precipitate HBV and/or HCV. Although, the World Health Organization (WHO) recommended initiation of HAART in HIV patients co-infected with HBV or HCV irrespective of value of CD4 count (WHO, 2009), unfortunately this cannot be achieved in most centers in Nigeria because free testing for HBV and HCV are not provided alongside free HIV testing and treatment in many health centers. The implication is that HIV patients whose CD4 counts are above 350cells/µl but who may be positive to HBV and/or HCV are not detected and do not have early commencement of HAART until they get to end-stage liver damage.

Bearing in mind that a number of factors such as geographic region, cultural practices, age, nutrition, infection etc influence CD4+ count level (Johnson and Kuritzikes, 1997; Alter, 2005; Wondimeneh, *et al*., 2013) it is of utmost important to clearly determine at what point to initiate prompt and adequate therapy against HBV and/or HCV co-infection in HIV positive patients in Nigeria.

In the present study, we evaluated the patterns of CD4+ cells and liver enzymes and their correlation with seroprevalence of HBV and HCV among HIV positive individuals at Orlu Hospital Counseling and Testing Unit, Imo state, South-East Nigeria.

Materials and Methods

Study area and population

This study was carried out at Orlu, Imo state, South Eastern Nigeria with patients' consent and after obtaining ethical committee approval. The study population comprises 846 HIV positive and 34 negative control patients who attended two major hospital counseling and testing (HCT) units in Orlu metropolis. The survey was conducted between March and September 2011. A total of 858 whole blood samples were collected and 12 were rejected due to insufficient blood sample. Informed consent was obtained from the patients through a structured questionnaire which captured socio-demographic data such as age, sex, marital status and occupation.

Data analysis was done using SPSS Version 16.0 (Chicago USA). The Chi-square test was used to evaluate the significance difference among the groups. P value ≤ 0.05 was considered significant. ANOVA was used to test the effects of the viral infection on mean $CD4^+$ count and liver enzymes on the patients.

Collection and processing of samples

Whole blood sample (10 ml) was aseptically collected from confirmed HIV-Positive patients using plain and EDTA vacutainer tube (5 ml in each tube) for the determination of HBV and HCV seroprevalence and CD4 and liver enzyme levels from each study participants. The blood specimen in the plain tube was centrifuged at 2500 RPM for 5 minutes to separate the serum which was used for determination of liver enzyme levels within one hour of separation.

The remaining serum kept in deep refrigerator (−40°C) until detection of HBV and HCV. The second tube that contains whole blood was used for the CD4 levels determination

The population was confirmed positive for HIV using the approved National serial Algorithm. The Determine rapid Kit, Unigold rapid kit and Stat-pak rapid kit were used in determining the discordant results. HBsAg and HCV were screened using CLINCOTECH and FICH TECH screening test strips. In addition, results of other test such as T-lymphocytes sub-set CD4+(CD4+Count) and liver enzymes (AST and ALT) tests were analyzed using flow cytometry (partec Cy-flow; SL-3Green) and spectrophotometer (Spectrum lab 23A) respectively. The manufactures protocols were strictly adhered to during each assay. HBV and HCV negative patients were used as control in ascertaining the impact of HCV and HCV on CD4+ count and liver enzymes of the HIV-positive patients.

Inclusion and exclusion criteria

During the study period, all HAART naïve adult HIV positive individuals who visited our center for CD4 and liver enzyme level determinations for their pre-ART follow up were included. But those who refused to give informed consent and who were already on ART follow up were excluded from the study. Also excluded are young adolescents below 18 years and those patients who had TB, malaria, leishmaniasis, chronic alcoholism, drug induced hepatotoxicity and other opportunistic infections were assessed and excluded according to the HIV management guidelines of Nigeria. (Federal ministry of health Abuja – Nigeria october 2010)

Results and Discussion

The effect of HBsAg and HCV on CD4+ count HIV patients

From the results, infected females had significant (P<0.05) high CD4+ count than males. Mean CD4+ count of healthy subjects (control) was found to be 1129.3 cells/µl for males and 1014.3 cells/µl for females.

Male patients having HIV mono-infection and HIV-HBsAg co-infection had significant (P<0.05) low CD4+ count of 345 cells/µl and 334.8 cells/µl respectively compared to healthy individuals. Similarly, HIV-HCV co-infected patients had significant (P<0.05) low CD4+ count (180.5 cells/µl) compared to HIV-HBsAg. Patients having triple infection (HIV-HBsAg-HCV) had the lowest value of CD4+ count which is highly significant (P<0.01) (Table 1).

Effects of HBsAg and HCV on liver enzymes (ALT and AST) of HIV patients

The mean serum AST and ALT levels of healthy subjects for males and females were different but not statistically significant (P>0.05). There was significant (P<0.05) elevation of serum AST and ALT levels of HIV mono-infected patients compared to uninfected individuals. Similarly, mean serum levels of AST and ALT in HIV-HBsAg and HIV-HCV co-infected patients were significantly (P<0.01) high compared to HIV mono-infected subjects. Patients having triple infection (HIV-HBsAg-HCV) also had significant (P<0.01) elevation of ALT and AST. However, this value is lower than those of HIV-HBsAg and HIV-HCV co-infected patients but not statistically significant. The elevation of ALT is highest in HIV-HCV co-infected patients and is statistically significant (P<0.05) when compared to HIV-HBsAg and HIV-HBsAg-HCV (Table 2).

This study investigated the patterns of CD4 cells and liver enzymes and their correlation with prevalence of HBV and HCV among HIV infected individuals who are HAART naïve.

The absolute CD4+ count is an important prognostic bio-marker that can be employed in establishing decision points for initiating appropriate therapy in HIV positive patients (National Institutes of Health, 1990; Fahey and Taylor, 1990; CDC, 1992). In the present study, there was statistical significant depletion of mean CD4+ value of HIV positive participants when compared to the healthy subjects. Also among the HIV co-infected participants, there was further statistical significant depletion of CD4+ count in

HIV-HBsAg, HIV-HCV and HIV-HBsAg-HCV co-infections when compared to HIV monoinfection. This is in confirmation that progressive depletion of CD4+ T cell is associated with an increased likelihood of clinical complication which if not detected early enough may lead to full blown AIDS (National Institutes of Health, 1990). Co-infection is associated with greater likelihood of disease progression and thus reduces immunity which can be confirmed by the reduced level of CD4+ count (WHO, 2009; Adewole, 2009).

The US public health service recommends that CD4+ T cell count be evaluated every six (6) months in all HIV positive cases (CDC, 1992b). However, this recommendation is scarcely adhered to in our centre at Orlu suburban area and many other communities in the south-east Nigeria. The US Department of Health and Human Services classified HIV positive people on the basis of absolute number of CD4+ T cells/µl (US Department of Health and Human Services, 2011). Again, it has been shown that antiretroviral therapy and antimicrobial prophylaxis are most effective within certain limits of immune system dysfunction (National Institutes of Health, 1990). Consequently, all HIV positive patients with less than 500 CD4+/µl are encouraged to start antiretroviral therapy (Fahey and Taylor, 1990, Dolan *et al*., 1993).

From our findings, this recommendation can be adopted in the sense that the mean CD4+ count of those with HIV mono-infection (345 cells/µl) is not statistically significant with those of HIV-HBsAg co-infection (334.8 cells/µl). This figure may be the bench mark for establishment of viral Hepatitis in HIV patients at Orlu suburban area. Statistically, it is reported that the rate of opportunistic infection in HIV positive patients with greater than 400 CD4+/µl is 1% within 1year whereas in patients with less than 400 CD4+/µl, this chance is increased to 21% within 1year (Dolan *et al*., 1993). In general, almost half of the HIV positive people with absolute CD4+ cells less than 100/ul will develop AIDS in less than 1year (Dolan *et al*., 1993).

On the other hand, following screening for viral hepatitis (HBV and HCV), the overall prevalence among the study participants was found to be very high (14.89%). The HIV-HBV co-infection rate was 9.8 % which is more or less comparable with that reported in western (11.9 %) and northern (11.5 %) Nigeria (Otegbayo *et al*., 2008; Adewole, *et al*., (2009). The prevalence of HIV-HBsAg co-infection was higher in females than males (2:1) which also corresponds with that reported by Otegbayo *et al*., 2008. The seroprevalence of HIV-HCV co-infection was 4.0 %. The seroprevalence of HIV-HBsAg-HCV triple infection was lowest (1.1 %) which is comparable with that reported in many parts of the world (Diop-Ndiaye, *et al*., 2008; Olufemi, *et al*., 2009; Alemayehu, *et al*., 2011).

Regarding liver enzymes, there was a significant increase in the serum liver enzymes (ALT and AST) of HIV mono-infected patients as against the healthy individuals. Similarly, these changes increased in HIV-HBsAg and HIV-HBsAg-HCV co-infected people and further increased in HIV-HCV individuals. This supports other studies conducted by Zhou *et al*., (2007) who reported that many people with chronic hepatitis have elevated liver enzymes levels. In a similar research in Northern Nigeria, Adewole *et al*., (2009) reported a high mean level of ALT among HIV patients co-infected.

Table.1 Effects of HIV, HBsAg and HCV on T-lymphocytes subset CD4+ of subjects examined

Patient profile	Sex	No. of infected/ Examined	Mean CD4+ Cells/µl
Healthy(None HCV/HBsAg/HIV)	M	14	1129.3
	F	20	1014.3
HIV(only)	M	206	345.3
	F	514	477.9
HIV &HBsAg	M	26	334.8
	F	57	363.1
HIV &HCV	M	15	180.5
	F	19	286.0
HIV,HBsAg&HCV	M	05	84.6
	F	04	129.0

Table.2 Effects of HIV, Hbsag and HCV on liver enzymes of subjects examined.

Patients profile	Sex	No. of Infected/ Examined	Mean ALT(IU/L)	Mean AST(IU/L)
Healthy(none HIV/HCV/HBsAg)	M	14	9.1	12.1
	F	20	13.0	14.1
HIV(only)	M	206	17.8	35.1
	F	514	27.3	38.2
HIV &HBsAg	M	26	33.3	58.3
	F	57	58.6	67.5
HIV &HCV	M	15	48.9	58.1
	F	19	72.4	74.8
HIV,HBsAg&HCV	M	05	23.3	47.0
	F	04	59.5	64.2

The highest level of liver enzymes observed among HIV patients co-infected with HCV in this present research may be as a result of the asymptomatic nature of HCV which leads to late diagnosis. At this stage, the patient might have developed most symptoms and thus, in chronic stage of the infection since 70-80% of people infected with HCV develop chronic infections (AIDS Info Net, 2010). Also, Boyer, (2001) reported that HCV is the leading cause of chronic liver disease in the United States.

Depletion of gastrointestinal tract associated CD4 lymphocytes is reported to contribute to this significant rise in the liver enzymes. This is achieved as the gastrointestinal mucosa become more permeable to microbial translocation and liberation of endotoxins (lipopolysaccharide (LPS)) which is pro-inflammatory and pro-fibrotic on the liver (Balagopal *et al*., 2008; Megan, *et al*., 2012).

In conclusion, it is recommended that CD4+ count of every HIV positive patients at Orlu and other suburban areas in the south eastern Nigeria be evaluated every six (6) months. A CD4+ count between 345 cells/µl – 400 cells/µl may be the set point for occurrence of viral hepatitis. For this reason, antiretroviral therapy and other antimicrobial prophylaxes are better commenced immediately should there be CD4+ count of this range. Screening for viral hepatitis should also be performed alongside HIV testing in order to detect it early for effective therapy and good prognosis.

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