

# The role of triple infection with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus (HIV) type-1 on CD4<sup>+</sup> lymphocyte levels in the highly HIV infected population of North-Central Nigeria

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*We set out to determine the seroprevalence of hepatitis B and C among human immunodeficiency virus type-1 (HIV-1) infected individuals in North-Central Nigeria to define the influence of these infections on CD4<sup>+</sup> lymphocytes cells among our patients as access to antiretroviral therapy improves across the Nigerian nation. The CD4<sup>+</sup> values of 180 confirmed HIV-1 infected individuals were enumerated using a superior fluorescence-activated cell sorter system. These patients were tested for the presence of hepatitis B surface antigen and anti-hepatitis C virus (HCV) using third generation enzyme-linked immunosorbent assays. Fifty (27.8%) patients had active hepatitis B virus (HBV) infection while 33 (18.3%) tested positive for anti-HCV antibody. Of these infections, 110 (61.1%), 37 (20.6%), and 20 (11.1%) had HIV only, HBV/HIV-only, and HCV/HIV-only respectively. A HBV/HCV/HIV coinfection prevalence of 7.2% (13 patients) was recorded. Patients coinfecting with HIV/HBV/HCV appeared to have lower CD4<sup>+</sup> counts (mean = 107 cells/ $\mu$ l; AIDS defining) when compared to HBV/HIV-only (mean = 377 cells/ $\mu$ l), HCV/HIV-only (mean = 373 cells/ $\mu$ l) and patients with mono HIV infection (mean = 478 cells/ $\mu$ l). Coinfection with HBV or HCV is relatively common among HIV-infected patients in Nigeria and should be a big consideration in the initiation and choice of therapy.*

Key words: human immunodeficiency virus - hepatitis B and C viruses - Nigeria

Human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) are devastating disease agents that share common modes of transmission (Santiago-Munoz et al. 2005), therefore HIV positive individuals are at risk of co-infection with HBV and HCV infections. With the advent of highly active antiretroviral therapy (HAART) regimens capable of dramatically prolonging the survival of HIV-infected patients, the impact of co-morbid infections such as HBV and HCV has come into focus (Petoumenos & Ringland 2005). Co-infection with HBV or HCV increases the risk for hepatotoxicity of HAART and likelihood of onset of an AIDS-defining illness, compared with infection with HIV-1 alone (Greub 2000, Feld et al. 2005). Although the HIV co-infection with HBV and/or HCV has been recognized worldwide in individuals exposed to blood-borne diseases, limited data are available on the extent of co-infection and effect of these viruses on the immune system in developing countries. Nigeria belongs to the group of countries highly endemic for viral hepatitis (Odemuyiwa et al. 2001). Few studies have been done on HIV, HBV, HCV separately in Nigeria but the knowledge about the interrelationship between these viruses and their effect on the immune system remains

unclear. This study was therefore carried out to estimate the prevalence of HBV and/or HCV seropositivity in a cohort of people living with HIV/AIDS in North-Central Nigeria and to investigate the effect of these viruses on CD4<sup>+</sup> lymphocytes in the HAART era in Nigeria.

One hundred and eighty plasma samples were randomly selected from confirmed HIV-1 positive samples stored at -24°C in the Virology laboratory of Innovative Biotech (IBL), Keffi, Nasarawa State, Nigeria. These samples were collected between June and December 2005 from clients who assessed IBL services for voluntary HIV counseling and testing or for other health needs. Before each client was bled, informed consent was obtained in accordance with IBL and international regulations. These samples were labeled with a serialized IBL code number that could not be linked to individuals. As patients were bled, a fluorescence-activated cell sorter system (Becton Dickenson FACSCount, Canada) was used to enumerate absolute values for CD4<sup>+</sup> cells for each sample according to manufacturers instructions. The second CD4<sup>+</sup> counts of clients who returned after HAART were also enumerated. The double antibody sandwich Shantest<sup>TM</sup> - HBsAg ELISA (Shantha Biotech Limited - India) was used for the detection of HBsAg in plasma following manufacturers instructions and the microplates read at a wavelength of 450 nm using the ELISA reader (BIO-RAD 2100, version 6.1, US). Anti-HCV antibodies were detected in plasma using the Anti-HCV-EIA-Avicenna (Avicenna Medical Center, Russia) which is a third generation quantitative ELISA that uses recombinant proteins and synthetic peptides derived from core and structural regions of HCV to detect the pres-

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ence of anti-HCV in plasma samples. The test was carried out and interpreted according to manufacturer's instructions. Demographic data (age and gender) for participants were retrieved from coded clients' electronic registration records maintained at IBL. To compare the proportion of HIV positive individuals co-infected with HBV and/or HCV, the chi-square test was used at 95% confidence level. The analysis was done after recording the data in a Microsoft Excel worksheet on a Windows-98 platform.

Of the 180 samples, 83 were from males and 97 from females. The age range of the patients in this study was 20-64 years. The seroprevalence of HIV/hepatitis viruses and the distribution of disease burden are as follows: HIV only = 61.1%; HBV/HIV only = 20.6%, HCV/HIV only = 11.1%, and HIV/HBV/HCV = 7.2%. The gender distribution and ages of the infected individuals are as shown: 110 HIV only (57 males: 53 females; mean age = 32 and 20 years respectively); 37 HIV/HBV (21 males: 16 females; mean age = 29 and 25 years respectively); 20 HIV/HCV (13 males: 7 females; mean age = 37 and 30 years respectively); 13 HIV/HBV/HCV (7 males: 6 females; mean age = 43 and 31 years respectively). The CD4<sup>+</sup> profile was not different from either the HBV (range = 5 to 523 CD4<sup>+</sup> cells/μl, mean = 377 CD4<sup>+</sup> cells/μl) or HCV (range = 46 to 791 CD4<sup>+</sup> cells/μl, mean 373 CD4<sup>+</sup> cells/μl) coinfecting individual. On the other hand, CD4<sup>+</sup> count values in HBV/HCV/HIV coinfecting patients were poorer (range = 4 to 442 CD4<sup>+</sup> cells/μl, mean = 107 CD4<sup>+</sup> cells/μl). The mean CD4<sup>+</sup> count value for patients infected with HIV only was 478 CD4<sup>+</sup> cells/μl (range = 8 to 755 CD4<sup>+</sup> cells/μl). Thirty-seven patients enrolled for HAART (nevirapine 200 mg, lamivudine 150 mg, stavudine 40 mg, and nutritive dietary supplement) during the period under review. The CD4<sup>+</sup> counts for 14 clients were enumerated four months post-antiretroviral therapy (ART). There was an average increase of 128 (range = 22 to 586) CD4<sup>+</sup> cells in all the category of patients. However, there was a post ART decline of 133 CD4<sup>+</sup> cells in a 35 years old female client coinfecting with HIV and HCV infections.

HAART has transformed HIV/AIDS from a uniformly fatal illness into a manageable chronic infection and has been shown to be able to restore CD4<sup>+</sup> cells in HIV infected patients (Rathbun et al. 2006). The gains of HAART could be compromised by coinfection with hepatitis viruses as they are known to have adverse effects on the prognosis of HIV and hepatitis infections (Feld et al. 2005). Consequently, increased attention has to be paid on coinfection of hepatitis viruses and HIV especially in the developing countries like Nigeria where these groups of viruses are endemic. In this study, 20.6% of HIV infected individuals were coinfecting with HBV. Our study correlates with a recent study in Jos (Uneke et al. 2005), that recorded that 25.9% of HIV-infected individuals were HBsAg seropositive. This study therefore confirms the endemicity of HBV infection and increased infection in HIV infected individuals. This calls for the need to strengthen the HBV vaccination program in Nigeria which is known to be able to considerably reduce the incidence of HBV infections. The average CD4<sup>+</sup>

count values for this group was 377 cells/μl which is lower than that for HIV mono-infection recorded (mean = 478 cells/μl). The natural history of HBV is known to be complicated by HIV-co-infection but the effect of HBV on the outcome of patients infected with HIV-1 is controversial (Rockstroh 2006). The reasons for the CD4<sup>+</sup> decline is not clear but it is known that there is an imbalance in peripheral blood T-lymphocyte subsets and turbulence in cellular immunity in the patients with chronic hepatitis B (Tian et al. 2005). Also, lamivudine resistant mutations in HBV treatment have adverse effects on treatment response in HIV infected individuals coinfecting with HBV.

We also found an HCV seroprevalence rate of 11.1% in the group of HIV-1 infected individuals sampled. Previous studies in Nigeria had reported an overall HCV prevalence of 2.9% among blood donors in Rivers state of Nigeria (Kaote et al. 2005). Agwale et al. (2004), had recorded an HCV seroprevalence rate of 8.2% among HIV infected Nigerians. There is a clear indication of increased HCV infection in HIV infected individual in Nigeria. It is known that HIV/HCV coinfecting individuals accelerate rapidly to end-stage liver disease, AIDS defining clinical event and death (Greub 2000, Monga et al. 2001). Unfortunately at this time, no effective vaccine has been developed against HCV infection. We also report a case of a 35 years old female client coinfecting with HIV and HCV who virologically failed therapy (CD4<sup>+</sup> decline from 199 to 66) after four months on HAART. We also record that, the rate of increase in CD4<sup>+</sup> cells post-HAART does not change in HIV and hepatitis coinfection but HCV appears to hinder virological response to therapy. Although there have been case reports of clearance of HCV viraemia after initiation of HAART (Ranieri et al. 2003), majority of available data indicates that HAART results in net increase in HCV viraemia (Chung et al. 2002). We have recorded that 7.2% of individual do have triple coinfection with HIV/HBV/HCV. As far as we know, this is the first report of HIV, HBV, and HCV triple combination coinfections in the same individuals in Nigeria. The CD4<sup>+</sup> values of these group was significantly poorer (mean = 107 cells/μl: AIDS defining) when compared with individuals who have HIV infection alone (mean = 478 cells/μl). Triple coinfecting individuals are more likely to present with lower CD4<sup>+</sup> counts and therefore reduced host immunity. Triple coinfection is therefore a growing problem in Nigeria and needs careful attention owing to its adverse effects on HIV treatment response.

We have demonstrated that co-infection of HIV and hepatitis viruses (HBV and/or HCV) is on the increase in Nigeria and appears to decrease the CD4<sup>+</sup> counts of patients who are coinfecting especially with triple coinfection of HIV, HBV, and HCV. Treatment of either hepatitis virus is complex because of pharmacokinetic interactions with components of HAART regimens. Thus, the phenomenon of HIV and hepatitis viruses coinfection is a cause for concern. The medical community in Nigeria therefore needs to be alert to this phenomenon as smart treatment options would need to be instituted in such individuals if treatment is to be meaningful.

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