



### Immunological and Virological Response to HAART in HIV-1 Patients Co-Infected with Hepatitis B and C Viruses

*Reponse Immunologique et Virale au Traitement Antiretroviral Hautement Actif Chez des Patients VIH-1 Co Infectes Par Les Virus de l' Hepatite B et C*

A. P. Okwuraiwe\*†, R. A. Audu†, O. B. Salu†, C. K. Onwuamah†, O. S. Amoo, F. A. Ige†, E. H. Meshack†, P. D. Jamda†, N. N. Odunukwe‡, D. I. Onwujekwe‡, O. C. Ezechi‡, E. O. Idigbe§

#### ABSTRACT

**BACKGROUND:** Among the countries highly endemic for viral hepatitis, Nigeria is found. Information on how triple infected persons (HIV, HBV, and HCV) fare on HAART in the country is lacking. Laboratory based investigation was carried out to assess the virological and immunological parameters of HIV-1 infected patients co-infected with Hepatitis B and C, accessing care at the Nigerian Institute of Medical Research. It was a case controlled study.

**OBJECTIVES:** The study aimed to compare the laboratory data of HIV-HBV-HCV patients seen between 2006 and 2009 with HIV-1 monoinfected patients in the same period, on HAART according to the national guideline and followed up for 12 months.

**METHODS:** Detection of Hepatitis B surface Antigen (HBsAg) and Hepatitis C Virus Antibody (HCVAb) were assayed using ELISA techniques (Bio Rad and DIA PRO respectively). The CD4 and HIV viral load were determined using the *Cyflow Counter/Kits* (Partec) and the *Amplicor HIV-1 Monitor Test V1.5* (Roche) techniques respectively.

**RESULTS:** Forty-one (0.4%) of the 10,214 HIV-1 patients seen during the period were co-infected with both HBV and HCV. Over the 12 month-period, median HIV-1 viral load and CD4 count reduced and increased respectively (12,205–200 RNA copies/mL; 210–430 cells/μL from baseline – 12<sup>th</sup> month), and for the HIV-1 monoinfected patients (36,794–200 RNA copies/mL [p=0.5485] and 206–347 cells/μL [p=0.7703] from baseline – 12<sup>th</sup> month).

**CONCLUSION:** There seems to be no significant influence of hepatitis B and C in HIV infection on HAART judging by the CD4 and viral load profiles which were similar in the two groups. *WAJM* 2012; 31(2): 124–128.

#### RÉSUMÉ

**CONTEXTE:** Le Nigeria est parmi les pays présentant une forte endémie à l'hépatite virale. Les informations sur les patients présentant la triple infection (VIH, HVB, et HVC) sous traitement antirétroviral hautement actif (TARHA) font défaut dans le pays. Des explorations au laboratoire ont été menées pour évaluer les paramètres virologiques et immunologiques des patients VIH-1 co-infectés par l'hépatite Virale B et C suivis à l'Institut Nigerian de recherche Médicale dans le cadre d'une étude cas témoins.

**OBJECTIFS:** Comparer les données de laboratoire de patients porteurs de VIH-VHB-VHC suivis entre 2006 et 2009 avec celles de patients mono infectés par le VIH-1 dans la même période sous TARHA selon les recommandations nationales sur un suivi de 12 mois.

**METHODES:** Les techniques ELISA Bio Rad et DIA PRO ont été utilisées respectivement pour détecter les antigènes de surface du VHB (Ag HBs) et les anticorps anti VHC (AC VHC). Le taux de CD4 et la charge virale ont été évaluées respectivement par les techniques du *Cyflow Counter/Kits* (Partec) et de l'*Amplicor HIV-1 Monitor Test V1.5* (Roche).

**RESULTATS:** Quarante et un (0.4%) des 10.214 patients porteurs de VIH-1 suivis durant la période étaient co-infectés par les VHB et VHC. Au cours de la période de 12 mois, la médiane de charge virale et le taux de CD4 avait baissé et augmenté respectivement pour les patients co infectés (12,205-200 copies d'ARN/mL; 210-430 cellules/μL du niveau basal au 12<sup>e</sup> mois), et pour les patients mono infectés par VIH-1 (36,794-200 copies d'ARN/mL [p=0.5485] et 206-347 cellules/μL [p=0.7703] du niveau de base au 12<sup>e</sup> mois).

**CONCLUSION:** Il ne semble pas y avoir d'influence significative des VHB et VHC sur l'infection au VIH sous TARHA si on tient compte du profile du taux de CD4 et de la charge virale qui sont similaires dans les 2 groupes. *WAJM* 2012; 31(2): 124–128.

**Keywords:** HBV, HCV, HIV, CD4, viral load, HAART.

**Mots clés:** VHB, VHC, VIH, CD4, Charge virale, TARHA.

\*Human Virology Unit, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria †Clinical Sciences Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria ‡Microbiology Department, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.

\*Correspondence: A. P. Okwuraiwe, Human Virology Unit, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. E-mail: azukaokwu@yahoo.com

**Abbreviations:** CD4, Cluster of Differentiation 4; HAART, Highly Active Antiretroviral Therapy; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; HIV, Human Immunodeficiency Virus.

## INTRODUCTION

Worldwide, hepatitis B virus (HBV) accounts for an estimated 370 million chronic infections, hepatitis C virus (HCV) accounts for 130 million and human immunodeficiency virus type 1 (HIV-1) accounts for 40 million.<sup>1,2</sup> Infection with HBV and HCV and the related liver damage is an important cause of mortality and morbidity among HIV-infected patients,<sup>3</sup> and often associated with severe forms of liver disease.<sup>4</sup> The principal routes for HIV transmission are similar to that followed by the hepatotropic viruses; consequently, infections with HBV and HCV are expected in HIV infected patients.<sup>4</sup> The prevalence of HBV infection varies throughout regions of the world, but is highly endemic in developing regions with large populations such as South East Asia, China, sub-Saharan Africa and the Amazon Basin where at least 8% of the population are HBV chronic carriers.<sup>5</sup> Globally, of the 370 million chronic infections, 2–4 million have HIV, which modifies the natural history of HBV infection.<sup>6</sup> The prevalence of HIV/HBV, HIV/HCV and HIV/HBV/HCV in Nigeria as observed in a North Central population study was 20.6%, 11.1% and 7.2% respectively.<sup>13</sup> However, more studies are needed to give a better picture of these co-infected patients in the country.

Chronic HBV/HCV infection is identified by the positivity of the HBV surface antigen (HBsAg) and the antibody to HCV (anti-HCV or HCVAb), which occurs in a sizable proportion of chronic hepatitis patients, and is generally considered a condition favouring the progression of liver fibrosis and the establishment of cirrhosis. It is an important risk factor for the development of hepatocellular carcinoma. *In-vitro* studies suggest that HCV is capable of suppressing HBV activity and this inhibitory effect is mediated by the HCV core protein.<sup>15</sup> Some *in-vivo* studies indicate a possible interplay between the two viruses, in some cases confirming a prevalent role of HCV, while other reports suggest a reciprocal interference or even a dominant effect of HBV.<sup>16–18</sup> Tenofovir has become a mainstay of treatment for HBV infection in HIV co-infection, along with

lamivudine and peg-interferon for HBV alone.<sup>7</sup>

In HCV therapy, a combination of interferon and the antiviral drug ribavirin is currently the treatment of choice and this rids the body of the virus in 40 to 80% of cases, but the virus has a high propensity for persistence.<sup>8</sup> In the United States, an estimated 30,000 cases of hepatitis C infection develop each year, and although some resolve spontaneously, 55 to 85% of all cases progress to chronic hepatitis. It is recognized that chronic hepatitis C patients of various racial groups have different outcomes, people of African descent generally exhibiting lower rates of spontaneous HCV clearance and poorer response to interferon-based therapy.<sup>9</sup> Manifestations of HCV infection are primarily non-hepatic and include membranoproliferative glomerulonephritis and necrotizing vasculitis of the skin.<sup>10</sup>

As HIV-infected individuals live longer, the effects of co-infection with chronic hepatitis B and C will likely become an increasingly relevant issue. HIV adversely affects the natural history of HBV and HCV.<sup>11</sup> HCV disease expression is related to viral expression: low levels of circulating HCV RNA are generally found in asymptomatic patients with normal ALT levels; elevations in serum ALT levels, often in a fluctuating pattern, are its most characteristic feature<sup>22</sup>

In a retrospective case controlled study, we set out to investigate the immunological (CD4) and virological (HIV1 viral load) indications of HBV/HCV co-infections in HIV-1 positive patients on HAART in relation to the more established indications of HIV-1 mono-infected patients on HAART for 12 months in a Nigerian health facility.

## SUBJECTS, MATERIALS AND METHODS

A total of 10,214 HIV-1 patients that visited the Human virology laboratory, NIMR, between 2006 and 2009, out of which 41 (17 male, 24 female) were found to be triple infected (HIV, HBV and HCV), as compared with the 8,976 HIV-1 only infected patients.

**Study Area:** The study was conducted

from January 2006 through December 2009. All patients who accessed care at the Nigerian Institute of Medical Research, (NIMR) Lagos, Nigeria, in that period, and placed on highly active antiretroviral therapy (HAART) according to the national guideline and followed up for at least 12 months were extracted for this study in the Human Virology Laboratory of the Institute.

**Data Collection:** Biodata information was extracted for each of the 10,214 patients containing details of their age and sex, and other demographics.

**Informed Consent/Ethical Approval:** Only data from patients who had given prior signed consent were included in this study; an IRB ethical approval was obtained from the NIMR Ethics committee.

**HIV Confirmation Assay:** Plasma from the 10,214 patients was confirmed HIV-1 positive using the Western blot technique by Immunetics (Boston, MA, USA).

**Hepatitis B Surface Antigen Assay:** Plasma from the 10,214 patients was assayed at baseline for presence of hepatitis B surface antigen (HBsAg) by Bio Rad (92430 Marnes-La-Coquette, France) following Manufacturer's instructions.

**Hepatitis C Virus Antibody Assay:** Plasma from the same 10,214 patients were assayed at baseline for presence of antibodies to HCV by a third generation enzyme-linked immunosorbent assay (ELISA) Kit, commercially available (DIA PRO Diagnostic Bioprobes, Milano, Italy) following manufacturer's instructions.

**CD4 Assay:** Whole blood of the same patients was used to perform CD4 assay at 3-month intervals using the Cyflow Counter and Kits (Partec, Germany). Manufacturer's instructions were followed.

**HIV-1 Viral Load assay:** The viral load assay was performed on plasma at 3-month intervals using the Amplicor HIV-

I Monitor Test V1.5 Kits (Roche Diagnostics GmbH, Mannheim, Germany) following manufacturer's instructions.

**Statistical Analyses:** Data analysis was done using *Epi info* version 2002 and test of significance was done using *Kruskal-Wallis* statistical test. Differences of  $p < 0.05$  were taken to be statistically significant.

**RESULTS**

Forty-one (0.4%; 95% CI) of the 10,214 HIV-1 positive patients seen during the period were co-infected with both HBsAg and HCVAb. Of the 41 patients, 17 were male and 24 female. The mean age of the patients in this study was 35 (range 28–60) years.

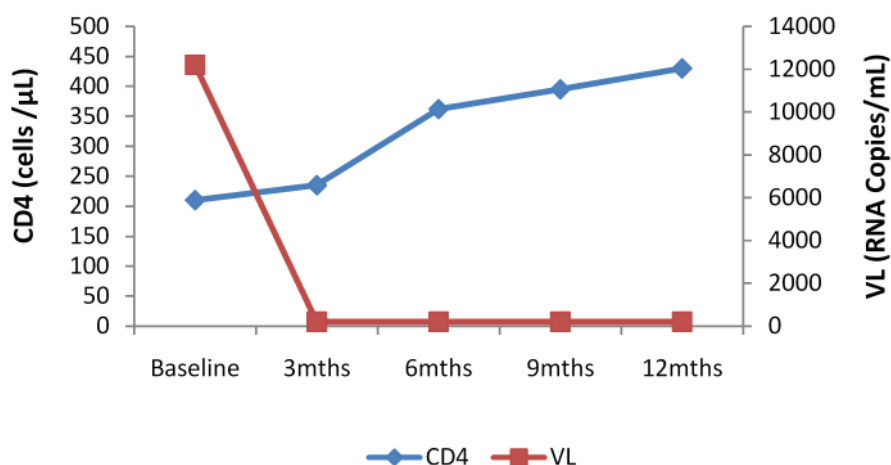
From this study 8,976 (87.7%) had HIV-1 monoinfection, 851 (8.3%) had HIV/HBV co-infection, 346 (3.4%) had HIV/HCV co-infection and 41 (0.4%) had HIV/HBV/HCV triple infection. The median immunological and virological response to HAART of the 41 triple infected patients is shown in Table 1 and Figure 1. Median CD4 and viral load was 210 cells/ $\mu$ L and 12,205 RNA copies/mL at

**Table 1: Median CD4 and Viral Load Response to Haart of HIV-HBV-HCV Tri-Infected Patients in 12 Months**

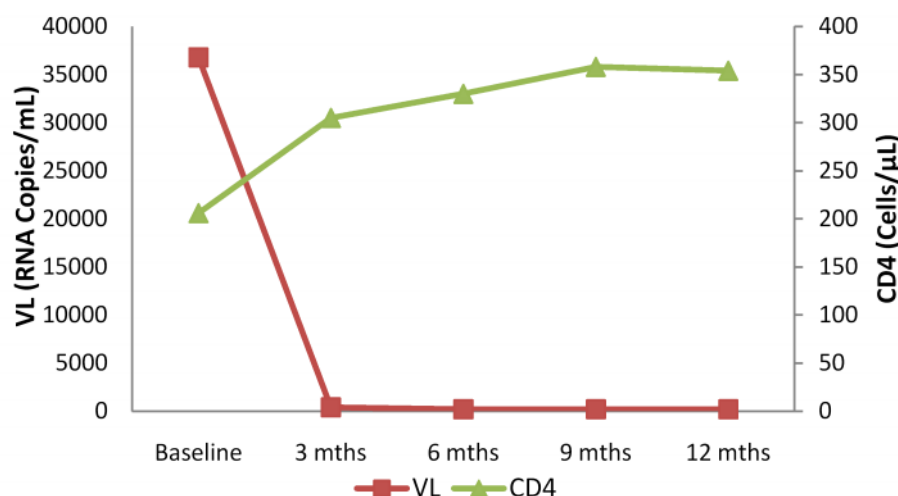
Time (Months)	Median CD4 (Cells/ $\mu$ L)	Median Viral Load (RNA Copies/mL)
0	210	12,205
3	235	200
6	362	200
9	395	200
12	430	200
<i>P values</i>	0.5485	

**Table 2: Median CD4 and Viral Load Response to Haart Of HIV-1 only Infected Patients in 12 Months**

Time (Months)	Median CD4 (Cells/ $\mu$ L)	Median Viral Load (RNA Copies/mL)
0 (baseline)	206	36,794
3	305	400
6	330	200
9	358	200
12	354	200
<i>P values</i>	0.7703	



**Fig. 1: Median CD4 and Viral Load Response to HAART of HBV-HCV Co-Infected HIV Patients in 12 Months**



**Fig. 2: Median CD4 and Viral Load Response to HAART of HIV-1 Patients in 12 Months**

baseline, and 430 cells/ $\mu$ L and 200 RNA copies/mL at the 12<sup>th</sup> month respectively. The median CD4 and viral load for the HIV-1 only patients was 206 cells/ $\mu$ L and 36,794 RNA copies/mL at baseline, and 354 cells/ $\mu$ L and 200 RNA copies/mL at the 12<sup>th</sup> month respectively.

The median CD4 and viral load profiles for the 8,976 HIV-1 mono-infected patients is shown in Table 2 and figure 2. The difference in CD4 cell recovery and HIV-1 viral load decline for the tri-infected patients was statistically insignificant ( $p$  values 0.5458 and 0.7703 respectively) as compared with the pattern observed in HIV-1 patients not coinfected with hepatitis B and C.

**DISCUSSION**

The impact of co-morbid infections such as HBV and HCV has come into focus.<sup>19</sup> Since the principal routes for HIV transmission are similar to that followed by the hepatotropic viruses, as a consequence, infections with HBV and HCV are expected in HIV infected patients.<sup>8</sup> Coinfection with HBV or HCV increases the risk of hepatotoxicity of HAART and the likelihood of onset of an AIDS-defining illness, compared with HIV-1 alone.<sup>20</sup>

In this study an assessment was carried out on patients with triple viral infection to see their response to HAART. This was necessary because the

gains of HAART could be compromised by co-infection with hepatitis viruses as they are known to have adverse effects on the prognosis of HIV and hepatitis infections.<sup>14</sup> Consequently, increased attention has to be paid on co-infection of hepatitis viruses and HIV especially in the developing countries like Nigeria where these groups of viruses are endemic.

The median CD4 level observed in this study at baseline (drug-naïve) was 210 cells/ $\mu$ L and 206 cells/ $\mu$ L for the triple infected and mono-infected patients respectively. These markers steadily increased to 430 cells/ $\mu$ L and 354 cells/ $\mu$ L respectively at the 12<sup>th</sup> month. This shows a fairly good immune reconstitution based on the drug intake. These results are at variance with that of Lincoln *et al*,<sup>21</sup> where they found that patients co-infected with HIV/HCV appeared to have a poorer response to HAART in terms of CD4 count changes, with a CD4 count increase of 32 cells/ $\mu$ L (95% CI 1-67) less than HIV-only patients. There has also been found to be an imbalance in peripheral blood T-lymphocyte subsets and turbulence in cellular immunity in patients with chronic hepatitis B.<sup>15</sup>

HCV infection does not cause fulminant hepatic failure, but, occurring in the setting of another chronic liver disease such as chronic HBV infection, may precipitate liver failure.<sup>12</sup> The profiles in Figures 1 and 2 show a normal virological suppression. It shows that the immunologic response improved in the first 12 months, and the viral load declined in the same period. However further studies for longer periods are needed as these findings are inconclusive due to the small sample size in this study. These observations are similar to the study by Smith *et al*.<sup>23</sup> However, it was reported that "although HAART increased the life expectancy in HIV infected patients, those with a chronic triple infection of hepatitis B, C and HIV as well as HCV-HIV co-infected patients still have an increased mortality risk".<sup>23</sup>

Median viral loads for the triple infected group was less than the HIV-1 monoinfected group (12,205 [4.1 log] < 36,794 [4.5 log] RNA copies/mL). The two groups on consistent HAART intake

had their viral load RNA reduced to near undetectable levels (200 [2.3] RNA copies/mL)

In conclusion, HIV-1 positive patients co-infected with hepatitis B and C maintained similar virological and immunological response to HAART as those not coinfected, in the first 12 months. It is thus fair to infer that hepatitis B and C virus coinfection do not seem to alter immunological and virological response of HIV to about 12 months after treatment commencement. Although this is at variance with studies by Smith *et al*, in terms of increased mortality of tri-infected individuals.<sup>23</sup> However, it is important to follow up these patients for a longer period, and for larger studies be undertaken to confirm these findings.

#### ACKNOWLEDGEMENT

We would like to acknowledge the President Bush's Emergency Programme for AIDS in Africa (Harvard PEPFAR) and the AIDS Prevention Initiative in Nigeria (APIN) for their collective effort in the management of HIV infection in Nigeria.

#### REFERENCES

1. Alter MJ. Epidemiology of hepatitis B in Europe and worldwide. *J Hepatol.* 2003; **39**: S64-S69.
2. Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. *J Hepatol.* 2006; **44**: S6-9.
3. Gang C, Wenyao L, Fumin S, Iloeje UH, Thomas WL and Alison AE. Past HBV Viral Load as Predictor of Mortality and Morbidity from HCC and Chronic Liver Disease in a Prospective Study. *The American Journal of Gastroenterology* 2006; **101**: 1797-1803.
4. Antipa C, Ruta S, Cernescu C, Copelovic Y, Strauss I and Iosipenco M. Immunological disorders of increased severity in children with AIDS associated with hepatitis B and C virus infections. *Romanian J. Virology.* 1995; **46**: 3-8.
5. Kim AY, Chung RT and Polsky B. HIV and hepatitis B and C co-infection. Pathogenic interactions, natural history and therapy. *AIDS Clin. Rev* 2001; **2**: 263-306.
6. Puoti M, Airoidi M and Bruno R. Hepatitis B virus co-infection in human immunodeficiency virus infected individuals. *AIDS Reviews* 2002; **4**: 27-35.
7. Piroth L, Birquet C, and Vergen M. The evolution of Hepatitis B virus serological patterns and the clinical relevance of isolated antibodies to hepatitis B core antigen in HIV infected patients. *J. Hepatol.* 2002; **36**: 681-686.
8. Saravanan S, Velu V, Kumarasamy N, Nandakumar S, Murugavel KG, Suniti S and Thyagarajan SP. Coinfection of hepatitis B and hepatitis C virus in HIV-infected patients in south India. *World J. Gastroenterol.* 2007. **37**: 5015-20.
9. Harris RA, Sugimoto K and Kaplan DE. Human leukocyte antigen class II associations with hepatitis C virus clearance and virus-specific CD4 T cell response among Caucasians and African Americans. *Hepatology* 2008, **48**: 70-79.
10. Manns MP and Rambusch EG. Autoimmunity and extrahepatic manifestations in hepatitis C virus infection. *Journal of Hepatology*, 1999, **17**: 1730-1733.
11. Apurva AM and Jordan JF. Viral Hepatitis and HIV in Africa. *AIDS Reviews* 2007; **9**: 25-39.
12. Hsu HH and Greenberg HB. Hepatitis C in Infectious Diseases. A treatise of infectious processes. 5<sup>th</sup> ed. *JB Lippincott Co, Philadelphia*, 1994: 820-825.
13. Forbi JC, Gabadi S, Alabi R, Iperepolu HO, Pam CR, Entonu PE and Agwale SM. The role of the 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. *Mem. Inst. Oswaldo Cruz*, 2007; **102**: 535-537.
14. Feld JJ, Ocama P and Ronald A. The liver in HIV in Africa. *Antivir Ther* 2005; **10**: 953-965.
15. Liu C, Chen P, and Chen D. Dual chronic hepatitis B virus and hepatitis C virus infection. *Hepatol Int.* 2009 **3**: 517-525.
16. Pontisso P, Ruvoletto MG, Fattovich G, Chemello L, Gallorini A, Ruol A and Alberti A. Clinical and virological profiles in patients with multiple hepatitis infections. *Gastroenterology* 1993; **105**: 1529-33.
17. Crespo J, Lozano JL, de la Cruz F, Rodrigo L, Rodriguez M, San Miguel G, *et al*. Prevalence and significance of hepatitis C viremia in chronic active hepatitis B. *Am J. Gastroenterology* 1994; **89**: 1147-51.
18. Zarski JP, Bohn B, Bastie A, Pawlotsky JM, Baud M, Bost-Bezeaux F, and Tran

- van Nhiew J. Characteristic of patients with dual infection by hepatitis B and C viruses. *J. Hepatol* 1998; **28**: 27–33.
19. Petoumenos K and Ringland C. Antiretroviral treatment among HIV, hepatitis B virus and hepatitis C virus co-infected patients in the Australian HIV Observational database. *HIV Medicine* 2005; **6**: 155–163.
20. Greub G. Clinical progression, survival, and immune recovery during anti-retroviral therapy in patients with HIV-1 and hepatitis C virus co-infection: the swiss HIV cohort Study. *Lancet* 2000; **356**: 1800–1805.
21. Lincoln D, Petoumenos K and Dore GJ. The Australian HIV Observational Database *HIV Medicine*. 2003, **4**: 241–249.
22. Houghton M. Hepatitis C viruses. In: Fields BN, Knipe DM, Howley PM, eds. *Fields Virology*, 3rd ed. Philadelphia, Lippincott-Raven, 1996: 1035–1058.
23. Smit C, Arends J, de Wolf F, and Hoepelman IM. Interaction between Hepatitis B and C in HIV Infected Patients; Risk of Dying among Patients with a Triple Infection. *44th Annual Meeting of the European Association for the Study of the Liver (EASL) 2009. Copenhagen, Denmark*. April 22–26.