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# EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ON CD4$^+$ CELL COUNT AND LIVER ENZYMES IN HIV INFECTION AT LOKOJA, NIGERIA

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**ABSTRACT**

**Aim:** To assess the effect of highly active antiretroviral therapy (HAART) on $CD_4^+$ cell count and liver enzymes in HIV-infected patients six months post-therapy based on age and sex distribution.

**Methods:** A cohort of 200 (66 males, 134 females) consenting HAART naïve patients, aged between 16 and 65 years with confirmed cases of HIV infection (by Western blot method) at Lokoja, Nigeria having a baseline $CD_4^+$ cell counts ≤350 cells/µL were initiated on HAART for six months. Two blood samples were collected, one at pre-HAART (baseline) and the other at post-HAART (follow-up) phase. $CD_4^+$ cells were counted and liver enzymes levels were evaluated using the Partec® Cyflow Counter and Reflotron® Plus Auto-Analyzer, respectively.

**Results:** The immunological outcome of 6 months HAART shows that there was slight increase (statistically, considered not significant, P>0.05) in the post-HAART mean±SEM$CD_4^+$ cell count in the different age and sex strata investigated, when comparison was made with the pre-HAART mean±SEM$CD_4^+$ cell count. Also, the post HAART liver function test show that the liver enzymes levels were not significantly (P>0.05) elevated when compared to the baseline values.

**Conclusion:** Six months of HAART repleted $CD_4^+$ cell counts of HIV-infected patients of different age and sex strata, with no risk of liver damage.

**Keywords**: HIV, HAART, Immune response, $CD_4^+$ cell, Liver enzymes

## INTRODUCTION

The introduction and widespread access to antiretroviral (ARV) drugs has revolutionized the management and treatment of HIV/AIDS resulting in a dramatic improved clinical course and survival in infected patients (Hogg et al., 1999). Current treatment guidelines recommend the use of a combination of at least 3 ARV drugs which include: 2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs) combined with 1 medication from either of the 2 remaining classes; the Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) or the Protease Inhibitors (PIs). When such drugs, typically three or four, are taken in combination, the approach is known as highly active antiretroviral therapy – HAART (Hogg et al., 1999; Miller et al., 1999). Increasingly, HAART has become the mainstay of treatment for those infected with HIV (Jayasuriya et al., 2007). Current treatment guidelines recommend that HIV-infected patients who have stage I or II clinical disease with a $CD_4^+$ cell count <350 cells/µL should start HAART, before it falls below 200 cells/µL (Kaplan et al., 2003; WHO, 2005, NGHA, 2010). And since progressive clinical and immunological deterioration of

HIV-infected patients correlate with a gradual reduction in $CD_4^+$ T-lymphocytes (Lederman et al., 1998; Lederman, 2001), enumeration of $CD_4^+$T-lymphocytes therefore aid in the diagnosis of HIV/AIDS, as well as in the assessment of response to HAART (Akanmu et al., 2001; Palella et al., 2003). Since its introduction in 1996, mortality rates in HIV-infected individuals in countries with widespread access to HAART have plummeted significantly, howbeit, the morbidity attributed to HAART has remained as a serious concern (Mocroft et al., 2003; Palella et al., 2006). Currently, Nevirapine (NVP) is the most affordable NNRTI available for HAART in resource-constrained areas. Unfortunately, one of the primary concerns regarding the use of NVP-containing HAART is the frequently reported risk of hepatotoxicities, following commencement of therapy, resulting in acute and chronic liver injury (Arminio-Monforte et al., 2000; Bica et al., 2001). Drug-induced hepatotoxicity is usually a common complication in HIV/AIDS patients undergoing NVP-containing HAART (Anthony, 2001), and approximately found in 6-30% of treated Subjects. In fact, 2-10% will need to interrupt HAART due to severe hepatic injury and marked elevation of liver enzymes (Rodriguez-Rosado et al., 1998; Spengle et al., 2002). The high prevalence of HIV infection in Nigeria (4.6%) and Kogi State in particular (5.1%) (FMOH, 2004), ordinarily calls for comparison of existing immunological markers used as methods for providing prognostic information in HIV-infected patients on therapy. Besides, the occurrence of HAART related hepatotoxicity among HIV-infected patients in Lokoja, has not been well documented, despite increased access to antiretroviral drugs. It therefore appears that there is dearth of information in this regard. This present study therefore seeks to investigate the effect of HAART on $CD_4^+$ cell count and liver enzymes (Alanine amino-transaminase – ALAT and Aspartate amino-transaminase – ASAT) levels in HIV-infected patients within six months of treatment based on their age and sex distribution. It is hoped that the outcome of this study will provide valid data that will both support and reinforce the continuous use of HAART as a safe and effective treatment option for HIV/AIDS and also give useful information about possible drug failure in patients after commencement of therapy.

## MATERIALS AND METHODS

### Study Area

This prospective cohort study was carried out at Lokoja, the state capital of Kogi state, Nigeria. It has a population of about 3, 087, 044 as at 2004 (UNDP, 2004).The main ethnic groups are Igala, Ebira and Okun. While agriculture is a major part of the economy, the state also has coal, steel and other miner industries.

### Study Design

A cohort of 200 (66 males, 134 females) HIV positive patients (confirmed by Western blot method), aged between 16 and 65 years were randomly recruited and prospectively studied for the effect of HAART on their $CD_4^+$ cell counts and liver enzymes levels post-therapy. Sampling was done by recruiting every $2^{nd}$ newly diagnosed HIV positive consenting individuals that visited the clinic. Recruitment was done over a seven-week period and only those who met the eligibility criteria were enrolled into the study. The purpose and procedures of the study were explained to the participants. 200 blood samples were collected at baseline of the study (pre-HAART) while 182 blood samples were collected at the end of six months of therapy (Post-HAART) as 18 of the 200 patients could not continue with the follow up leaving only 182 patients to complete the study.

### Informed Consent

Informed consent was obtained from each patient and all participants were requested to voluntarily sign the consent forms in their own handwriting as proof of willingness to provide samples for the tests.

### Eligibility Criteria

Only patients, either male or female age between 16–65 years who were HIV-seropositive, with no history of HAART (HAART naïve), had $CD_4^+$ cell count <350 cells/μL and baseline liver enzymes (ASAT or ALAT) levels normal before the treatment were enrolled for the study.

### Data Collection

Information was obtained from the participants through administration of questionnaires. Interpreter was provided for translation in local dialect where necessary. The first part of the questionnaires contained the biodata of the patients e.g. name, sex, age etc. Second part includes history of HIV Infection and HAART.

The study groups were stratified by sex and age. For reasons of privacy, all data were kept confidential in accordance with World Medical Association declaration of Helsinki (WMA, 2008).

**HAART Regimen**

After the baseline assessment, the patients were then placed on first line therapy in accordance with the WHO Guidelines on the Treatment of HIV/AIDS for adults and adolescents, adopted for the national ART program by their clinicians (Kaplan et al., 2003; WHO, 2005, NGHA, 2010). The HAART regimen used in this study consists of: Zidovudine (400–600 mg/d, divided, i.e., administered bid) + Lamivudine (150 mg bid) + Nevirapine (200 mg once daily for 2 weeks, then increase to 200 mg bid) combination. The HAART regimen lasted for six months.

**Follow Up**

The follow-up of the cohort was done monthly and at 6 months. The prescribed HAART were renewed during each monthly visit. During each visit, patients were attended to by their clinicians, trained nurses, adherence counselors, and pharmacists. Adherence Counseling, pill counting and recording of treatment adherence data alongside other clinical parameters were carried out to ensure adherence to HAART medications. At 6 months post-HAART, the patients were reassessed for $CD_4^+$ cell count and liver enzymes levels. Those who failed to return for their scheduled appointment were contacted on cell phone or via e-mail.

**Collection of Specimen**

Blood specimen was collected twice: first at pre-HAART (baseline) and second, at post-HAART (after six months of therapy) from the same patients who have an evidence of adherence to the prescribed HAART. On each occasion, the arm of each patient was tied with a tourniquet to make the veins prominent. The median antecubital vein was then selected and the area was disinfected with 70% alcohol. The vacutainer needle was introduced into the vein and the first half of the blood collected was transferred into a clean EDTA- vacutainer tube (thoroughly mixed) ready for evaluation of $CD_4^+$ cell count, while the second portion was transferred into plain bottle and allowed to clot and subsequently centrifuged at 2000 resolution per minutes (rpm) for 5 minutes to obtain serum component for evaluation of serum level of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT).The serum was stored in a cryovial at $-80^0$C until they were needed. Specimens were properly labeled for each participant with a serialized code number that could not be linked to individuals except for the researchers.

**Preparation of Specimen for $CD_4^+$ Cell Count**

To a 20 µL of CD4 MAb (monoclonal antibody) already pipette into a durhan tube, 20 µL of well mixed EDTA blood collected within 6 hours was added, properly mixed by gentle tapping and incubated in the dark for 15 minutes after which 800 µL of CD4 buffer was added and mixed thoroughly to avoid bubbles.

**Laboratory Evaluation $CD_4^+$ Cell Count**

$CD_4^+$ cell count was estimated using Partec® Cyflow Counter (Germany), as described by PCC (2006). The Cyflow Counter was operated as instructed in the user's operational manual.

**Definition of Immunological Reconstitution (IR)**

We defined immunological reconstitution as a >25% increase in $CD_4^+$ cell count from baseline to 6 months. This is mid-way of 20-30% purported by Smith et al., (2003) and Yeni et al., (2004).

**Liver Enzymes Evaluation**

The serum level of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were evaluated photometrically with Reflotron® Plus auto-analyzer (Boehringer, Mannheim, FRG, Roche, Germany) according to the method as described by Deneke and Rittersdorf (1984, 1985). The auto-analyzer was operated as instructed in the user's operational manual.

**Statistical Analysis**

All numerical results were collated from the 200 (pre-HAART) and 182 (post-HAART) samples collected from confirmed HIV-infected patients. Data generated in this study are presented as mean±SEM and analyzed using Statistical Packages for social Scientists (SPSS) Version 18.0 (SPSS Chicago Inc., IL, and U.S.A.). Krusta-Wallis Test (Non-parametric ANOVA) and Dunn's Multiple Comparisons Test were performed. P. values $< 0.05$ were considered to be statistically significant.

## RESULTS

The immunological outcome of 6 months HAART, shows that the younger cohort within the age range of 16-25 and 26-35 years, with >95% adherence had ≥50% increase in $CD_4^+$ cell count while the older Cohort within the age range of 36-45 and 46-65 years, with ≤95% adherence, had <50% increase in $CD_4^+$ cell count. There was increase (statistically, considered not significant, P>0.05) in the post-HAART mean±SEM $CD_4^+$ Cell Count and liver enzymes (ALAT and ASAT) levels in all the age range investigated: 16-25 years (564±47Cell/µL, 12.37±1.7U/L and 11.57±2.6U/L), 26-35 years (471±20Cell/µL, 9.72±0.5U/L and 8.94±0.6U/L), 36-45 years (398±20Cell/µL, 9.01±0.8U/L and 10.9±1.18U/L) and 46-65 years (325±96Cell/µL, 11.8±2.0U/L and 12.1±2.9U/L), respectively, when comparison was made with their pre-HAART mean±SEM $CD_4^+$ Cell Count and liver enzymes (ALT and AST) levels: 16-25 years (281±33Cell/µL, 10.42±1.2U/L and 9.07±1.2U/L), 26-35 years (212±11Cell/µL, 9.11±0.4U/L and 8.15±0.5U/L), 36-45 years (206±13Cell/µL, 7.78±0.6U/L and 8.55±0.7U/L) and 46-65 years (169±43Cell/µL, 6.76±0.6U/L and 8.09±1.2U/L), respectively, (Table 1). Also, the post-HAART mean±SEM $CD_4^+$ cell count of the female cohort (480±20Cell/µL) was slightly higher than that of the male cohort (430±24 Cell/µL), although this difference was not statistically significant (P>0.05). The female cohort with >95% adherence, achieved >50% increase in $CD_4^+$ cell count, while their male counterparts with 95% adherence, achieved 50% increase in $CD_4^+$ cell count post-HAART. However, there was no significant difference (P>0.05) between the female (10.28±0.61U/L and 10.15±0.80U/L) and male (10.95±0.87U/L and 9.64±0.93U/L) post-HAART liver enzymes (ALT and AST, respectively) levels (Table 2).

## DISCUSSION

The relationship that exists between HAART and the immunological reconstitution of HIV/AIDS individuals is well documented (Lederman et al., 1998; Gea-Banacloche and Clifford, 1999). Several cohort studies and clinical trials have shown that the $CD_4^+$ cell count is the strongest predictor of subsequent disease progression (CDC, 1992) and also, a reliable marker for treatment outcome (Miceli and Parnes, 1993). The present study evaluated the pre-HAART (baseline) and post- HAART (follow-up) $CD_4^+$ cell counts and liver enzymes (ALAT and ASAT) levels of HIV-infected patients who were placed on HAART over a period of six months. It was observed that the patients responded to HAART as evident by the increase in the post-HAART $CD_4^+$ cell counts (although considered statistically, 'Not Significant', P>0.05). The result of this present study further underscores the immune-reconstituting capacity of HAART and is in agreement with other previous works by Miller et al., (1999), Staszewski et al., (1999), Lederman (2001) and Palella et al., (2003). The outcome of all these studies shows that the immune system of HIV/AIDS patients can actually be reconstituted after HAART. Furthermore, the hypothesis that age and sex differences exist in immunological responses to HAART is plausible (Johnson and Kuritzikes, 1997; Smith et al., 2004). It was noted in this study, that the post-HAART $CD_4^+$ cell counts and immunological reconstitution of younger cohort within the age range of 16-25 and 26-35 years appear to be higher, than that of the older cohort within the age range of 36-45 and 46-65 year, although these differences were not statistically significant (P>0.05). This seems to agree with a retrospective case-control study by Skiest et al., (1996) who compared the $CD_4^+$ cell counts of an older cohort (aged >55 years) with a matched younger cohort (aged <45 years). They found out that the older cohort had lower $CD_4^+$ cell counts than the younger cohort (205 versus 429 cells/$mm^3$). Another study by Tumbarello et al., (2003), also compared the $CD_4^+$ cell counts of a group of HIV infected older patients (aged >50 years) with a group of younger patients (aged 20–35 years) placed on HAART. The older cohort had a slightly lower mean $CD_4^+$ cell counts than the younger cohort (108 versus 187 cells/$mm^3$). The outcome of all these works weakly underscores the link between age and immune recovery, as no significant differences were observed across age strata on short term monitoring of HAART. Considering treatment outcome based on sex distribution, the post-HAART $CD_4^+$ cell counts and immunological reconstitution of the female cohort appears to be higher (statistically, considered not significant, P>0.05) than the male cohort. This partly agrees with the work of Patterson et al., (2005), who purported that women may achieve virological suppression at a faster rate and have a better immune response than men on starting therapy most likely due to levels of endogenous female hormones, or other

sex-linked factors. Sex differences in CD4 cell and clinical responses to HIV infection pre-HAART initiation (Prins et al., 1999; CASCADE Collaboration, 2003) and post-HAART initiation have been reported in some previous studies (Moore et al., 2002; Giordano et al., 2003a, 2003b; Mocroft et al., 2000), but not all (Mocroft et al., 2003; Moore et al., 2003). Interestingly, the work of Patterson et al., (2007), show that immunological responses to HAART among previously antiretroviral-naïve patients appear to be similar across sex and age strata in the initial 6 months. However, longer term monitoring of HAART outcomes by age and sex is indicated, when biological and behavioral factors may become more apparent. Although, there is no agreement on the definition of better immune response, but in general, an average rate of immune reconstitution of up to 20-30% of the $CD_4^+$ T-cell number from baseline value in the first 6 months of HAART has been considered positive and it anchors on adherence (Smith et al., 2003; Yeni et al., 2004). In this current study, it was observed that the younger and female cohorts had >95% adherence, while the older and male cohorts had ≤95% adherence. This may in part, explains why the younger and female cohorts achieved a $CD_4^+$ cell count increase of ≥50 cells/µL (IR ≥50%) on one hand, howbeit, the older and male cohorts achieved a $CD_4^+$ cell count increase of ≤50 cells/µL (IR ≤50%) on the other hand. The outcome of this study further strengthens the claim that, "for HAART to be effective, high rate of adherence (≥95%) is essential" (Reiter et al., 2000). Regarding the impact of antiretroviral therapy on the liver; on completion of HAART in this present study, liver enzymes (ALAT and ASAT) levels were evaluated and they appear to be slightly elevated above the baseline (although still within the normal range, 0-47U/L). Comparison between the pre-HAART and post-HAART ALAT and ASAT levels shows that the increase was not statistically significant (P>0.05) in all the age strata investigated and the mean difference between the male and female cohorts was not significant as well (P>0.05). Hence, hepatotoxicity as initially anticipated; was absent. Therefore, the use of HAART in the treatment and management of HIV infection can be considered both safe and non-hepatotoxic, irrespective of age or gender. The outcome of this study does not however agree with the findings made by Rodriguez-Rosado et al., (1998), Carton et al., (1999) and Spengle et al., (2002), who reported significant elevated liver enzymes levels in their works following long-term exposure to HAART. On this note, we must point out that our study had two limitations. First, if patients who experienced toxicity were lost to follow-up, the study might have underestimated toxicity. Second, laboratory data (liver function test) were only measured up to 6 months and not beyond. Thus, we were unable to assess the long-term effects of HAART initiated. Therefore, the cumulative length of treatment with HAART, which are known to be toxic to the liver and the need for withdrawal or dosage adjustment of HAART based on plasma level in all phases of HIV/AIDS therapy, must be considered in order to optimize it use. This is a continuous challenge for the present and further generations of hepatologists (Spengler et al., 2002).

## CONCLUSION

The use of HAART proved to be both effective and reliable in the treatment of HIV-infected patients of different age and sex strata without risk of liver damage.

### Competing Interests

The authors declare no competing interest.

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**Table 1:** The Pre-HAART and Post-HAART Age Distribution of $CD_4^+$ Cell Counts and Liver Enzymes Levels in HIV Patients

| AGE (Yr) | N1 = 200 | N2 = 182 | AD (%) | IR (%) | $CD_4^+$ CELL COUNT (No. of cells/µL) 1 | $CD_4^+$ CELL COUNT (No. of cells/µL) 2 | ALAT LEVEL (UL) 1 | ALAT LEVEL (UL) 2 | ASAT LEVEL (UL) 1 | ASAT LEVEL (UL) 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 16-25 | 31 | 30 | 98 | 50 | 281±33 | 564±47$^{A,B}$ | 10.42±1.2 | 12.37±1.7$^{A,B}$ | 9.07±1.2 | 11.57±2.6$^{A,B}$ |
| 26-35 | 115 | 107 | 97 | 55 | 212±11 | 471±20$^{A,B}$ | 9.11±0.4 | 9.72±0.5$^{A,B}$ | 8.15±0.5 | 8.94±0.6$^{A,B}$ |
| 36-45 | 43 | 38 | 95 | 48 | 206±13 | 398±20$^{A,B}$ | 7.78±0.6 | 9.01±0.8$^{A,B}$ | 8.55±0.7 | 10.9±1.1$^{A,B}$ |
| 46-65 | 11 | 7 | 93 | 48 | 169±43 | 325±96$^{A,B}$ | 6.76±0.6 | 11.8±2.0$^{A,B}$ | 8.09±1.2 | 12.1±2.9$^{A,B}$ |

All values are expressed in Mean ±SEM**,** P<0.05 is considered to be significant.

**Key:** 1 = Pre-HAART (Before HAART, i.e Baseline), 2 = Post-HAART (After Six months of HAART, follow-up), 1 = Pre-HAART Number of Sample Size, N2 = Post-HAART Number of Sample Size, AD = Adherence, IR = Immunological reconstitution, ALAT = Alanine Amino-Transminase, ASAT = Aspartate Amino-Transminase, SEM = Standard Error of Mean, A = Not Significant (P>0.05) comparison between Pre-HAART and Post-HAART values of all the age ranges, B = Not Significant (P>0.05) comparison of post-HAART values within age ranges

**Table 2:** Pre-HAART and Post-HAART Sex Distribution of $CD_4^+$ Cell Counts and Liver Enzymes Levels in HIV Patients

| GENDER | N1= 200 | N2= 182 | AD (%) | IR (%) | $CD_4^+$ CELL COUNT (CELLS/µL) 1 | $CD_4^+$ CELL COUNT (CELLS/µL) 2 | ALAT LEVEL (UL) 1 | ALAT LEVEL (UL) 2 | ASAT LEVEL (UL) 1 | ASAT LEVEL (UL) 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| Male | 66 | 61 | 95 | 50 | 215±16 | 430±24$^{A,B}$ | 9.23±0.70 | 10.95±0.87$^{A,B}$ | 9.05±0.78 | 9.64±0.93$^{A,B}$ |
| Female | 134 | 121 | 98 | 54 | 221±11 | 480±20$^{A}$ | 8.73±0.39 | 10.28±0.61$^{A}$ | 8.05±0.45 | 10.15±0.80$^{A}$ |

All values are expressed in Mean ±SEM**,** P<0.05 is considered to be significant.

**Key:** 1 = Pre-HAART (Before HAART, i.e Baseline), 2 = Post-HAART (After Six months of HAART, follow-up), N1 = Pre-HAART Number of Sample Size, N2 = Post-HAART Number of Sample Size, AD = Adherence, IR = Immunological reconstitution, ALAT = Alanine Amino-Transminase, ASAT = Aspartate Amino-Transminase, SEM = Standard Error of Mean, A = Not Significant (P>0.05), comparison between Pre-HAART and Post-HAART values in both sexes, Not Significant (P>0.05), comparison between male and female post-HAART values

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