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THERAPEUTIC DRUG MONITORING STUDY ON COFORMULATED 600-MG EFAVIRENZ, 300-MG TENOFOVIR DISOPROXIL FUMARATE AND 300-MG LAMIVUDINE AMONG HIV-POSITIVE PATIENTS WITH IMMUNE SUPPRESSION

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ABSTRACT

Background: Combination of three antiviral drugs are used to treat adult and adolescent HIV infection. These antiretroviral drugs stop an enzyme (reverse transcriptase) that HIV uses to replicate itself. By doing this, they stop the infection from spreading. Objectives: the study is aimed at evaluating therapeutic drug monitoring on co-formulated 600-mg efavirenz, 300-mg tenofovir disoproxil fumarate and 300-mg lamivudine among HIV-positive patients. Methods: A Retrospective longitudinal study was carried out from October, 2014 to November, 2015 at Madonna Catholic Referral Hospital, Umuahia, Abia State, Nigeria. All volunteer antiretroviral therapy (ART)-naïve HIV-infected individual's age above 18 years who were initiating ART were enrolled consecutively and followed for six months. Socio-demographic data such as gender, age, and occupational status, were recorded using a structured and pre tested questionnaire. The samples were run using BD FACSCount analyzer (Becton Dickinson, USA). Results were expressed as number of cells/µl. Reference range for CD4+ were taken as 600-1200cells/µl. CD4 T cells and CD8 T cell counts were enumerated using FACS (Fluorescent Antibody Cell Sorter, Becton Dickinson). At baseline, 74 (49.0%) HIV-naïve cohorts had CD4 Count of <200 cells/µl, 67 (44.4%) had CD4 count of > 200 cells/µl and 10 (6.6%) had CD4 Count of >500 cells/µl. After six months of their highly active antiretroviral therapy (HAART), 78 (51.7%) of HIV/AIDS patients on HAART had CD4 count of >500 cells/µl followed by 73 (48.3%) of HIV/AIDS patients on HAART had CD4 count of > 200 cells/µl (t = -22.794; p = 0.000). Findings showed that the cohorts on HAART responded positively with increased in their CD4 count after 6 months of their antiretroviral drugs. The study also supports the evidence by World Health Organization guideline that recommend a Fixed–Dose Combination ART containing (non)-nucleoside reverse transcriptase inhibitors (TDF), Lamivudine (3TC), and efavirenz (EFV) as first-line HIV treatment.

KEYWORDS: *CD4 Count, Naïve patients, HAART patients, First-line HIV treatment, FACSCount analyzer*

INTRODUCTION

Over 30 antiretroviral (ARV) drugs with eight functional classes are available in U.S today (CDC, 2022). These eight classes are nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), a fusion inhibitor, a CCR5 antagonist, a CD4 T lymphocyte (CD4) post-attachment inhibitor, and a gp120 attachment inhibitor (CDC,2022). Furthermore, two drugs, Ritonavir (RTV) and Cobicistat (COBI) are used as pharmacokinetic (PK) enhancers (or boosters) to improve the PK profiles of PIs and the INSTI elvitegravir (EVG) (CDC, 2022). Initial ARV treatment regimen for a person with HIV generally consists of two NRTIs, usually abacavir/lamivudine (ABC/3TC) or either tenofovir alafenamide/emtricitabine (TAF/FTC) or tenofovir disoproxil fumarate/emtricitabine (TDF/FTC), plus a drug from one of three drug classes: an INSTI, an NNRTI, or a boosted PI (CDC, 2022). Numerous studies suggest the strategy for initial treatments result to drastically reduction of HIV replication and CD4 count increases in most people with HIV (Lee *et al*., 2014; Eshleman *et al.*, 2022). Evidence data now support the use of the two-drug regimen dolutegravir/lamivudine (DTG/3TC) for initial treatment of some people with HIV (Cahn *et al*., 2020).

Efavirenz is known as Sustiva® and it is one of the non-nucleoside reverse transcriptase inhibitors (NNRTI) and also highly active antiretroviral therapy (HAART) used for the management of human immunodeficiency virus (HIV) type 1 (Ren *et al*., 2002; John, 2007; Robertson *et al*., 2005). It inhibits the activity of viral RNA-directed DNA polymerase such as reverse transcriptase (Michaud *et al*., 2012).

Tenofovir disoproxil fumarate is a derivative of tenofovir, a product of Gilead Sciences with the trade name *Viread*, belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs). This drug is prescribed in combination with other drugs for the management of HIV infection as well as for Hepatitis B therapy (Gilden, 2001; Miller *et al*., 2001; Uglietti *et al*., 2012; Uma *et al*., 2011). Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analog reverse transcriptase inhibitors (NtRTIs), which block reverse transcriptase, an enzyme necessary for viral production in HIV-infected individuals (Gazzard, 2001). This enables the management of HIV viral load through decreased viral replication (Thompson, 2002).

3TC has been studied in children with HIV both alone and in combination with other ARV drugs. Extensive data have demonstrated the safety of 3TC and have shown that this drug is associated with clinical improvement and virologic response. It is commonly used in children with HIV as a component of a dual nucleoside reverse transcriptase inhibitor (NRTI) backbone (Scherpbier *et al*., 2006; Green *et al*., 2007). Findings demonstrate the efficacy of NRTI background components, the combination of 3TC plus abacavir (ABC) was superior to zidovudine (ZDV) plus 3TC or ZDV in achieving long-term virologic efficacy (Green *et al*., 2007). WHO recommends that countries consider a combination of

approaches to monitor antiretroviral drug toxicity and promote patient safety. This includes surveillance of safety in pregnancy, as well as active and routine toxicity monitoring in all populations, including adults, adolescents and children (WHO, 2022).

However, ARTs often recommended for HIV/AIDS Patients in Nigeria include the combined therapy of efavirenz, tenofovir disoproxil fumarate and lamivudine. In some cases, the combined therapy of Zidovudine (AZT), Lamivudine (3TC), Nevirapine (NVP) are also used for the management of HIV/AIDS patients in Nigeria. Furthermore, this study evaluated the therapeutic drug monitoring on co-formulated 600-mg efavirenz, 300-mg tenofovir disoproxil fumarate and 300-mg lamivudine among HIV-positive patients with immune suppression

METHODS

Study Design and Area

This is a longitudinal study which was conducted from October 2014 to November, 2015 at Madonna Catholic Referral Hospital, Umuahia, Abia State, Nigeria that is one of FHI360 sponsored ART referral centers at Umuahia town, the capital of Abia state, Nigeria.

Study Population

All volunteer ART-naïve HIV-infected individuals age above 18 years who were initiating ART were enrolled consecutively and followed for six months. During enrolment for ART, socio-demographic data such as gender, age, and occupational status, were recorded by ART trained physicians using a structured and pre tested questionnaire. Participants were asked when they were first tested for HIV and the reason why they were tested.

HIV Screening

HIV screening was done to determine the HIV status of the control subjects using Determine HIV-1/2 test kit, Uni-Gold kit (Trinity Biotech) and STAT-PAK (Chembio Diagnostic System, Inc.) The test group were known HIV sero-positive subjects attending out- patient clinic at Madonna Catholic Hospital, Umuahia, Abia State, Nigeria. The control subjects were screened to determine their HIV status using immunochromatographic technique. BD FACSCount method was used for CD4+ count.

CD4+ t- Cell Count

The samples were run using BD FACSCount analyzer (Becton Dickinson, USA). Results were expressed as number of cells/µl. Reference range for CD4+ were taken as 600-1200cells/µl. At initiation of ART and after 6 months of treatment, 4 ml venous blood was collected from each participant using K3 EDTA vacutainer tube. CD4 T cells and CD8 T cell counts were enumerated using FACS (Fluorescent Antibody Cell Sorter, Becton Dickinson).

Procedure

In the laboratory, the venous blood samples collected were sent immediately and analysed using BD FACSCount analyzer. BD FACSCount CD4 reagents tube was brought to ambient temperature and vortexed upright for 10 seconds before it was opened for use. Fifty (50) µl of whole blood was added to the CD4 reagent tube containing CD3/CD4 PE monoclonal antibody (Becton Dickinson, USA). The tube was incubated in the dark for 30 minutes at room temperature and 50µl of fixative (5% formaldehyde in PBS) was added and vortexed before reading on Becton

Dickson FACS machine according to manufacturer's instruction using CD4 Absolute and CD4% count software (Beckton, 2008).

Statistical Analysis

Data were entered and analyzed using SPSS window version 26. Categorical variables were calculated using mean±standard deviation. Data also tested by paired t-tests and p- values <0.05 were statistically significant.

Ethical Considerations

Ethical approval was obtained from Madonna Catholic Referral Hospital Management Board. Informed consent was obtained from each study participant.

Quality Control

To ensure reproducibility and precision of CD4 count testing, daily quality controls were run for the BD FACSCount. The BD FACS count machine quality control pack (BD Biosciences, San Jose, CA) was analyzed by running low, medium and high bead count following manufacturer's procedures (Beckton,2008). The outcome reading of "passed control" indicated the testing process was under control such as reagents, equipment, personnel and standard operation procedures was followed before clients' sample were tested. For BD FACSPresto, daily quality control was ensured by analyzing CD Chex Plus BC low and normal control (Streck, Omaha, NE).

RESULTS

One hundred and fifty-one patients with mean age range of 38±.08 were recruited. At baseline, 74 (49.0%) HIV-naïve cohorts had CD4 Count of <200 cells/µl. 67 (44.4%) had CD4 count of > 200 cells/µl while 10 (6.6%) had CD4 Count of >500 cells/µl whereas, after 6months of their highly active antiretroviral therapy (HAART), 78 (51.7%) of HIV/AIDS patients on HAART had CD4 count of >500 cells/µl while, 73 (48.3%) of HIV/AIDS patients on HAART had CD4 count of > 200 cells/µl (t = -22.794; p = 0.000) (figure 1).

At baseline, 43 males (26.5%) and 31 females (20.5%) accounted for CD4 count <200 cells/µl, 40 males (26.5%) and 27 females (17.9%) had a CD4 count > 200 cells/µl while, 5 males (3.3%) and 5 females (3.3%) had a CD4 count >500 cells/µl (figure 2a). After being on HAART treatment, 42 males (27.8%) and 31 females (20.5%) accounted for CD4 count >500 cells/µl (figure 2b).

At baseline, CD4 counts of <200 cells/µl was found in 21(13.9%), 12 (7.9%), 16 (10.6%), 6(4.0%) and 19 (12.6%) of Artisans, Businessmen, Civil servants, Students, and Traders respectively. CD4 counts of >200 cells/µl was found in 18 (11.9%), 6(4.0%), 10 (6.6%), 13 (8.6%), and 20 (13.2%) of Artisans, Businessmen, Civil servants, Students, and Traders respectively (figure 3a).

CD4 count of >500 cells/µl was found in 3 (1.9%), 1 (0.6%), 2 (1.3%), 3 (1.9%) and 1 (0.6%) of Artisans, Businessmen, Civil servants, Students, and Traders respectively. CD4 count of >500 cells/µl was found in 22(14.6%), 7 (4.6%), 12(7.9%), 16 (10.6%) and 21 (13.9%) of Artisans, Businessmen, Civil servants, Students, and Traders respectively (figure 3b).

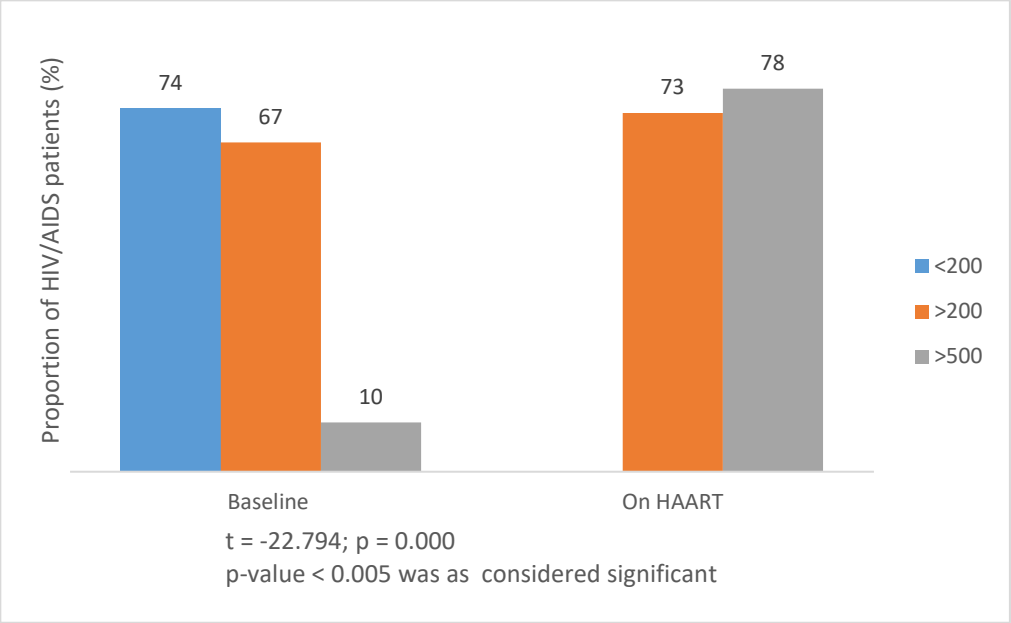

Fig. 1: CD4 count on baseline and 1st quarter of HAART

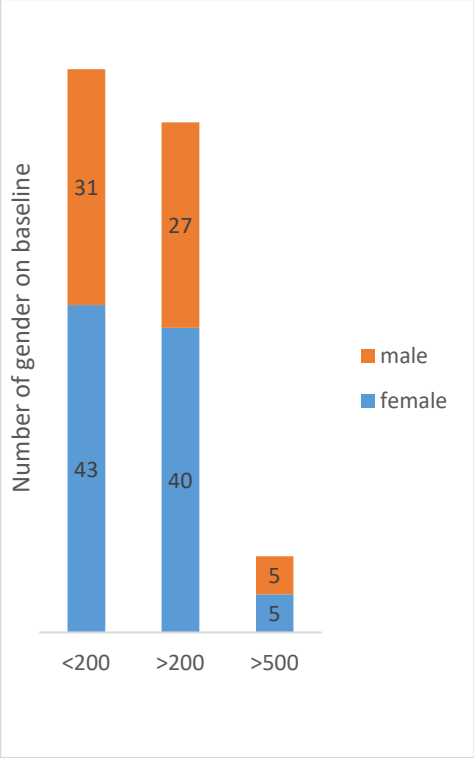

Fig. 2a: CD4 Count on Naive HIV/AIDS

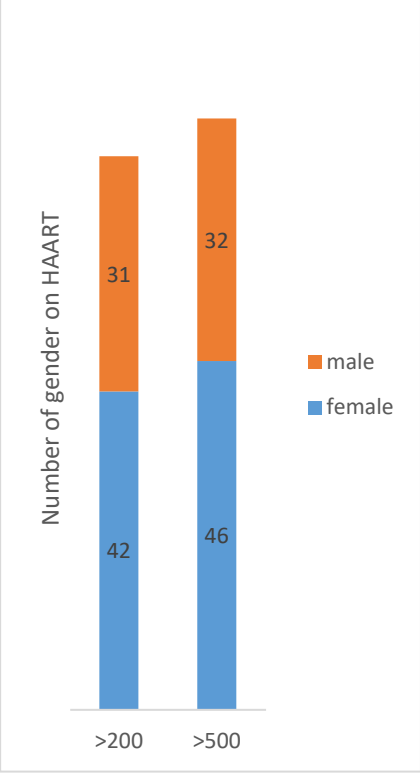

Fig. 2b: CD4 Count on HAART HIV/AIDS

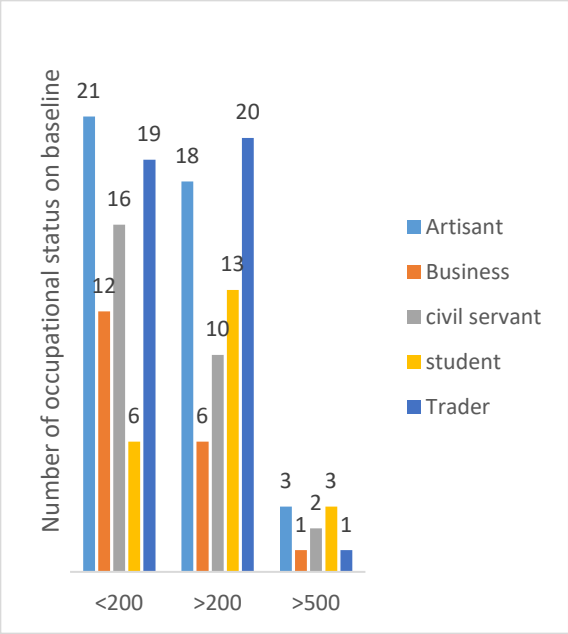

Fig. 3a: CD4 Count on Naive HIV/AIDS

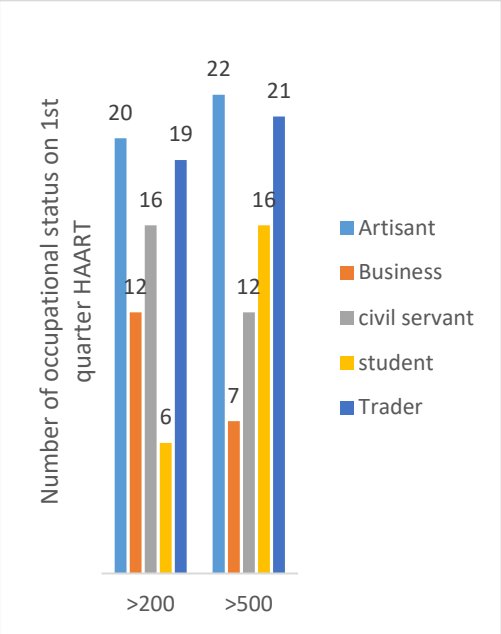

Fig. 3b: CD4 Count on HAART HIV/AIDS

DISCUSSION

In this study, all the HAART cohorts with mean age range of 38±.08 responded positively to their medications. The majority of the participants were in their active young age and the increased in their CD4 count were age dependent. The literature's assertion that immune function declines with age is supported by this data (Tegegne, 2021). Previous studies have shown that patients above the age of 40 had decreased rates of CD4 growth over the course of the cohort, which is linked to poor HAART adherence (Alok *et al*., 2014). Findings indicate that patient age is closely connected to retention in antiretroviral drug adherence (Tegegne, 2021). Similar studies show that age and medication retention are therefore inversely connected, although the dependent variable (HAART adherence competency) and medication retention are positively correlated (Tegegne, 2021). Age and HAART adherence, competence are significantly correlated, according to recent studies (Tegegne, 2021). So, as a patient's age increased, their ability to adhere to HAART reduced (Adams and Luguterah, 2013).

The counting of CD4+ T-cells (CD4) is a reliable indicator of the development of AIDS and a way to monitor antiviral medication (ART) (Badri *et al*., 2008). It would be possible to gain insight into how patients respond to treatment and how successful treatment is over time by understanding how CD4+ cell counts fluctuate over time (Badri *et al*., 2008).

This study clearly demonstrated the value of early treatment. A patient's current CD4+ cell count after starting ART was demonstrated to be highly influenced by their initial CD4+ cell count. An improved rate of recovery for patients receiving ART would be the outcome of a larger baseline CD4+ cell count. This supports the findings of Kulkarni *et al*. (2011) and Viviane *et al*. (2009).

Women had a higher CD4 cell response than males did among antiretroviral-naive patients who suffered virus suppression after following HAART for six months, even after controlling for baseline CD4 cell count and virus load. Women are more likely than men to experience nevirapine-related rash (Antinori *et al*., 2001), and nucleoside analogue-related pancreatitis (Bersoff-matcha *et al*., 2001).

These higher risks could be explained by gender-specific pharmacokinetic variations depending on various mean body mass indices, nutritional statuses, or other variables. Because of pharmacokinetic differences, women might have had higher antiretroviral medication concentrations, which resulted in greater levels of virus suppression and a larger rise in CD4 cell count.

This study is supported by data from numerous studies (Staszewski *et al*., 1999; Bennett *et al*., 2002) that show a higher CD4 cell response in individuals who respond to therapy more virologically. The clinic does not measure patient heights or drug levels, so data to test this hypothesis were not available. In general, regardless of whether they are HIV-positive or not, women have greater CD4 cell counts than men (Prin *et al*., 1999; Maini *et al*., 1996).

Based on the differences in CD4 cell response seen in the current study, women appear to more quickly replenish their peripheral CD4 cells after viral suppression than do males. In response to declines in viral load brought on by HAART, women might have greater peripheral redistribution of memory CD4 cells from lymphoid tissue. Another hypothesis is that women may produce more thymic production of naive CD4 cells in response to HAART than males do (Pido-lopez *et al*., 2001), which is a crucial factor in later rises in CD4 count. However, women can progress at higher CD4 cell counts than men, and it is difficult to predict who will achieve viral suppression and who will not. Only larger studies with clinical endpoints could determine whether women are safely able to withhold antiretroviral therapy until they achieve lower CD4 cell counts than men.

The high CD4 count at baseline was observed among artisan and student followed by civil servant and the least was found in businessmen and trader. Also, the high CD4 count on HAART patients was observed among artisan followed by trader and the least was found in businessmen. However, there is no association existed between CD4 count and occupational status of the participants. Furthermore, the previous studies report that the association between occupational status and HIV/AIDS patients with opportunistic infections was high in artisan when compared with other occupational status (Urama *et al*., 2018). This is a clear indication that CD4 count as low as <200 cells/µl would predispose HIV/AIDS individuals to opportunistic infections.

CONCLUSION

The result showed that those in HAART cohorts using their antiretroviral drugs responded significantly to the treatments at 6 months. The study also becomes supportive evidence as World Health Organization guidelines recommend

fixed-dose combination ART containing (non)nucleoside reverse transcriptase inhibitors (TDF), lamivudine (3TC) and efavirenz (EFV) as first-line therapy for the management of HIV/AIDS patients.

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