Effect of Simultaneous Administration of Antiretroviral Therapy and Nutritional Supplements on Cd4 Count of HIV-Infected Persons in Selected Communities of South Eastern Nigeria

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Abstract

The simultaneous effect of highly active anti-retroviral therapy (HAART) and nutritional supplements on cluster of differentiation4 (CD4)count of 100 people living with HIV/AIDS (PLWHA) was investigated. CD4 cell count was determined using the Cyflow SL-3. Individuals with baseline CD_4 counts less than 200 cells/µl were placed on HAART for a period of 8 weeks at 4 weekly intervals. Total white blood cell (TWBC), haemoglobin (Hb)/ packed cell volume (PVC) were estimated using Sysmex KX-21N automated machine; Body mass index (BMI) calculated using World Health Organization's BMI Reference Standards. Dietary intake and feeding history were recorded, followed by a 24 hourly dietary recall with Recommended Dietary Intake [RDA] of ELERON nutritional supplement. Eighty-one percent of treated subjects had increased CD4 count (P<0.05): TWBC had 24% increase; mean value (P<0.05). Haemoglobin and PCV showed significant increase (P < 0.05) at 5% or 10% levels. Concomitant effect of HAART and nutritional supplements on the CD4 count, Hb/PCV, BMI were significant at 5% or 10% levels. Mean BMI value was highly significant (20.23 kg/m2) compared with 16.40 kg/m^2 (P=0.004). Immunological reconstitution of patients was observed following improved nutritional supplementation with HAART as effective adjunctive therapy/or clinical outcome-enhancer for PLWHA in rural and resource poor communities.

Keywords: Antiretroviral therapy, CD4 Count, Nutritional Supplement, HIV/AIDS

Introduction

Antiretroviral therapy (ART) reconstitutes the immune system and improves survival of the people living with HIV/AIDS. Access to antiretroviral therapy (ART) is currently expanding in low and middle-income countries, especially the sub-Saharan Africa, where it has become a cost-effective public health intervention (Valdez *et al.,* 2001). Plasma viral load (VL) monitoring, the gold standard used in advanced countries for diagnosing virological failure, is not available in many under-developed and developing countries due to the constraint of infrastructure and high expense. WHO however, has recommended an alternative; the use ofCD4cellcount measurements and clinical outcomes for monitoring ART in the absence of viral load monitoring (WHO, 2006). CD_4, a measure of CD_4 cells in the blood, serves as a direct monitor of the progression of HIV as well as the response of the immune system to treatment with antiretroviral drugs (ARD) (Brooks 2007; Willey *et al*, 2008; Biron, 2010).

Advanced HIV disease (AIDS), compromises the gastrointestinal tract (GIT) with severe infestation by several opportunistic parasitic pathogens (Cheesbrough, 2006), leading to imbalance in the micro and macro nutrients; with increased HIV progression to AIDS. Nutritional deficiencies chiefly vitamins and micronutrients (VMD) decrease epithelial integrity, weaken antibody responses and facilitate changes in the body's normal flora (Willey *et al.*, 2008; Thurnham, 2010). Nutritional supplements in forms of haematinins (multivite, fesolate and folate), coupled with balanced diet, fruits, vegetables and others, which contain the necessary micro and macro nutrients improve the nutritional status and hasten immune reconstitution of HIV infected persons. The nutritional status of the patients can be assessed using anthropometric methods such as body mass index (BMI) calculated from a person's weight and height as weight/$height^2$. BMI , which correlates with direct measure of body fat such as underwater weighing and dual energy x-ray absorptiometry (DXA), is a fairly reliable indicator of body fatness for most people (FAO/WHO, 2006). The study evaluated the concomitant effect of antiretroviral therapy (art) and nutritional supplements on CD_4 count of people living with HIV/AIDS (PLWHA) in selected rural and resource limited communities of Nigeria, with the view to establish the immunologic, metabolic, and clinical effect or outcome in HIV-infected patients taking highly active antiretroviral therapy (HAART). The micronutrient supplement may hold promise as an adjuvant therapy in the management of PLWHA.

Materials and Methods

Study population: The study population consisted of 280 individuals referred for HIV screening and counseling by physicians at the Enugu-Ezike District Hospital, a HIV Referral Centre designated for HIV voluntary testing and counseling (VTC) and for antiretroviral therapy and counseling for people living with HIV/AID in the area. Participants were selected on the basis of their presenting clinical symptoms, including wasting syndrome, oral thrush, dermatological conditions. Out of the total number screened, one hundred (100) were HIV positive and these constituted the study population (picked on

the basis of their HIV seropositive status). These were then subjected to regular visits and monitoring at their different homes from onset of study. All the subjects, 60.8% males, and 39.2% females, (median age, 38 years [range, 18-60 years]) who were naive to antiretroviral therapy, commenced the antiretroviral cum nutritional supplement treatment during the study period with intensive follow-up visits. Demographic characteristics of subjects including age, gender, occupation and education were obtained from questionnaire administration. The benefits, confidentiality and voluntary participation features of the study were explained and written informed consent obtained from the subjects followed by approval by the ethical committee of the various participating clinics and hospitals as well as the Research Ethics Committee of the University of Nigeria, according to the Declaration of Helsinki (World Medical Association and Council for International Organizations of Medical Sciences (CIOMS), prior to commencement of study. Eligibility criteria were restricted to subjects between the age ranges of 19 to 60 years.

HIV screening: A 5-ml sample of blood was collected from each patient by venipuncture into a Vacutainer EDTA bottle and screened for HIV-1 antibodies using the *Genie HIV*-1/2 *kit* (Sanofi Diagnostics Pasteur, Montreal, Quebec), a synthetic-peptide solid-phase enzyme immunoassay according to Manufacturer's instructions. The lower limit of detection of this assay was 400 copies per milliliter.

CD4 cell count: HIV positive samples were assayed for CD4 by the Cyflow technique using the Cyflow counters (Partec, Germany), for both counting and analyses of particles and cells. Blood samples were stained with fluorescent dye, and allowed to stand for about 5 mins for the dye molecules to be imbibed by the cells (this was illuminated at defined wavelength). The color intensity for each labeled cell was measured by a ploidy analyzer; the intensity of emitted light was proportional to its CD4. The concentration or volume of fluorescent cell was measured at 0.2 ml by the volume detector, while the ploidy analyzer determined the number of cells per ml. CD4 cell counts were monitored at 6 month intervals. Overall treatment efficacy was defined as an increase of the CD4 cell count by at least 50% of the baseline value, while AIDS was defined as clinical stage C of the 1993 classification system (CDC, 1993).

Baseline characteristics: "Baseline" CD4 cell count was date of HAART initiation and the levels were nearest the HAART initiation within 6 months before HAART. BMI was calculated using height and weight values within 30 days to 1 year before HAART initiation. No deaths were observed during the study period; only 5 dropouts were recorded before the commencement of the study, and these were among the 180 HIV seronegative cases that were not considered eligible for the study, hence, were excluded.

Antiretroviral therapy: Highly active antiretroviral therapy (HAART), combination therapy was administered to subjects following the World Health Organization (WHO) guideline, as follows:
Zidovudine (AZT): 300 mg twice daily;
Lamivudine (3TC): 150 mg twice daily;
evirapine (NVP): 200 mg twice daily for first 14 days;
Efavirenz (EFV): 600 mg once daily (at bedtime);
Stavudine (D4T): 40 mg every 12 hours for adults of > 60kg and 30mg every 12 hours for adults < 60kg;
Didanosine (DdI): 400 mg once daily for adults > 60kg and 250mg daily for adults < 60kg; Tenofovir (TDF): 300 mg once a day.
Lopinavir (LPV): This was variable: adult dosing was dependent on formulation or treatment experience.

Time was measured from the date of starting treatment to the date of the last follow-up visit (June 2, 2009 to June 30, 2010). Patients were maintained in the original regimen for the period of study. Exclusion criterion included pregnant women as a result of difference in their HAART regimen requiring that women not already taking treatment, but requiring HAART, should defer commencement until second trimester after the period of organogenesis and when symptoms of morning sickness are likely to have settled. Gestational period was not determined among the pregnant women, hence their illegibility for the study. Mega-HAART, involving 3 or more antiretroviral drugs (for treatment failure) was avoided.

Haematological tests: An automated Coulter counter T540 machine **(**Beckman/Coulter T540, USA**),** standardized against a 4C plus blood control was used for haematological parameter estimation. The machine automatically diluted 29.6 µl whole blood samples, lysed, counted and printed out the result of absolute numbers of White Blood Cells, Red Blood Cells and lymphocyte. Potassium, Magnesium and Calcium, Urea, Creatinine and Albumin or Transferrin were however not measured.

Investigation of anaemia in HIV/AIDS: As HIV infection has an effect on red blood cells, decreasing their lifespan and preventing their maturation and replacement by the bone marrow thereby engendering severe anaemia, haematologic indicators of HIV-associated anemia namely, iron deficiency or low haemoglobin content measured as HB and/or packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) indicative of severity of infection were therefore investigated. Nutritional imbalance, another cause of HIV-associated anaemia was similarly investigated. Other causes of anaemia such as opportunistic infections, vitamin B_{12} deficiency, autoimmune destruction of red blood cells as well as other causes of low or elevated white cell counts were however not determined.

Selection for administration of nutritional supplement: Measurement of anaemia: The selection for nutritional supplement administration was, in addition to HIV status (seropositivity) based

on evidence of malnutrition and associated anaemia, assessed by haemoglobin content or PCV, poor appetite, as well as physical examination for indicators of malnutrition such as the following:

1. Weight loss (wasting syndrome) compared with body mass index
2. Neurologic symptoms, including weakness/fatigue, gait abnormalities pain and depression
3. Mouth appearances such as angular stomatitis, papillar atrophy, cheilosis and other breakdown in oral mucosa
4. Skin texture and appearance including overt dryness, pealing, pallor, hyper or hypopigmentation

Assessment of nutritional imbalance or malnutrition: Nutritional imbalance or malnutrition and related poor dietary intake from deficiency, food intolerance or aversion were assessed from dietary history during oral interviews. Poor diet was reported as:
1. Poor food choices, intolerance and allergies
2. Skipping meals
3. High sugar intake and high intake of refined foods, high alcohol consumption
4. Low fruit and vegetable intake

Anthropometric measurements for establishing relationship between body weight (weight loss) and HIV infection: Another index of nutritional status namely, anthropometric measurements of height and weight: Body Mass Index (BMI), an indirect measurement of body composition, was used to assess level of weight loss (indicator of poor nutritional status, and typified by a low BMI) common in HIV infection. The values obtained from the BMI were compared to the World Health Organization's Reference Standard for healthy individuals as follows:
- Less than 18.5 underweight for individual height;
- 18.5 to 24.9, an ideal weight for individual height;
- 25 to 29.9, over the ideal weight for individual height;
- 30 and above, obesity (WHO, 2000).

Body weights and heights were measured using a WEYLUX scale and a Stadiometer while observing standard precautions (RNIS, 2000). The BMI was calculated as: weight (kg) / [height (m)]^2

Administration of nutritional supplements: Micronutrients: Micronutrients were provided in forms of fruits and vegetables. The diet plan or menu of the study population was fortified or supplemented with a cup of egg plant leaf (*Solanum manocarpum*) water or Ugwu, pumpkin (*Telfairia occidentale*) water three times daily in addition to filling half their plates with half cooked vegetable soup made from a variety of local vegetables including spinach, *Vernonia amygdalina* (onugbu or bitter leaf), ugwu, waterleaf, tomatoes, red pepper and any other African vegetable available at each period. Patients were in addition encouraged to have a variety of local fruits such as oranges, guava, avocado pear, soursop pawpaw, mango, (*Mangifera indica*), watermelon, and other seasonal fruits as much as possible during the day, and especially after meals as a compulsory part of their diet plan. Those in very bad condition (AIDS patients) with little or no appetite, were put on soft fruits such as avocado, banana, papaya or boiled vegetables such as carrot, or pumpkin. Compliance with additional intake of vegetables and fruits was achieved by follow up visits and regular counseling on the immense benefit of strict adherence to their diet plan for their survival.

Administration of nutritional supplements: Nutritional supplements in form of ELERON Capsule [Iron (III) Hydroxide polymaltose Complex] containing elemental iron (100 mg), 550 mcg of folic acid and B-complex vitamin (Recommended Dietary Intake [RDI] of 2 tablets daily) were administered to individuals with evidence of malnutrition and associated anaemia, indicated by both low BMI, HB and/or PCV below normal ranges.Clinical symptoms of anaemia were established prior to medication from both the full blood count including the haemoglobin (HB) tests and the packed cell volume (PCV) from haematocrit measurement according to the WHO's threshold for the definition of anaemia (WHO, 2005). Other clinical indicators or markers used included shortness of breath(respiratory symptom), heart palpitation and arrhythmia (cardiac symptom), unsteady gait and postural stability, especially in the dark.

ELERON administration was based on WHO'S Hemoglobin thresholds for the definition of anaemia (1 g/dL = 0.6206 mmol/L), in accordance with the daily recommended dose approved by National Agency for the Control of AIDS, Nigeria (NACA). Selection for the medication was as indicated earlier, on the basis of seropositivity and other measures of anaemic condition. This regimen was fortified with increased intake of vegetables and fruits at each meal as indicated above. Routine medications commonly used in Nigeria for PLWHA including Vitamin E, selenium and zinc were also administered on daily basis, though the effect of these micronutrients which were considered minimal was not monitored.

Efficacy of drug administration and nutritional supplementation was determined by comparison of the initial (baseline) and final CD4 mean values, indicators of immune reconstitution or otherwise hence no control group was employed for the efficacy assessment. Overall compliance with medication was evaluated subjectively by oral interviews by investigators and medical personnel in attendance, and questions were on treatment and adherence. Problems associated with medication and compliance were resolved during home visitations and follow-up exercises.

Statistical analysis: All analyses were carried out on an intent-to-treat with basis. Continuous and categorical variables were analyzed using the Student *t*-test, Chi-square, one or two ways analysis of variance and regression models where appropriate. Available data were therefore grouped into dependable and undependable variables before application of the statistical tools. Significant

differences level were put at 5% and not 10% as indicated. Repeated measures models were used to examine the concomitant impact of the treatment (HAART and nutritional supplementation) **(**Steel and Torrie, 1960). The primary end point of the study was to examine the effect of antiretroviral therapy and nutritional supplementation on immunologic parameters (CD4 cell count).

Results and Discussion

Result of a total of 100 subjects assigned to a combination HAART and nutritional regimen following baseline HIV and CD4 count are shown in Table 1. Most subjects showed evidence of a CD4+ T cell increase after HAART initiation. Significant increase in the mean CD4 cell count of patients was observed following drug combination therapy compared with baseline (P<0.05).

Table 1: Baseline and final CD4 count with mean difference following HAART administration

Drug	Mean Before	Mean After	Mean Difference	% Mean Difference	Total	p-value
AZT, 3TC, NVP	118.4286	183.2857	64.85714	54.76476	4.164	0.001
D4T, 3TC,NVP	142.5000	230.2500	87.75000	61.57895	3.483	0.040
ddI, TDF, LPV	123.7000	201.9000	78.20000	63.21746	3.503	0.007
D4T, 3TC, NVP	93.1667	164.5000	71.33333	76.56526	2.235	0.076
D4T, 3TC,EFV	146.6667	242.8333	96.16667	65.56817	4.246	0.008
D4T, AZT, NVP	87.1667	180.5000	93.33333	107.0745	3.585	0.016
AZT, 3TC, EFV	125.5000	225.0000	99.50000	79.28287	2.774	0.069

AZT = Zidovudine; 3TC = Lamivudine; NVP = Nevirapine; EFV = Efavirenz; d4T = Stavudine; ddI = Didanosine; LPV = Lopinavir ; TDF = Tenofovir. The results were significant at either 5% level.

The D4T, 3TC, EFV combination was the highest in effectiveness, and markedly increased the CD4 count of the subjects compared to the baseline (242.83cells/µl cf 146.67 cells/µl; P = 0.008). Next was the D4T, 3TC, NVP, with effective activity of 230.25 cells/µl cf 142.50 cells/µl (P = 0.04); AZT, 3TC, EFV and ddI, TDF, LPV, had activity of 225.00 cells/µl cf 125.50 cells/µl, (P = 0.007), and 201.90 cells/µl cf 123.70 cells/µl (P = 0.007), respectively. The combination of AZT, 3TC, NVP resulted in mean increase of CD4 count of the subjects (183.29 cells/µl, cf 118.43 cells/µl, P = 0.001) compared with the baseline, while D4T, AZT, NVP had (180.50 cells/µl cf 87.17 cells/µl, P = 0.016). The D4T, 3TC, NVP combination had mean increase of 164.50 cells/µl cf 93.17 cells/µl (P = 0.076) (Fig. 1).

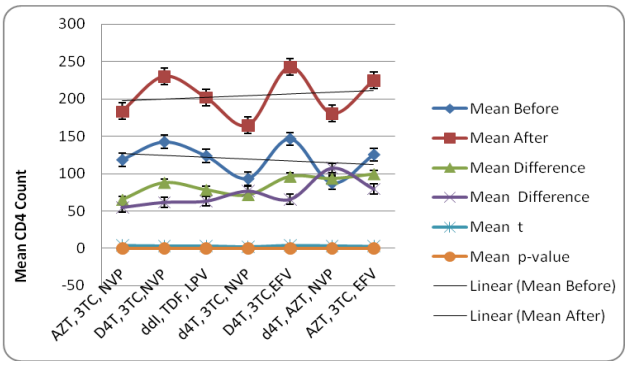

Fig. 2: Administration Profile: Effect of different HAART drug combination on the CD4 count of the subjects

AZT = Zidovudine; 3TC = Lamivudine; NVP = Nevirapine; EFV = Efavirenz; d4T = Stavudine ; ddI = Didanosine; LPV = Lopinavir ; TDF = Tenofovir.; cf = compared with. The results were significant at either 5% or 10% level.

Effect of HAART on TWBC: On the effect of combination of different HAART on TWBC (Fig. 2), available results indicated that ddI, TDF, LPV administration had significant (P<0.05) increase on patients' TWBC with per cent increase of 24%. Furthermore, d4T, 3TC, NVP showed 21% increased on the TWBC at 5% significant level; D4T, 3TC, NVP had minimal increase on the TWBC (5%). However, some drug combinations such as AZT, 3TC, NVP; D4T, 3TC, EFV; d4T, AZT, NVP and AZT, 3TC, EFV had no effect on subjects' TWBC levels (P> 0.01); per cent mean differences were 0.00%, 13%, 14% and 5%, respectively.

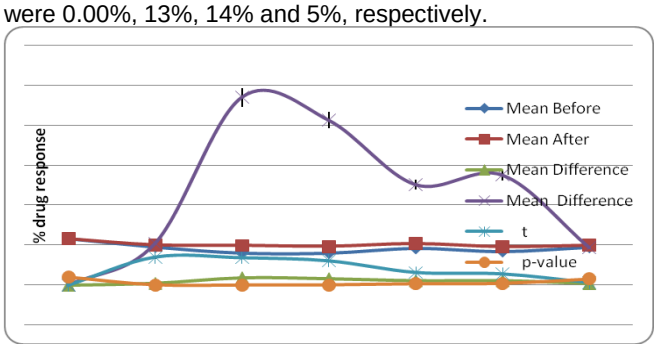

Fig. 2: Effect of combination of different HAART on TWBC

AZT = Zidovudine; 3TC = Lamivudine; NVP = Nevirapine; EFV = Efavirenz; d4T = Stavudine ; ddI = Didanosine; LPV = Lopinavir ; TDF = Tenofovir.; cf = compared with. The results were significant at either 5% or 10% level.

Effect of different HAART combination on haemoglobin levels: The activity of several HAART combinations on the haemoglobin level of subjects was significant (P< 0.05) (Fig. 3). However, no activity was observed in the administration of D4T, 3TC, EFV on the Hb of the subjects (P>0.05); per cent mean difference was 5%.

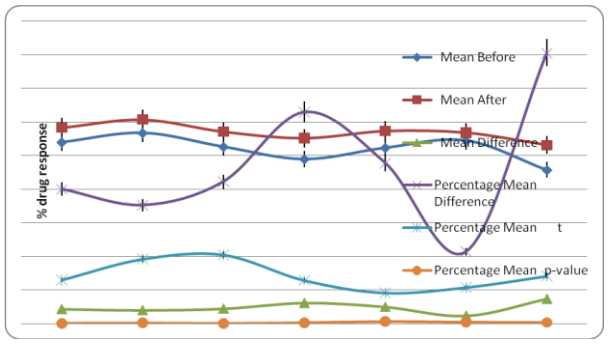

Fig. 3: Effect of combination of different HAART on Haemoglobin levels

AZT = Zidovudine; 3TC = Lamivudine; NVP = Nevirapine; EFV = Efavirenz; d4T = Stavudine ; ddI = Didanosine; LPV = Lopinavir ; TDF = Tenofovir.; cf = compared with. The results were significant at either 5% or 10% level.

Effect on PCV: The Effect of various combinations of used HAART on subjects PCV was variable: while AZT, 3TC, NVP; D4T, 3TC, NVP, DDI, TDF, LPV; D4T, 3TC, NVP and AZT, 3TC, EFV combinations exerted significant increase on the PCV levels (P<0.05); per cent mean differences, 7%, 6%, 7%, 10% and 12%, respectively; no observable effect was shown by D4T, 3TC, EFV and D4T, AZT, NVP combinations (Fig. 4), (P>0.05).

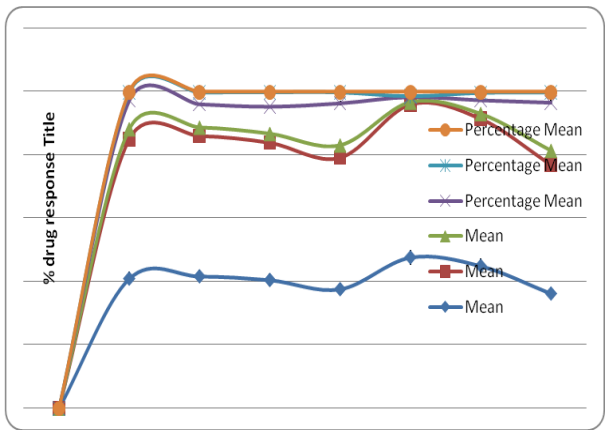

Fig. 4: Effect of different ART drug combinations on PCV

AZT = Zidovudine; 3TC = Lamivudine; NVP = Nevirapine; EFV = Efavirenz; d4T = Stavudine ; ddI = Didanosine; LPV = Lopinavir ; TDF = Tenofovir.; cf = compared with. The results were significant at either 5% or 10% level.

Effect of nutritional supplements: The efficacy of nutritional supplements on investigated parameters: CD4 count, Hb, as well as the BMI of subjects, was remarkable (Table 2). Concomitant administration of the various combinations of HAART and nutritional supplements (including increased intake of fruits and vegetables) had significant difference in the CD4 count, at 5% level of significance; the mean Hb was similarly at 5% level of significance.

Table 2: Concomitant effect HAART and nutritional supplements on the CD4 count, Hb and BMI of the Subjects

Parameter	Mean HAART	Mean HAART+	Mean Difference	T	p-value
CD4 Count	198.6600	300.7857	102.1257	3.072	0.003
Hb	11.4360	10.6500	0.78600	1.769	0.082
BMI	17.55kg/m^2	20.23kg/m^2	2.68kg/m^2	1.636	0.065

ART = Antiretroviral therapy; CD4 = Cluster of differentiation; Hb = Haemoglobin; BMI = Body Mass Index. The results above were significant at 5% level.

Observed mean BMI value of HIV patients on antiretroviral medication and nutritional supplements was higher than those who were neither taking antiretroviral drugs nor nutritional supplements (20.23kg/m2 cf 16.40kg/m^2, P = 0.004). Negative correlation (- 0.17096) was however established between age and final CD4 count (0.004332 cf 0.704519) following HAART administration and nutritional supplementation. Data on nutritional assessment of the HIV positive subjects, giving details of their BMI and weight evaluation at baseline, expressed as either an underweight, ideal weight, overweight or obese, based on their BMI (index of their nutritional status) indicated that most of patients (72%) were underweight; 14% had ideal weight, 11% were overweight while 3% were obese (Fig. 2). Significant weight loss was therefore typified by low BMI in the absence of HAART and nutritional supplements. However, observed mean BMI value of HIV patients on antiretroviral medication and nutritional supplements (ELERON Capsule supplemented with improved food intake of vegetables and fruits) was higher than those who were neither taking antiretroviral drugs nor nutritional supplements (20.23kg/m^2 cf 16.40kg/m^2, P = 0.004). Negative correlation (- 0.17096) was nevertheless found between age and final CD4 count (0.004332 cf 0.704519) following HAART administration and nutritional supplementation.

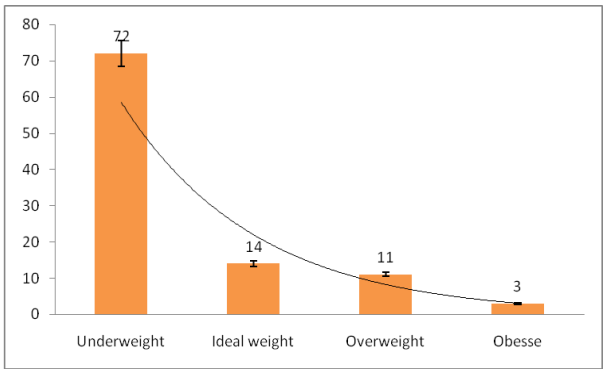

Fig. 5: BMI and weight evaluation of HIV positive subjects

UW =Underweight; IW = Ideal weight
OW = Overweight; O = Overweight

The present study aimed at investigating the concomitant effects of antiretroviral therapy cum nutritional supplementation on CD4 cell count of immunocompromised, but treatment naïve individuals. Significant changes in average CD4 percent and in the rate of change of CD4 were observed among 81% of subjects following HAART administration. Our results confirm the beneficial effects of HAART in increasing CD4 levels, and are in consonance with several evidence-based reports on the efficacy of HAART in CD4 increase and subsequent viral load reduction. Increase in CD4 cell count reflects reconstitution of the immune system, and this delays development of opportunistic infections (WHO, 2006). An abrupt decline in CD4 after acute HIV infection followed by a rise, and subsequent return to normal range (close to pre-infection baseline) after recovery from acute infection: a biphasic increase in CD4; 50-120 in first 3 months, was attributable to redistribution of memory CD4 cells from lymphoid tissue, followed by average increase of 2-7 cells/m^3 through an expansion of naive CD4 cell population; viral enzyme functions were reported to be inhibited, their number reduced, with resultant increase in CD4 cell as compared to initial number (baseline) before the administration of ART (Joel and Hoffmann, 2011).

In a study in a Swiss cohort reduced disease progression and mortality with HAART was reported; an average increase of more than 100 CD4 cells/m^3 were observed in patients who received triple or quadruple therapy continuously and who were also the most immune suppressed prior to HAART (Egger *et al., 1997*).The present study similarly observed the efficacy of HAART, a combination therapy with different classes of antiretroviral drugs; this phenomenon has been attributed to its unique mechanism of action, which is mostly by blocking the action of enzymes that are important in replication and functioning of HIV.

Observations from the present study therefore, establishes the efficacy of HAART in immune system restoration and consequently, boosting of CD4 cells. This view is supported by the reports indicating that by targeting multiple steps in HIV replication, HAART successfully turns HIV infection into a chronic treatable disease unlike mono-therapy which results in the rapid emergence of drug resistant mutants of HIV (Brooks *et al*., 2007). Results of this study are also in consonance with several HAART therapeutic trials which have provided strong evidence that highly active antiretroviral combination therapy is effective at halting immunologic and clinical progression of HIV; achieving a reduction in viral loads, an increase in CD4 cell counts.

Result of the effectiveness of HAART combinations on CD4 count was further expressed in their mean percentage values. By evaluation, some drugs exerted higher activity on CD4 cells by achieving more increase in mean values of the CD4 count compared to the baseline. Available result underscores the fact that CD4 expressed as a mean percentage is a better prognostic marker than the absolute CD4 counts, since it is not affected by changes in total and differential leucocyte counts (not estimated in the study) as compared to the absolute counts in dual-platform technology as earlier reported (Amatya, 2004). The use of CD4 cell count in monitoring of rate of immunosuppression as well as indicator of ART effectiveness is herein established. The observed increase in mean CD4 count is an indication of HAART efficacy, and establishes a direct relationship between HAART administration and subsequent increase in mean CD4 cell. This view is supported by previous studies indicating that CD4, a measure of CD4 cells in the blood, serves as a direct monitor of the response of the immune system to treatment with antiretroviral drugs (ARD) as well as the progression of HIV (Brooks *et al., 2007;* Willey *et al.,* 2008; Biron, 2010). Though the cut-off values for CD 4 counts reported in the present study were not deduced from ROC curves (ROC curve Acronym for receiver operating characteristic curve; a graphical method of assessing the characteristic of a diagnostic test) previously determined for the estimation of CD4 (DeLong *et al.,* 1988). A large individual variability in CD4 measurement, a reflection of the method of determining CD4 count by calculation from 3 measured variables: WBC, % lymphocytes, and % lymphocytes that are CD4+ (CD4%) was reported (Joel and Hoffmann*, 2011*).

Result of this study observed low CD4 cell in spite of HAART regimen among some subjects. This could be related to several factors including individual immunologic or genetic factors, lack of adherence to drugs, decreased activity or failure of the thymus which functions in the maturation of CD4 cells to lack of compliance which results in drug resistance. Similar resistance paradigm in their AIDS adherence training report in which adherence, which is the response (dose and time of intake) of an individual to drugs, was considered the most important factor in the success of ART was reported; resistance was hence attributed to lack of adherence to drug regimen and high rate of viral replication. For ART to be effective, there should be greater than 95% adherence (WHO, 2006).

The observed trend in theTWBC values in the present study is remarkable. Some ARD combinations, ddI, TDF, LPV; d4T, 3TC, NVP and D4T, 3TC, NVP markedly increased the TWBC values ($P< 0.05$) while others did not. The reported positive response (significant increase in TWBC) following the combination therapy was demonstrated in an earlier study, which attributed the apparent increase to the subjects' innate immune responses, disease conditions and/or effects of nutritional supplements or both (Raxit, 1995;Chandra, 1999). On the other hand, the observed non response to ARD correlates with the report which showed that TWBC could be used to investigate infections and monitor treatments and that many infections can lead to disorders of the white blood cells which can be an increase in leucocytes numbers (leucocytosis) in response to parasitic, bacterial or viral infections or absolute decrease in leucocyte numbers (leucopenia) caused by infections especially HIV (Cheesbrough, 2006).The argument was that since TWBC is the main component of the immune system, any

alteration in its values will therefore adversely affect the patient's immune responses. Such incidence of low response was also alluded to cytotoxic drugs, drug reaction, folate and vitamin B_{12} deficiencies (Delves *et al.,* 2006; UNAIDS/WHO/UNICEF, 2008),

The complications of HIV infections are due to the interplay of malnutrition, mal-absorption, metabolic alteration and nutrients depletion associated with the HIV infection. These nutrients are many including Zn, Se, Cu, Mn, sulphated amino acids (Thios) (Rice *et al.*, 1995). Development of HIV-related opportunistic infections and malignancies which lead to decreases in CD4 count also results in reduction in food intake resulting from reduced appetite or taste disturbances or painful mucosal lesion of the mouth, pharynx or oesophagus (Smith *et al.,* 1992). The efficacy of concomitant administration of HAART and nutritional supplement in increasing CD4 cell count and consequently reducing rate of HIV progression to active disease, AIDS, has been emphasized in this study: CD4 cell count, TWBC, HB, PCV and BMI of the people living with HIV/AIDS were significantly increased compared to the baseline values, thus buttressing the importance of nutritional supplement in the management of HIV/AIDS infections. This opinion is in agreement with the FAO/WHO recommendation of micronutrients for PLWHA which is 10 – 20% above RDA (FAO/WHO, 2010; Thurnham, 2010). In a similar report, a correlation between nutrient supplementation and CD4 increase was further established; showing that low serum B_{12} is a predictor of disease progression, and those with low serum concentrations of retinol, B_{12} and zinc had significant decreases in CD4 count while those that developed normal serum levels of these micronutrients had an appreciable increase in CD4 counts (Thurnham, 2010). Studies have similarly shown that PLWHA are associated with low serum concentrations of Vitamin A, B_6 , B_{12}, C, E, folate, carotenoids as well as Se, Zn, and Mg (Lacy *et al.,* 1996). The Na/K ratios and other biomarkers of inflammation for example, anaemia of chronic inflammation (ACI) with Hb values in the range of 9.3–9.5g/l where haemoglobin and red blood synthesis is markedly depressed can inhibit iron absorption and re-utilization (Chandra, 1999). Available results further corroborates with the reports on the micronutrient supplementation and subsequent increase of CD4 count in HIV-infected individuals on HAART (Semba, 1997).

The present study succinctly establishes the fact that increasing dietary supplementation of nutrients in form of ELERON Capsule (Iron (III) hydroxide polymaltose Complex) containing elemental iron 100mg and 550mcg of folic acid and B-Complex vitamin (RDI of 2 tablets daily) in conjunction with recommended doses of HAART markedly improved the health conditions of the HIV+ patients by increasing the CD4 counts compared with the baseline thereby delaying the progression of HIV to AIDS. This view finds credence in the randomized controlled trial (RCT) of HIV infected persons was reported in which a daily supplement of 800 IU vitamin E and 1000 mg of vitamin C given over three months period greatly reduced oxidative stress and was associated with appreciable increases in CD4 counts compared to the placebo (Allard *et al.,* 1997). Failure of response to HAART and nutritional supplement by some subjects was however observed from this study. The nutrient-nutrient and nutrient-drug interactions is patient's specific and could account for the minimal or non-response of some subjects to ART drug administration as observed in the present study, for example the abnormal Na/K and Ca/P ratios of subjects can affect the absorption and re-utilization of these minerals due to imbalance in the body homeostasis (Dimtry *et al.,* 2010).

Furthermore, HAART treatment failure has been described as an emerging global challenge, especially in developing countries where HIV infection is still endemic and the use of HAART is being scaled up (Hawkins and Murphy, 2009). Nevertheless, results of the present study confirm the beneficial effects of combination therapy and nutritional supplementation despite the minimal compliance and adherence rates, which could further be attributed to either non-compliance to drugs, immune composition of the patient involved or the genetic makeup of the individual. The preferential effect of the concomitant administration of HAART and nutritional supplement in immunological reconstitution of HIV infection, is apparent from the study, which further reports that provision of nutritional supplements can be an easy and cost effective adjunctive therapy to boost immune status of HIV infected persons, decrease the side effects of HIV drug regimen and enhance clinical outcomes among the PLWHA in rural communities facing the difficult ties of poor living standards.

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