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# Effect of Multiple Micronutrient Supplementation on CD4 T Cell levels of Clinically Stable HIV Patients on Highly Active Antiretroviral Therapy; A Randomized Controlled Crossover Trial

**Anthony C. Iwu[1], Chukwuma B. Duru[2,*], Kenechi A. Uwakwe[2], Christopher N. Obionu[3], Kevin C. Diwe[2], Abah O. Steve[4], Prosper O. Adogu[5], Irene A. Merenu[2], Nathan C. Nwaimo[6], Uche R. Oluoha[1], Ikechi Ohanle[1], Emmanuel U. Ndukwu[1]**

[1]Department of Community Medicine, Imo State University Teaching Hospital, Orlu Imo State, Nigeria
[2]Department of Community Medicine, Imo State University, Owerri, Imo State, Nigeria
[3]College of Medicine, University of Nigeria Nsukka, EnuguState, Nigeria
[4]College of Medicine, Ambrose Ali University Ekpoma, Edo Nigeria
[5]Department of Community Medicine,NnamdiAzikiwe University, Anambra State, Nigeria
[6]Department of Chemical Pathology, Imo State University Teaching Hospital, Orlu Imo State, Nigeria
*Corresponding author: duruchukwuma16@yahoo.com, iwuchinedu@yahoo.com

**Abstract** **Background:** Human Immunodeficiency Virus (HIV) infection produces a chronic and potentially fatal disease of the immune system. In the pre HAART (Highly Active Antiretroviral Therapy) era, micronutrient supplementation was associated with improvement in the immune status of HIV infected individuals but in the post HAART era, this association has been unclear with conflicting results. **Objective:** To determine the effect of multiple micronutrient supplementation on the CD4 T cell levels of clinically stable HIV patients on HAART for at least one year. **Methods:** A randomized cross over intervention trial was used to determine the CD4 T cell effect of a 12 week daily consumption of multiple micronutrient supplement on 50 clinically stable HIV infected participants receiving treatment from the Imo State University Teaching Hospital. The participants were purposively selected based on certain inclusion criteria and were randomized into two groups to receive the supplement at different periods after a washout period. CD4 T cell measurements were taken at baseline, 12, 20 and 32 weeks. **Results:** The mean age of the participants studied was 43.8±10.8 years with an average duration on HAART of 3.2±1.5 years. At baseline, 55.3% of the participants were severely immunodeficient, 51.1% were either overweight or obese and 45% were hypertensive. The intervention results, revealed that there was no statistically significant difference in CD4 T cell count levels with micronutrient supplementation compared to no supplementation [t=0.78, p=0.438]. **Conclusion:** Micronutrient supplementation in the post HAART era especially in patients on long term HAART appear not to have any significant effect on the CD4 T cell counts.

***Keywords:*** *micronutrient supplementation, CD4 T cells, HIV patients, HAART*

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## 1. Introduction

The Human Immunodeficiency Virus (HIV) Infection produces a chronic and potentially fatal disease of the immune system targeting a specific type of white blood cell known as T-lymphocyte which is part of the infection and cancer fighting system of the body and is measured in the blood as the CD4 T cell count. [1]

The CD4 T cell count is a strong predictor of HIV disease progression and survival which is closely linked to HIV morbidity and mortality. [2] In resource poor settings, it is the main indicator used in establishing the stage of HIV disease, its progression, prognosis, treatment guide and response to therapy due to the high cost of viral load testing. [3]

Before the introduction of Highly Active Antiretroviral Therapy (HAART), HIV infection resulted in wasting, metabolic disorders, nutrient deficiencies with poor nutritional states and low CD4 T Cell counts. [4] In a randomized trial in Tanzania, micronutrient supplementation in ART naïve HIV patientswas associated with improved clinical outcomes and a significant increase in CD4 T cell counts. [5]

In 2003, World Health Organization's (WHO) reporton nutrient requirement in HIV/AIDS, noted that dietary

intake of micronutrients at recommended daily allowance (RDA) levels may not be sufficient to correct nutritional deficiencies in HIV infected individuals and therefore advocated for micro nutrient supplementation.[6]

However, with the introduction of HAART, micronutrient supplementation as routine management of HIV/AIDS is still emphasized for the improvement of immune health especially in resource poor regions even though HAART alone has been associated with the halting of the devastation and rapid progression of the disease. Following this, some post HAART era studies were done on micronutrient supplementation which revealed conflicting outcomes with pre HAART era studies with respect to their effects on CD4 T cell levels. [7-13]

Nevertheless it is important to consider micronutrient supplementation effect especially in developing countries in the context of HAART where vitamins and mineral deficiencies are high withthe associated impact of HIV disease on nutrition and vice versa.

Consequently, there is a need to explore the effect of micronutrient supplementation in relation to CD4 T cell levels within the post HAART era in order to determine if the role of micronutrient supplementation is still significant in improving CD4 T cell levels in patients who have been on HAART for a considerable length of time.

# 2. Methodology

## 2.1. Study Area

The study was conducted at the adult HIV clinic of Imo State University Teaching Hospital situated in Orlu Local Government Area (LGA) of Imo State, South East, Nigeria. Imo State is made up oftwenty seven Local Government Areas and the major tribe is Igbo. The State lies within latitudes 4°45'N and 7°15'N, and longitude 6°50'E and 7°25'E and covers an area of about 5,100sq km. The study area is predominantly rural with a population density varying from 230-1400 persons per sq. km.

The study centre is a Teaching Hospital with an accredited centre for Antiretroviral Treatment (ART) which was activated in 2008. The project is operated in collaboration with the Imo State Government and the Centre for Clinical Care and Research, Nigeria (CCCRN). The HIV clinic has a total enrolment of 4769 patients out of whom 3565 are on ART with 3330 patients above 18 years old. It offers comprehensive outpatient HIV care services to about 900 patients monthly, comprising those residing within and outside the State.

## 2.2. Study Population

The study population comprised adult HIV infected patients accessing HAART from the HIV clinic of Imo State University Teaching Hospital, who were either on the first or second line drug regimen consisting of Zidovudine, Lamuvidine, Emtricitabine, Tenofovir, Abacavir Nevirapine, Efavirenz, Atazanavir and Lopinavir/Ritonavir.

## 2.3. Study Design

The study design was a randomized single blinded controlled AB/BA crossover interventional trial. The study participants were randomized into two groups AB and BA. The AB received micronutrient supplement and BA received no supplement in the first intervention period (0-12 weeks),following a washout period of 8 weeks, the groups were crossed over with the BA group now receiving micronutrient supplement and AB receiving no supplement in the second intervention period (20-32 weeks). The intervention was a multiple micronutrient supplement which is commercially available as Immunance tablets given once daily for 12 weeks.

## 2.4. Study Intervention Product

The study intervention product was a multiple micronutrient supplement that is commercially available as Immunace™ which was developed by Meyer/Vitabiotics of Meyer healthcare PVT Ltd to contribute to the normal function of the immune system. It provided a comprehensive range of vitamins and minerals in the form of tablets which was consumed daily for 12 weeks along with food and ART. The Immunace tablets were gluten free and do not contain any drugs, hormones, yeast, lactose or any preservatives and it is produced in accordance with Good Manufacturing Practice(GMP) standards of quality control. Drug constituents as shown in Figure 1.

| Nutritional Information | Average per tablet |
|---|---|
| Vitamin A | 5000 IU |
| Vitamin D3 | 800 IU |
| Vitamin E | 25 mg |
| Vitamin K | 90 µg |
| Vitamin C | 150 mg |
| Vitamin B1 (Thiamine) | 10 mg |
| Vitamin B2 (Riboflavin) | 7.5mg |
| Vitamin B6 | 3 mg |
| Folic Acid | 1500 µg |
| Vitamin B12 | 15 µg |
| Iron | 10 mg |
| Magnesium | 100 mg |
| Zinc | 15 mg |
| Iodine | 150 µg |
| Copper | 1.5 mg |
| Manganese | 4 mg |
| Selenium | 200 µg |
| Chromium | 75 µg |
| L-Cystine | 33 mg |
| L-Carnitine | 30 mg |
| Calcium pentothenate | 20 mg |
| Betacarotene(NaturalCarotenoids) | 50 mg |

**Figure 1.** List of Drug Constituents

## 2.5. Sample Size Estimation and Sampling Technique

In calculating the minimum sample size, the within patient standard deviation of 10 cells/mm$^3$ each for both treatment A and B was assumed based on a published study by Picon*iet al* [14] with immunological non-responders. Therefore, the variance for treatment A and B was $10^2$ (100) each. The minimum mean detectable treatment difference target was set at 12 cells/mm$^3$ based on the study result by Wilkin *et al*.[15]

The minimum sample size was calculated using the approximate approach.

$$\mathbf{N} = \left(Z_{\alpha/2} + Z_{1-\beta}\right)^2 \sigma^2 / \left(\mu A - \mu B\right)^2$$

When N= minimum sample size, $Z_{\alpha/2=}$ Standard normal deviate corresponding to the probability of type I error, $Z_{1-\beta=}$ Standard normal deviate corresponding to the probability of making type II error,
$\boldsymbol{\sigma^2}$ = Variance of the estimated treatment means difference and μA−μB= minimum effect size. The following were the components imputed into the sample size formula above**:**

$Z_{\alpha/2} = 1.96$ $\sigma_{AA} = 100$

$Z_{1-\beta} = 1.28$ $\sigma_{BB} = 100$

$\mu A - \mu B = 12 \text{cells} / \text{mm}^3$ $\sigma^2(\sigma_{AA} + \sigma_{BB}) = 200$

$N = (1.96 + 1.28)^2 \text{ X } 200 / 12^2 N = 15.$

So a minimum sample size of 15 participants per group using the crossover study design convention allowed us predict a mean treatment difference (CD4 T cell count) of 12cells/$mm^3$ between supplementation and no supplementation with a power of 90% and a type 1 error of 0.05.

In anticipation of a possible significant carryover effect which may necessitate the setting aside of the second treatment period data in order to analyse only the first treatment period; the researchers increased the total number of participants in the study to 50 to accommodate this possibility but3 participants were lost to follow up(AB group=25and BA group=22).

The 47 participants that completed the study were selected using purposive sampling technique based on the following selection criteria.

## 2.6. Selection Criteria

The section criteria were based on the following; having CD4 T cell counts of 350 cells/ul or less, receiving HAART for at least one year, with a minimum clinic attendance of 95% and being clinically stable i.e. having no fever, diarrhoea or cough. Patients on current or previous micronutrient supplement use within the last three months, pregnant women or women intending to get pregnant and breastfeeding mothers were excluded from the study.

## 2.7. Data Collection and Analysis

All clinical measurements such as weight, height and blood pressure were done in the HIV clinic consulting rooms with the standard measurement scales and blood pressure apparatus. They were carried out by the nursing officers. The blood CD4 T Cell count were measured by flow Cytometry using the CyFlow green (Partec GmbH, Münster, Germany) which is a single-parameter, single-platform volumetric flow cytometer which provides direct CD4 T Lymphocyte counts using a single phycoerythrin conjugated-monoclonal antibody to CD4.Data was collected from case files and CD4 T cell measurements were taken at baseline, 12 weeks, 20 weeks and 32 weeks.

The data collected from the AB/BA groups were analysed with SPSS version 20 and compared using Independent sample t test with a two sided hypothesis and a p-value set at 0.05.In analysing the intervention effect on CD4 T cell count, the within subject differences in CD4 T cell count of the AB group at 12 and 32 weeks were compared with the within subject differences in CD4 T cell count of the BA group at 12 and 32 weeks. In analysing for carryover effects, the within subject sums in CD4 T cell count of the AB group at 12 and 32 weeks were compared with the within subject sums in CD4 T cell count of the BA group at 12 and 32 weeks.

## 2.8. Ethical Approval

Ethical approval was obtained from the Ethics Committee of Imo State University Teaching Hospital (IMSUTHEC) before proceeding for the study. Also written informed consents were obtained from the participants after the reason for this study was duly explained to them. All authors hereby declare that the study has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

## 2.9. Limitations to Study

This study basically assessed the influence of multiple micronutrients intake on the level of CD4 cell counts in clinically stable HIV patients on HAARTS over a long period of time. We did not asses its effect on naïve patients and patients with obvious symptoms of acute disease. Also other laboratory parameters where not assessed because this research was conducted in a poor research setting where CD4 count is used primarily to monitor clinical progress in patients on HAARTS. Based on these reasons, there should be caution in generalizing the findings of this work

# 3. Results

Fifty participants were recruited to the study but three participants dropped out from the study after randomization with the AB and BA groups having 25 and 22 participants respectively. The two groups were similar with respect to their socio-demographic characteristics. More than half of the participants were females (55.7%), married (53.2%) and were traders (57.4%) with majority of the participants (70.3%) within the ages of 30-49 years old and having either a primary or secondary school education as their highest level of educational status (89.4%). The average number of years the participants were on HAART was 3.2±1.3 years with the majority (85.1%) receiving Zidovudine, Lamivudine and Nevirapine combination therapy. There was no statistically significant difference in socio-demographic characteristics of the groups, p>0.05. (Table 1)

At baseline the two groups were similar with respect to CD4 T cell counts, Body Mass Index and Blood Pressure measurements. More than half of the participants were severely immunodeficient, (55.3%) and were either overweight or obese, (51.1%). About 44.7% of the participants had high blood pressure measurements. (Table 2).

**Table 1. Distribution of Sociodemographic Characteristics**

| | A-B Grp(n=25) | B-A Grp(n=22) | | | | |
|---|---|---|---|---|---|---|
| | **Freq** | **Freq** | **Total(%)** | **Statistic** | **df** | **p-value** |
| **Age(Yrs)** | | | | | | |
| 30-39 | 9 | 11 | 20(42.6) | t=1.96 | 45 | 0.057 |
| 40-49 | 6 | 7 | 13(27.7) | | | |
| 50-59 | 4 | 3 | 7(14.9) | | | |
| 60-69 | 6 | 1 | 7(14.9) | | | |
| X±SD | 46.6±11.5 | 40.6±9.1 | 43.8±10.8 | | | |
| **Gender** | | | | | | |
| Male | 12 | 9 | 21(44.7) | $\chi 2$ =0.24 | 1 | 0.626 |
| Female | 13 | 13 | 26(55.3) | | | |
| **Occupation** | | | | | | |
| Trading | 13 | 14 | 27(57.4) | $\chi 2$ =0.88 | 3 | 0.831* |
| Business | 7 | 4 | 11(23.4) | | | |
| Artisan | 3 | 2 | 5(10.6) | | | |
| Civil | | | | | | |
| Servant | 2 | 2 | 4(8.5) | | | |
| **MaritalStatus** | | | | | | |
| Single | 1 | 6 | 7(14.9) | $\chi 2$ =5.38 | 2 | 0.068* |
| Married | 15 | 10 | 25(53.2) | | | |
| Separated | 9 | 6 | 15(31.9) | | | |
| **Education** | | | | | | |
| Primary | 14 | 7 | 21(44.7) | $\chi 2$ =3.59 | 1 | 0.166* |
| Secondary | 8 | 13 | 21(44.7) | | | |
| Tertiary | 3 | 2 | 5(10.6) | | | |
| **Years HAART** | | | | | | |
| 1-2 | 11 | 8 | 19(40.4) | t=1.25 | 45 | 0.218 |
| 3-4 | 11 | 7 | 18(38.3) | | | |
| 5-6 | 3 | 7 | 10(21.3) | | | |
| X±SD | 3.0±1.3 | 3.5±1.7 | 3.2±1.5 | | | |

*Likelihood ratio used when >20% cells have expected values <5.

**Table 2. Distribution of baseline CD4 and Physical Parameters**

| | A-B Grp(n=25) | B-A Grp(n=22) | | | | |
|---|---|---|---|---|---|---|
| | **Freq** | **Freq** | **Total(%)** | **Statistic** | **df** | **p-value** |
| **CD4** | | | | | | |
| **cells/ul** | | | | | | |
| ≤350>200 | 11 | 10 | 21(44.7) | t=0.93 | 36 | 0.360 |
| ≤200 | 14 | 12 | 26(55.3) | | | |
| X±SD | 182.4±63.6 | 204.8±96.6 | 192.9±80.6 | | | |
| **Blood Pressure** | | | | | | |
| Normal | 14 | 12 | 26(55.3) | $\chi 2$ =0.01 | 1 | 0.920 |
| High | 11 | 10 | 21(44.7) | | | |
| **BMI kg/m$^2$** | | | | | | |
| 18.5-25.0 | 14 | 9 | 23(48.9) | t=2.95 | 45 | 0.769 |
| >25-29.9 | 7 | 10 | 17(36.2) | | | |
| >30 | 4 | 3 | 7(14.9) | | | |
| X±SD | 24.8±4.3 | 25.2±3.9 | 25.0±4.1 | | | |

At 12 weeks after supplementation of the AB group, there was no statistical significant difference in the mean CD 4 T cell count between the AB and BA groups. (p>0.05) At 32 weeks after supplementation of the BA group, there was no statistical significant difference in the mean CD 4 T cell count between the AB and BA groups.(p>0.05) Overall, the within subject differences between the 12$^{th}$ and 32$^{nd}$ week of the AB group did not differ significantly from the within subject differences of the BA group. (p>0.05)Also the within subject sums between the 12$^{th}$ and 32$^{nd}$ week of the AB group did not differ significantly from the within subject sums of the BA group. (p=0.05). (Table 3)

**Table 3. Distribution of mean CD4 T cell counts**

| | AB Grp (n=25) | BAGrp (n=22) | t | df | p-value |
|---|---|---|---|---|---|
| 12wks | 223±74 | 226±107 | -0.13 | 37 | 0.901 |
| 32wks | 230±76 | 226±99 | 0.17 | 45 | 0.869 |
| 12/32wks | | | | | |
| *Within subdiff | -7.4±35 | 0.3±32 | -0.78 | 45 | 0.438 |
| 12/32wks | | | | | |
| **Within sub sum | 453±146 | 452±204 | 0.02 | 38 | 0.987 |

*Within sub diff-The group mean of the individual participant CD4 measurement difference of 12$^{th}$ and 32$^{nd}$ week **Within sub sum- The group mean of the individual participant CD4 measurement sum of the 12$^{th}$ and 32$^{nd}$ week.

## 4. Discussion

The results suggest that multiple micronutrient supplementation had no statistically significant effect on the level of CD4 T cells in patients who have been on HAART for at least one year as observed in the within subject differences of the $12^{th}$ and $32^{nd}$ week between the AB and BA groups. This was emphasized by the fact that there was no crossover effect as observed in the insignificant differences of the within subject sums of the $12^{th}$ and $32^{nd}$ week between the AB and BA groups on the level of CD4 T cell count. This analytical approach of crossover designs was based on the publication by Welleket al[16] who considered the two randomized groups within the sample as independent, therefore analysing the intervention and crossover effects based on within subject differences and sums respectively using Independent sample t tests. Without consideration to the within subject differences of each group, it was also observed that there was no statistically significant difference between the AB and BA groups with respect to the CD4 T cell levels at $12^{th}$and $32^{nd}$ week, where only the AB group had received supplementation from baseline tothe $12^{th}$ weekand only the BA group had received supplementation from the $20^{th}$to $32^{nd}$week after the washout period.

A number of studies reported similar CD4 T cell outcome of no significant effect with micronutrient supplementation. [7,8,9,10] On the contrary, a study by Kaiser et al,[11] a randomized trial of Adult HIV patients with distal symmetrical polyneuropathy receiving HAART, reported an increase in the CD4 T cell count in the micronutrient supplementation group (p=0.01). Even though there were challenges of comparison with these studies, the Kaiser study had some similarities to an extent with respect to the patient population, micronutrient composition and duration of intervention. The design of the present study was different from that used in the Kaiser study because we were of the opinion that the crossover design as against the parallel design was more appropriate due to the challenges of the marked individual variability of CD4 T cell counts and also in identifying appropriate controls as a result of this variability. So participants serving as their own controls would provide a higher valid result of the effect of micronutrient supplementation. Furthermore the crossover design also separated the period effects from the intervention effects. The participants in this study were those with persistently low CD4 T cell count with more than half of the participants having severe immunodeficiency whose viral loads and drug resistance status were unknown though were clinically stable. As a result, substantial levels of viral loads if present, apart from their improved nutritional status, may have contributed to the no significant effects of the micronutrient supplementation observed in the present study. In addition, the level of treatment adherence in this study was based solely on clinic attendance and not on drug levels and therefore clinical drug resistance due to non-adherence generally associated with chemotherapeutic and virologic failure in HIV patients on antiretroviral therapy (ART) [17] could be a possible explanation of the observed lack of significant effect of micronutrient supplementation on CD4 cell count.

The introduction and adherence to HAART for relatively long periods seem to have had an effect on the nutritional status of the participants in the present study. It was observed that none of the study participants had a BMI of less than $18.5kg/m^2$ but there were slightly more participants especially the females with BMI greater $25kg/m^2$. This pattern is similar to what has been reported by Amorosa et al., with overweight and obesity appearing to be more common than wasting in this therapeutic era [18].

Drain et al., [19] suggested that HAART initiation may improve some nutrient deficiencies seen in HIV patients. This may also explain the 'no significant effect' of micronutrient supplements on CD4 T cell counts seen in the present study unlike the significant effects that were generally observed in the pre HAART era amongst ART naïve HIV patients.

## 5. Conclusion

The role of micronutrient supplementation had in improving immune status during the pre HAART era may be limited, as more people living with HIV disease are accessing and adhering to HAART for longer periods with associated improved nutritional states. So the management emphasis of micronutrient supplementation especially in resource limited regions should move towards proper and adequate dietary nutrition for these stable HIV patients on long term HAART.

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## Authors' Contributions

All the authors participated in the study.

## Competing Interest

The authors hereby declare that there are no competing interests.

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