

Effects of Selenium and Zinc on Weight and CD4+ T -Cell Changes of HIV-Infected Persons in Federal Capital Territory, Abuja, Nigeria

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Abstract: Human immunodeficiency virus (HIV), the primary cause of Acquired Immunodeficiency Syndrome (AIDS), is responsible for millions of deaths worldwide. As of 2022, Nigeria has a prevalence rate of 1.4%, with approximately 1.9 million individuals infected, contributing to about two-thirds of the deaths attributable to this illness in sub-Saharan Africa. Micronutrient deficiency is a common issue among individuals living with HIV, exacerbating immune suppression, negatively impacting prognosis, and accelerating the progression of the infection. Therefore, this research aimed to investigate the effects of various doses of selenium and zinc supplements on the weight and immune function of HIV-infected individuals through weight and CD4 monitoring, with the goal of providing a solution for improved management of HIV. A total of 30 subjects (25 HIV-infected individuals and 5 healthy controls) were selected and divided into six groups, with five individuals per group; groups 1 and 6 served as negative and positive controls, respectively. Different doses of selenium and zinc, or combinations thereof, were administered to the groups for 12 weeks while assessing the outcomes through weight measurements and CD4 count analysis at 28-day intervals. The generated data were analyzed using two-way ANOVA. The results demonstrated a significant difference in mean weight and CD4 counts across the different groups (p -value < 0.05). This indicates that selenium and zinc supplements are viable options for enhancing antiretroviral therapy (ART) in the treatment of HIV..

Keywords: Micronutrient supplement, CD4 count, HIV/AIDS, zinc, and selenium.

I. Introduction

Human immunodeficiency virus (HIV) infection continues to pose a significant global health challenge, with new cases reported worldwide, including in Nigeria (Awoyemi and Olusegun, 2018). HIV is the primary cause of acquired immunodeficiency syndrome (AIDS) (Awoyemi and Olusegun, 2018). As of 2022, Nigeria has a prevalence rate of 1.4%, with approximately 1.9 million individuals infected, contributing to about two-thirds of deaths attributable to HIV in sub-Saharan Africa, according to the UNAIDS report for 2021/2022. The use of antiretroviral therapy (ART) has been globally recommended for the management of HIV/AIDS. Regular monitoring of blood plasma viral load and CD4+ T-cell counts is also advised to assess the effectiveness of ART, as the primary goal of effective ART is the long-term suppression of plasma viral load. Evidence from cohort studies, observational studies, and mathematical modeling (Nxasana *et al.*, 2023) suggests that effective ART may be a promising strategy for reducing HIV transmission within populations. Significant advances have also been made in understanding the biology of the infection; however, the role of host nutrition in the pathogenesis and progression of the disease remains a major challenge in scientific knowledge (Sharon *et al.*, 2020).

Micronutrients are essential nutritional components that the body requires in minute quantities. They play a crucial role in maintaining good health; however, some micronutrients can be toxic when consumed in excessive amounts (Alebel *et al.*, 2022). Studies have demonstrated (Sashindran and Takur, 2020; De Pee and Semba, 2010; Childs *et al.*, 2019) that micronutrient deficiencies are prevalent among individuals infected with HIV, particularly among disadvantaged and undernourished patients (Kudakwashe *et al.*, 2017). Furthermore, it has been established (Felice *et al.*, 2023) that undernourishment and micronutrient deficiencies in HIV-infected individuals contribute to worsening immune suppression, accelerated HIV replication, increased oxidative stress, and depletion of CD4+ T cells. The deficiency of antioxidant micronutrients in people living with HIV (PLHIV) is often linked to heightened utilization of these nutrients due to increased oxidative stress, rather than inadequate dietary intake or malabsorption (Filteau *et al.*, 2015). The benefits of nutritional interventions are well-documented, underscoring their significant role in health improvement. It is widely recognized that evidence of the role of micronutrients in childhood infections has led to the development and implementation of preventive and therapeutic measures that reduce infectious disease morbidity and mortality among children in developing and impoverished countries (Kudakwashe *et al.*, 2017). Additionally, existing

information and data emerging from ongoing and future research could result in interventions aimed at improving micronutrient intake and status, which may contribute to reducing the magnitude and impact of the global HIV epidemic (Kabalimu *et al.*, 2018). It has been reported (Habtamu *et al.*, 2016) that malnourishment in an HIV-positive individual leads to greater susceptibility to infections and a relatively poorer prognosis. However, it is often challenging to demonstrate which specific nutritional deficiency is responsible for poor clinical outcomes (Negessie *et al.*, 2019). Essential trace elements such as zinc and selenium have been found to offer a wide range of benefits for HIV-positive patients, including increased survival, improved management of oxidative stress, reduced hospitalization, increased weight gain, enhanced birth outcomes and infant immune status, and decreased mother-to-child transmission (Sainz *et al.*, 2020).

With the spread of the HIV/AIDS pandemic in developing countries such as Nigeria, where nutritional deficiencies are prevalent, there is an urgent need to explore all potential interventions aimed at achieving zero transmission of HIV infection. Identifying and addressing micronutrient deficiencies is crucial for halting the continued spread of the virus and for improving the health, quality of life, and survival of those already infected. Therefore, this study aimed to identify effective strategies for mitigating the effects of HIV through micronutrient supplementation by examining the impact of selenium and zinc supplements on weight and CD4 cell counts in individuals living with HIV.

II. Materials and Methods

Study design: This study was conducted with volunteer persons living with HIV and are residents in the six Area Councils of the Federal Capital Territory Abuja Nigeria, after informed consent. Study analysis was carried out at the Human Virology Laboratory of National Institute for Pharmaceutical Research and Development, Idu, Abuja. Normal uninfected persons were included as positive controls. It was segregated among male and female volunteers aged 18-60 years on antiretroviral therapy. Different doses of zinc and selenium were administered for 3 months, ensuring full compliance with given guidelines among participants. Study assessment was carried out monthly through CD4 count analysis and weight measurement.

Sample size: sample size was obtained based on the likely population of observational study using human subject, this is usually from ten (10 and above) volunteer subjects. A small randomly selected study population of 30 volunteers was adopted after Luciano *et al.* (1992), who examined “Blood zinc status and zinc treatment in 11 HIV-infected persons.”

Volunteers were drawn from the participants attending the study site outpatient clinic or admitted in selected study site hospitals.

Inclusion criteria: Persons who tested HIV positive, following Nigerian HIV National Testing Algorithm and have AIDS-defining illness (except those used as positive controls) .

Exclusion criteria: Persons who tested HIV negative, following Nigerian HIV National Testing Algorithm. Persons already on multivitamins/zinc supplementation in the previous three (3) months before this study enrollment. Persons who refused their consent

Collection of blood samples: Blood samples were collected for CD4 analysis using 100 mL EDTA-coated bottles through venipuncture technique and coded appropriately using aseptic techniques.

Micronutrient administration: Volunteers in this study were grouped into six, according to micronutrient type, concentration, and dosage. Group 1 received no micronutrients throughout the study compared to Group 6, which was healthy, without HIV infection, and not on ART but received Se (50 µg) and Zn (40 mg). The major test categories: group 2, 3, 4, and 5 were on Se (25 µg) /ART, Zn (40 mg)/ART, Se (50 µg) /Zn (40 mg)/ART, and Se (25 µg) /Zn (20 mg)/ART, respectively.

Sample processing and analysis: All blood samples were processed, and CD4 count analysis carried out using a CD4 enumeration machine (ParteCyflow Sysmex, Germany) according to Njoku *et al.* (2003), while weight changes were measured using a digital floor scale.

Ethical approval: FCT Health Research Committee, FCTA Secretariat, Garki Abuja, Nigeria issued the approval for this study.

Statistical analysis: Obtained data were analyzed using arithmetic mean to a common outcome from individual variations of either weight or CD4 count changes. A two-way analysis of variance (2-way ANOVA) was used to test the significance of treatment dosage and duration of treatment.

III. Results

In this study, volunteers administered with single dose supplement demonstrated more weight and CD4 gain compare to the ones administered with combined dose. Positive control volunteers demonstrated more weight and CD4 gain compared to negative control volunteers. The results are summarized in tables and bar charts; every 4 bars represents a study group, each bar represents interval of follow up interval and the height of each bar represents weight (kg) or CD4 count

Effect of selenium and zinc on weight:. Se (25 µg) /ART had a resultant effect of 5.8kg (10%) weight gain, the highest among all groups. This was followed by the resultant effect of Zn (40 mg)/ART with 4.4kg (8%) weight gain. Se (50 µg) /Zn (40 mg)/ART resulted in 4.2kg (7%) weight gain while Se (25 µg) /Zn (20 mg)/ART resulted in 2.2kg (3%) weight gain. Positive control resulted in 3.2kg (4%) weight gain while negative control resulted in 1.6kg (3%)weight gain after 12 weeks of study. Table 1 summarizes weight changes after micronutrient administration while Figure 1 compares the effect of each micronutrient

dose within 12 weeks of study. Using 2-way ANOVA, the data showed a significant difference in the weight changes observed across different groups (Cal. F df5, 15 = 95.69 > Tab F = 2.90; p < 0.05). There was no significant difference in weight changes across different intervals of treatment (Cal Fdf3, 15 = 2.50 < Tab F = 3.29; P > 0.05).

Table 1: Percentage weight changes after micronutrients administration (Kg)

| | Weight change (Kg) | Percentage change |
|---------------------------------------|----------------------------------|----------------------------|
| Grp 1: (ART only) | 1.6 | 3% |
| Grp 2: (ART+25µg Se) | 5.8 | 10% |
| Grp3: (ART+40mg Zn) | 4.4 | 8% |
| Grp4: (ART+50µg Se/40Zn) | 4.2 | 7% |
| Grp5: (ART+25µg Se/20mg Zn) | 2.2 | 3% |
| Grp6: (50µg Se/40mg Zn/no ART) | 3.2 | 4% |
| $\alpha=0.05$, Df 5, 15 | F-tabulated=2.90,F-ratio = 95.69 | P-value = 0.00001 (<0.05). |

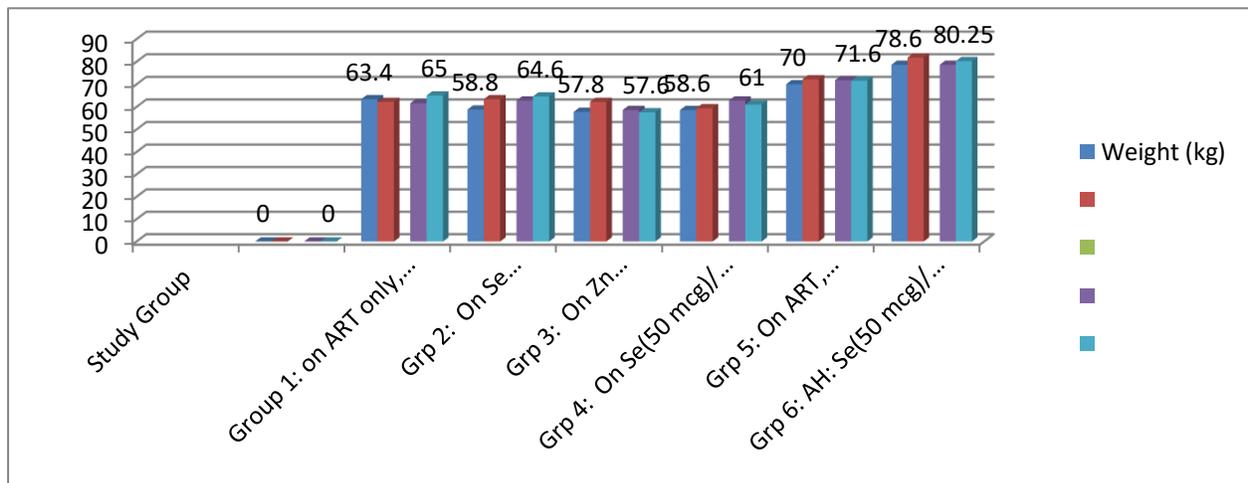


Figure 1: Effect of selenium and zinc treatment on volunteers' weight

Effect of selenium and zinc on CD4 (Cells/uL): Zn (40 mg)/ART resulted in 226 CD4 cell increase (largest number of CD4 gain) while Se (25 µg) /ART had a resultant effect of 194 CD4 cell increase. Se (50 µg) /Zn (40 mg)/ART had a resultant effect of 149 CD4 cell increase while Se (25 µg) /Zn (20 mg)/ART resulted in -114 CD4 cell decline. Positive control resulted in 36 CD4 cell increase while negative control resulted in -214 CD4 cell decline from initial CD4 count after 3 months treatment. Table 2 summarizes the percentage difference in CD4 after 12 weeks supplement administration while Figure 2 compares the effect of different doses on CD4. Using a two-way ANOVA, the data showed significant difference in CD4 cell count across different groups (Cal. F. df. 5, 15 = 27.29 > Tab. F. df. 5, 15 = 2.9; p < 0.05). There was no significant difference in CD4 cell count across different treatment intervals (Cal Fdf3,15 = 0.47 < Tab F = 3.29; p > 0.05)

Table 2. Percentage CD4 changes after micronutrients administration (Cells/uL)

| | CD4 change | Percentage change |
|---------------------------------------|----------------------------------|----------------------------|
| Grp 1: (ART only) | -214 | 39% decrease |
| Grp 2: (ART+25µg Se) | 194 | 59% increase |
| Grp3: (ART+40mg Zn) | 226 | 68% increase |
| Grp4: (ART+50µg Se/40Zn) | 149 | 55% increase |
| Grp5: (ART+25µg Se/20mg Zn) | -114 | 14% decrease |
| Grp6: (50µg Se/40mg Zn/no ART) | 36 | 4% increase |
| $\alpha=0.05$, Df 5, 15 | F-tabulated=2.90,F-ratio = 95.69 | P-value = 0.00001 (<0.05). |

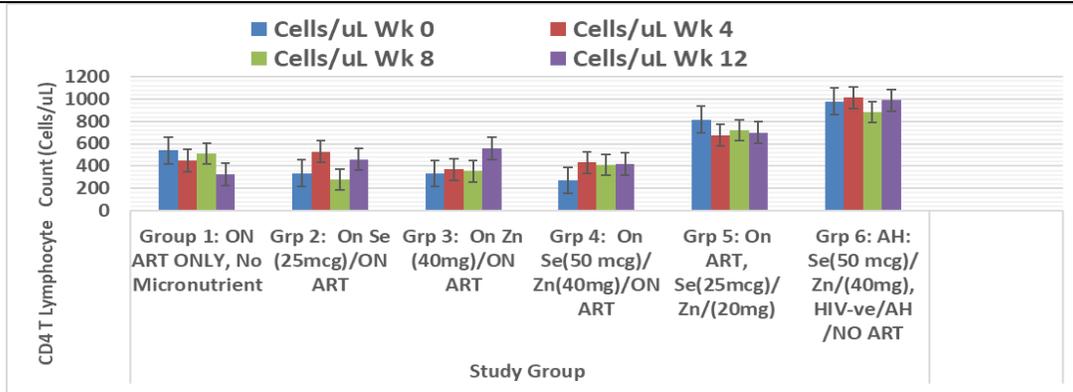


Figure 2: Impact of Selenium and Zinc Treatment on Volunteers' CD4 Count

IV. Discussion

This study tends to find more effective ways of mitigating the effect of HIV through micronutrient supplementation by investigating the impact of selenium and zinc supplements on weight and CD4 in persons living with HIV. On the basis of this, single and combined doses of selenium and zinc supplements were tested against weight and CD4 for 3 months. The results showed that selenium and zinc significantly impacted the weight across different groups, implying that different doses are responsible for higher weight gain demonstrated by volunteers in all major test groups.

The different weight changes observed across major test groups in Table 1 are indications that every dose exerted its own effect on weight, since weight differences in Table 1 represent the resultant effects of every treatment administered on each group. On the basis of effect, it could be observed that single-dose selenium was the best dose for weight improvement across the study population, followed by single-dose zinc, before co-supplements. The major weight difference observed between major test groups (groups 2, 3, 4, and 5) and the negative controls in group 1 is an indication that the introduction of zinc and selenium as supplements, irrespective of dosage, triggered a higher effect on weight compared to the effect of ART alone in the negative control. The observed weight difference between volunteers who received standard dose co-supplement in group 4 and positive controls is an indication that although the administered micronutrient could trigger weight gain, higher weight gain is triggered by a combination of ART and micronutrient supplement. Ordinarily, positive controls are supposed to gain more weight compared to group 4 since they are healthy and received the same standard dose, but the presence of ART in group 4 made the difference. Tracking the progress of different groups from Figure 1, it could be seen that the introduction of selenium and zinc triggered a weight increase seen at week 4 (indicated by the bar heights) in all major test groups compared to the negative control with weight loss at week 4, although the assessment interval was so short (4 weeks) to notice a substantial weight change in the assessment interval of the negative control and major test groups. Figure 1 also showed that more overall weight was gained by volunteers in major test groups (indicated by the difference in bar height between week 12 and 0) compared to the negative control, indicating that the addition of zinc and selenium supplements to ART triggered more weight gain compared to the lone effect of ART on weight in group 1.

Many scholars have reported different results in similar studies, although very few investigated the effect of combined doses of selenium and zinc on weight. This finding is in agreement with Boaz *et al.* (2021), who reported a significant weight gain of 2.5 kg when HIV-positive pre-puberty treatment-naïve individuals were administered 50 µg of selenium for six months. The weight gain of 2.5 kg could be compared to the weight gain of 5.8 kg in group 2 of this study. The difference in dosage and the inclusion of ART were responsible for the higher weight gain recorded in this present study. This asserts the earlier claim that “ART and micronutrient supplements trigger more effect on weight or CD4 compared to the effect of ART or trace elements alone” since Boaz *et al.* volunteers were treatment naïve.

This also agrees with Mocchegiani *et al.* (1995), who reported a significant increase in weight when 200 mg of zinc was used as a supplement for 30 days in 57 HIV-infected persons. Although it was reported by some scholars that high-dose zinc may be safe within a short period of administration, it has been known to hinder the absorption of copper and also cause other complications like vomiting. So it may improve weight as reported above but could cause other problems in the longer run, thereby discouraging its usage in longer HIV treatment.

In contrast, Osuna-Padilla *et al.* (2024) reported no significant change in weight when 30 mg of zinc and 200 µg of selenium were used as supplements on 2 groups (18 and 13 individuals) for 6 months. The dosage of trace elements or ART regimen may be responsible for the insignificant effect reported by Osun-Padilla *et al.* Also, a review done by the World Health Organization reported that 25 mg of zinc per day given to Tanzanians from pregnancy until six weeks postpartum had no effect on the fetal and neonatal mortality, duration of pregnancy, birth weight, maternal T-cell count, or viral load when compared to the control.

The results of this study also demonstrated a significant impact on CD4 count across different groups. This indicates that administered doses are responsible for CD4 count changes witnessed in all major test groups. It could be observed from Figure 1 that negative controls recorded the highest CD4 loss compared to other tested groups; this was demonstrated by the height

difference between week 12 and week 0 bars. This indicates that the addition of selenium and zinc to ART, triggered a higher CD4 count in major test groups compared to the lone effect of ART in the negative control, since bar heights represent CD4 counts. The difference in CD4 change observed in Table 2 in all major test groups (2, 3, 4, and 5) indicates that each dose exerts its own effect on CD4 count since CD4 changes in Table 2 represent the resultant effect of different doses on CD4. Also, on the basis of effects exerted on CD4, single-dose zinc was the best supplement dose across all study groups. The observed difference between the mean CD4 count of group 4 and positive controls is an indication that ART and micronutrient supplements could trigger more CD4 cells compared to lone ART or only micronutrient, since positive controls were not administered ART.

These results totally agree with Kamwesiga *et al.* (2015), who reported a significant reduction (43%) in the CD4 declining rate when 200 µg of selenium was administered daily to ART-naïve participants for 24 months. The increase in CD4 could be compared to the results of this study, but the presence of ART was the major difference in both study design and treatment effect. The lone effect of selenium in their study was only able to reduce the CD4 declining rate by 43%, while the use of ART in combination with micronutrients totally halted the decline of CD4 and initiated a progressive trend in CD4 count in this present study. This further asserts the claim that “higher CD4 count could be achieved by supplementing ART treatment with micronutrients rather than micronutrients alone.

Asdamongkol *et al.* (2013) also observed in a randomized trial that administering 15 mg of chelated zinc for 6 months increased the CD4 of ART-treated HIV patients with low baseline plasma zinc. Though the dose and study period differ, the result totally agrees with the present study, going by the significant increase in CD4.

Baum *et al.* (2010) also reported a similar result when they observed that supplementing ART-treated HIV men and women with 15 mg and 12 mg of elemental zinc for 18 months could reduce immunological failure (defined by reaching a CD4 count <200 cells/mm³) and lower the rate of diarrhea. This further establishes that different doses of zinc could impact the CD4, provided it is used to augment ART effect.

It partially agrees with Osuna-Padilla *et al.* (2024), who reported in a 6-month study that 30 mg of zinc significantly impacted CD4 of 18 HIV participants; selenium (200 µg) had no impact on CD4 of 13 HIV participants, and a combination of selenium/zinc (200 µg/30) had no impact on CD4 of 13 participants when compared to control. The difference in study design and dosage may be responsible for the disparity in results between Osuna-Padilla *et al.* and this present study.

Lodha *et al.* (2014) reported contrasting results when they observed that zinc supplementation with 20 mg of zinc for 24 months in 52 children aged 6 months and above who newly initiated HAART had no difference in CD4 of the tested group and that of the placebo. This is suggesting that supplementation at ART initiation time or very close to the ART initiation period may not be effective compared to supplementation done months after ART initiation. This may be as a result of ART composites struggling for binding space with micronutrients, thereby limiting the effect of the micronutrients.

Baum *et al.* (2013) also reported a different result, but in 220 ART-naïve HIV patients, when they observed that supplementing with 200 µg of selenium/day for 24 months had no difference in CD4 compared to placebo. This further supported the claim that the effect of micronutrient supplements is limited in the absence of ART, while ART presence complements it. The use of high single-dose selenium may also be responsible for the insignificant effect on CD4 recorded by Baum *et al.*

We also compared the effect of multivitamins like vitamins A, B complex, C, and E on weight and CD4, taking into cognizance their long- and short-term effects as well as high- and standard-dose effects when compared to the effects of selenium and zinc. Some scholars have reported that high doses of multivitamins are safe and could be helpful in patients CD4 and weight recovery in the absence of ART. Others reported that multivitamins are antagonistic in the presence of ART and struggle for binding space with ART composite. Some scholars who reported significant impact on weight and CD4 in ART treatment supplemented with multivitamins had very little study sample size and a short study period, making generalized conclusion with their result very difficult. Guwatudde *et al.* (2015) reported that the administration of a standard dose of multivitamins on HAART-treated patients for 18 months had no impact on CD4 and weight. Fawzi *et al.* (2003) reported that the administration of multivitamins to pregnant ART-naïve HIV women affected their children by reducing the likelihood of diarrhea and increasing the CD4 by 151 cells/uL when compared to children whose mothers were not given multivitamins. Isanaka *et al.* (2012), who reported earlier that a high-dose multivitamin is safe and could reduce disease progression through increased CD4 and weight gain in ART-naïve patients, also observed in a later study that a high-dose multivitamin has no effect on CD4 and weight of ART-treated patients and even caused elevated alanine transaminase in their 24-month study. This has further approved the claim that the “interaction between micronutrients in a multiple setting still remains a mirage in the scientific knowledge.” This further agrees with the earlier claim that different doses of micronutrients in ART treatment could trigger different result outcomes. The findings of Isanaka *et al.* could be compared to the result of this present study, where it was noticed that the effect of a single dose of selenium was higher than the effect of co-supplements. This suggests that micronutrient antagonism would be more pronounced in ART treatment supplemented with multimicronutrient. This has further suggested that single-dose supplemented ART treatment would be more effective compared to the multisupplement ART effect, just as it was seen in this present study, but in ART-naïve cases, multinutrient supplements could be more effective than single doses.

From the results of this study and the results from other reviewed literature, it could be established that ART regimens supplemented with single doses of micronutrients tend to be more effective than ART regimens supplemented with multimicronutrients, as a result of reported antagonisms that may exist in multinutrients in the presence of ART, though some

ART regimens may prove otherwise. This was proved by the observed effect of single doses compared to combined doses on weight and CD4 in this study. Though the sample size and study period made it difficult to make a generalized conclusion using our results, other studies with longer periods and larger samples proved likewise. Supplementation with high-dose multimicronutrients may be safe and may also be more effective than single and low-dose supplements in the absence of ART, but in ART treatment, the reverse may be the case, since reports from other studies proved otherwise. The introduction of supplements months after the initiation of ART may be more effective than initiating supplementation alongside ART, since the competition for binding space may be high, according to some studies.

The results of this study contribute insight with regard to an easier way of managing HIV infection using micronutrient supplements, especially in a location with a high prevalence of micronutrient deficiency. It is easily arguable that the obvious implication of this study is that it proposes an alternative way of HIV management using zinc or selenium. This study may have established that the use of selenium and zinc as supplement in HIV ART treatment could trigger higher weight gain and CD4 count, which could translate to better immune status compared to lone effect of ART. The study also demonstrated that the use of selenium and zinc as supplement could trigger quicker response in times of time taken to acquire a significant weight and CD4 which could translate to quicker recovery when compared to ART effect alone; this means that the use of zinc and selenium supplement could trigger better health and quicker recovery in HIV infection compared to the lone effect of ART. It may have also established that low and standard doses of selenium and zinc would be safe for human consumption since there was no complication recorded in the course of this study. This study has also revealed that a single dose of selenium and zinc may reduce the complications associated with HIV treatment, giving a glimmer of hope to HIV patients who are in poor and developing countries that meeting their daily dietary requirement poses a big challenge. Though the sample size and study period have made it very difficult to generalize the findings in this study, but backups from similar studies carried out with a larger study sample and longer period have made the result relevant and could also be consolidated. The aim was never to substitute the use of antiretroviral therapy but rather to complement its effects by ameliorating some of its side effects and triggering a quick and massive response, which would be evidenced in parameters like CD4 and weight increment, thereby reducing frequent hospitalization and complications associated with HIV ART treatment. This study has revealed that supplementing HIV ART treatment with a single dose of selenium or zinc (either low dose or standard dose) may improve the health status of HIV patients through increased weight and CD4. This will be of great relief to HIV patients in countries like Nigeria, where the prevalent HIV infection and nutritional deficiency are very high. The low cost of zinc and selenium made it even more relevant, so instead of sourcing scarce resources for secondary treatment that could enhance ART, 40 mg of zinc or 25 µg or 50 µg of selenium could easily be used instead. This could also alleviate the complications that may arise in the use of multimicronutrients. When ART becomes inaccessible, multimicronutrients or even high-dose multimicronutrients may be used since some studies deemed it safe and helpful in CD4 and weight recovery.

Limitations :Sample size and study period were the major limitations of this study. The study size of 30 participants and 3 months study period may not be enough to make a generalized and authoritative conclusion on the effect of zinc and selenium on weight and CD4 of HIV patients because a higher or lesser effect may be observed in a longer run, though backups from similar studies made it relevant. Also, being unable to investigate the effect of zinc and selenium on ART-naïve HIV patients so as to suggest whether zinc or selenium could substitute ART was also a limitation.

Challenges: This study also encountered some challenges, like unavailability of power source (electricity) and lack of funds. Unavailability of needed funds made it unrealistic to recruit and sustain a large number of volunteers over a long period of time, thereby limiting our choice to a small sample size and short study period since this was a self-sponsored study. Therefore, this study should be replicated with a larger study sample and a longer study period using a quantitative statistical test (post hoc) so as to enable generalized conclusions.

V. Conclusion

Selenium and zinc supplements have proven to significantly improve the weight and CD4 of HIV participants in this 3-month study. Their effect on weight and CD4 differs and is dependent on dosage and concentration.

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