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Impact of carrot-ginger blend on micronutrient status and CD4$^+$ cell-counts of HIV-positive-patients on antiretroviral therapy in Kaduna, Nigeria

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ABSTRACT

Micronutrients deficiency is a major challenge especially in Human Immunodeficiency Virus (HIV) infected patients on antiretroviral therapy (ART). This study investigated the impact of natural micronutrient supplements (Vitamins A, C, and E, Selenium and Zinc) from Carrot-ginger (75:25) blend on the micronutrient status and counts of T CD4 + lymphocytes of HIV-infected patients on ART. Ninety HIV-infected-patients attending Special Treatment Clinic, Kafanchan General Hospital were randomized into three groups of thirty patients each: Group 1 are control group and received ART alone, Group 2 are standard group and received ART + Ready to use commercial micronutrient supplements Registered (SelACE® supplement), while Group 3 are supplement group and received ART + Carrot-ginger blend for a period of 90 days. Vitamins A, C and E, selenium, zinc, CD4$^+$ T-cell counts and Body Mass Index (BMI) were assessed using standard methods at baseline (0 day), 30 days, 60 days and 90 days respectively. The results indicated that patients on carrot-ginger blend and SelACE® supplements had significant ($p < 0.05$) increase in BMI, CD4$^+$ T-cell counts, serum vitamins A, C, E, selenium and zinc from zero day. However, there was no significant ($p > 0.05$) difference on patients treated with ART alone when compared to their baseline values. In addition, patients on SelACE® supplement revealed significant ($p < 0.05$) difference in their mean BMI, CD4$^+$ T-cell counts, serum vitamins A, C, E, Selenium and Zinc when compared to patients on carrot-ginger blend after 90 days. The result further indicated a strong positive relationship ($p = 0.00$) between CD4+ T-cell counts, micronutrients status and BMI after 90 days of micronutrient supplementation. Micronutrients supplementation is important during ART treatment and carrot-ginger blend could be a beneficial adjunct to patients on ART due to its potential towards improving the immune system and strengthened nutritional status in patients with HIV infection.

1. Introduction

Human Immunodeficiency Virus (HIV) infection is described as an acute syndrome associated with the primary infection, which is followed by a prolonged asymptomatic condition and subsequently leading to advanced HIV disease [1]. Most individuals with HIV infection after about 3–6 weeks experience a febrile sickness lasting for few weeks with anorexia, nausea and diarrhea and then loss of weight [2]. Throughout the acute HIV syndrome period, the viral load peaks and is represented with very low CD4$^+$ T-cell counts which sporadically results in opportunistic infections [2]. The CD4$^+$ T-cell counts then returns to nearly normal amounts whereas the viral load stabilizes.

Nigeria ranks second largest in HIV epidemic worldwide [3]. In 2018, 1.9 million people were living with HIV in Nigeria [4] where six states account for 41% of people living with HIV including Kaduna State [3]. As of 2017, approximately 150,000 people died from acquired immunodeficiency syndrome (AIDS) related illnesses in Nigeria [4] and Kaduna State has HIV prevalence of 9.2% [5].

Malnutrition notably micronutrient deficiencies and anaemia are common complications associated with HIV diseases [6,7]. Poor nutritional status is independent risk factor linked with weakened immune system, opportunistic infections, and shorter survival in patients with HIV infection despite being on antiretroviral therapy (ART) [8,9]. A vicious cycle has been envisaged in which undernourished HIV-infected

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persons have micronutrient deficiencies, leading to further immune-suppression and oxidative stress and subsequent acceleration of HIV replication and $CD4^+$ T-cell depletion [10]. Persistent chronic inflammation due to increased release of reactive oxygen and nitrogen species formed by activated phagocytes are the major link identified in the pathophysiology of malnutrition during HIV infection [11,12].

Several studies have reported that micronutrients deficiencies are common among HIV-infected individuals, particularly those in low and middle income countries [13,14] and can contribute to the pathogenesis of HIV infection via increased oxidative stress and compromised immune system [15]. Interestingly, micronutrient supplements have been shown to delay HIV disease progression and reduce mortality in HIV-infected patients not receiving ART [16]. Vitamins B, C and E and trace elements such as selenium are essential nutrients required for maintaining a responsive immune system and have been used to manage HIV infection [39]. Micronutrient supplements containing multi-vitamins and selenium were reported to be harmless and significantly reduced the risk of immune deterioration and morbidity in HIV-infected adults [17].

Therefore, micronutrient supplementation represents a promising, effective and beneficial approach to defer the initiation of expensive, potentially toxic and lifelong ART in the management of HIV disease. However, micronutrient supplementation has been challenged with high cost especially in developing countries and possibility of non-adherence to the supplements. These could affect the treatment outcome of HIV infected patients on ART. Hence, natural source of micronutrients which are readily available, accessible, sustainable and cost-effective could be beneficial.

Carrot and Ginger are rich in nutrients such as vitamins, minerals and antioxidants which fight inflammation and boost immune system. Carrots are good source of carotenoids which have immune-boosting effects and the antioxidants lutein and zeaxanthin [18,19]. Ginger root contains active compounds called gingerols which provides many health benefits and exhibits anti-inflammatory and antioxidant properties [20,21]. Hence, this study therefore investigated the effects of carrot-ginger blend as natural source of micronutrients on nutritional status and $CD4^+$ T-cell counts of HIV-infected-patients on ART in Kaduna, Nigeria.

2. Materials and methods

2.1. Description of study area

This study was conducted at the HIV/AIDS Special Treatment Clinic (STC) in Kafanchan General Hospital, Jama'a Local Government Area (LGA) of Kaduna State, North-Western part of Nigeria. Kafanchan being the headquarter of Jama'a LGA is one of the oldest LGA in Southern senatorial district of Kaduna State with a population of 375,500 according to the National Population Commission, 2016 population projection. Jama'a is located between latitude 9° 11′ and 9° 30′ N and longitude 8° 00′ and 8° 30′ E (Fig. 1). The LGA is bounded in the East by Kagoroand Kaura LGA, in the North by Zonkwa and Agwan Rimi District of Zango Kataf LGA, to the West by Jaba LGA and in the South by Nasarawa State and in the South-East by Sanga LGA respectively. Jama'a LGA has 11 political wards, the people are mostly commercial farmers and their cash crops include ginger, sorghum, millet, maize and finger millet.

2.2. Study design

The research was a prospective cohort study that involved 90 patients purposively randomly selected volunteers already diagnosed and confirmed to be HIV-infected and were attending outpatients HIV/AIDS STC in Kafanchan General Hospital of Jama'a LGA, Kaduna State. The 90 patients were randomized into three groups consisting of 30 patients each. All patients received ART (Zidovudine (Retrovir)) for 90 days; Group 1 were the control group and received ART alone, Group 2 were standard group and received ART and SelACE® (Ready to use commercial micronutrient supplements Registered) for 90 days and Group 3 were the treatment group which received ART and locally formulated micronutrients supplements from Carrot-Ginger (75:25 ratio) blend for 90 days.

2.3. Selection criteria

HIV-infected-patients with $CD4^+$ T-lymphocytes counts within 350–400 cells/μl, about to commence ART therapy and within 18–49 years were included in the study. Patients with pregnancy and those with any metabolic syndrome (as reported by the patients or confirmed

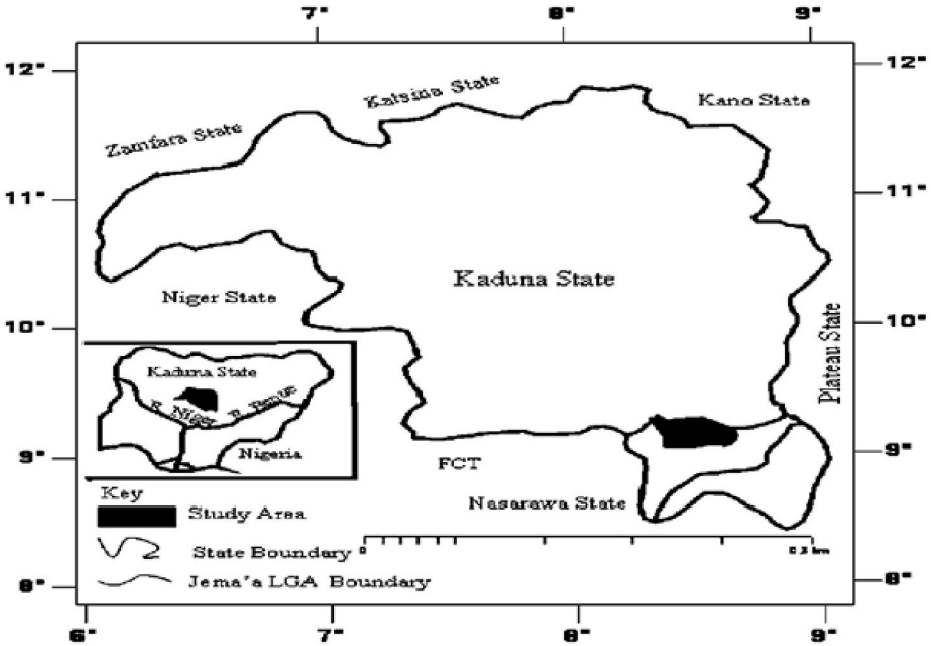

Fig. 1. Map of Nigeria and Kaduna State showing the study area.

from patient's medical history) were excluded. On enrollment, a validated semi-structured questionnaire was administered and information on patient's socio-demographic characteristics was assessed. Blood samples were also collected at four different phases.

2.4. Collection of blood samples

About 10 ml of blood samples were collected by a trained phlebotomist using venipuncture; immediately 1 ml was transferred into an Ethylene Diamine Tetraacetic Acid (EDTA) tube and about 9 ml into a plain tube. The blood samples were collected at different phases of the study (day 0, day 30, day 60 and day 90). After collection, the samples were delinked from donor's information except that concerning age and sex. Blood samples in EDTA tubes were used for the $CD4^+$ T-cell counts while samples in plain tubes were allowed to clot and then centrifuged at 1200 g for 5 min to obtain sera which were used to analyze for Vitamins A, C and E, Zinc, and Selenium using standard methods.

2.5. Formulation of carrot-ginger blend

Carrot-Ginger supplement was formulated using the method described previously [22]. Fresh carrot and ginger were obtained from farms in Zaria and Kafanchan Kaduna State Nigeria, respectively. The carrot and ginger were washed with distilled water to remove dirty and other contaminants and sliced into smaller size particles of 0.3 mm. The samples were sun dried for 2 h in a stainless tray to reduce moisture contents followed by oven drying at 40 °C for 10 h. This was followed by grinding of the dried samples and passed through 0.01 mm sieve to obtain a fine powder. Then, 75:25 of the carrot-ginger powder were mixed together in an electric stainless blender to obtain consistency. About 10,000 mg of the formulation was sealed in a polythene sachet using electric manual sealer and taken for micronutrients analysis.

2.6. Study regimen

The study regimen used was a commercially formulated micronutrient supplement which contained 5 ingredients; these are provitamin A (β-Carotene-150 μg), Vitamin C (Ascorbic acid-500 mg), Vitamin E (α-Tocopherol-12.06 μg), Selenium-250 μg and Zinc-50mg. It has a trade name SelACE® and was procured from Meyer PVC organics, India and distributed in Nigeria by Meyer Vitabiotics Ikeja, Lagos. The micronutrients supplement in local formulation from carrot-ginger contains provitamin A (β-Carotene-748.44 μg), Vitamin C (Ascorbic acid-3.87 mg), Vitamin E (α-Tocopherol-6.07 μg), Selenium-3.56 μg and Zinc-11.58 mg. The subjects were randomly assigned to receive either one tablet of SelACE® micronutrient supplement daily or 2 sachets of Carrot-ginger (75:25) blend containing 10,000mg/sachet after meal for 90 days. The participants were not allowed to use another micronutrient or natural health product. Compliance with the study regimens was assessed according to previously published methods [23,24] and adopted by Ref. [25]. The participants were asked to bring the unused SelACE® tablets back in the next clinic visit. Participants also exchanged a used bottle with a new bottle that contains SelACE® tablets. Compliance with the SelACE® supplement was calculated as the number of SelACE® tablets absent from the returned bottles. This was used as the indicator of the subject's compliance to the study medication.

2.7. Determination of $CD4^+$ T-cell counts

This was determined by the flow cytometry technique, using Cyflow Counter machine (PARTEC GmbH, Germany). Cyflow counter flow cytometer was used to determine $CD4^+$T-cell count. Sheaths fluid bottle was filled to 800 ml mark and air was expelled from filter before corked tightly. The fluid was discarded in waste bottle and rinsed with 10% hydrochloride solution and corked tightly. The sample was prepared as follows: into a Rohren test tube, 20 μl of $CD4^+$ T-cell count PE mAb was added and 20 μl of well mixed EDTA with whole blood that was collected within 6 h was added, mixed and incubated in the dark for 15 min at 25 °C. Exactly 800 μl of $CD4^+$ T-cell count buffer was added, mixed and read on the cyflow. The prepared samples was then plugged to the sample port of the cyflow and wait for acquisition and displayed of data.

2.8. Measurement of anthropometric parameters

Anthropometric parameters were measured at day 0, day 30, day 60 and day 90 respectively using standard methods. The patients were weighed with minimum clothing to the nearest 0.1 kg by using a regularly calibrated weighing health scale (Model ZT 120 SecaGmbh and Co., Germany). While the height was measured using a calibrated Standiometer, model 220 (SecaGmbh and Co., Germany) and then BMI was calculated using the formula as follows: BMI = Weight (kg)/Height (m^2). These procedures were repeated twice and the mean value was recorded.

2.9. Ethical consideration

Ethical approval for the study was obtained from the Health Research Ethics committee, Ministry of Health, Kaduna State (MOH/ADM244/VOL.1/520). Informed written and/or verbal consent was sought from each patient before inclusion into the study.

2.10. Data analysis

Statistical package for the social sciences (SPSS) version 20.0 a product of Microsoft incorporation was used for data analyses. Data were represented as frequency (percentage) or mean ± standard deviation as appropriate. Descriptive statistics were performed to summarize the socio-demographic data. One-way analysis of variance and Tukey's multiple range post-hoc test was used to analyze variations between the groups. Pearson's moment correlation was also used to determine relationships between the duration of treatments and concentrations of micronutrients, $CD4^+$ T-cell counts, and BMI and p-value <0.05 was considered statistically significant.

3. Results

3.1. Socio-demographic characteristics of HIV-infected-patients

The general socio-demographic characteristics of HIV-infected-patients enrolled in this study revealed that 62 (68.9%) of the patients were females and 28 (31.1%) were males (Table 1). Thirty-nine (43.3%) of the patients were within the age range of 18–28 years while 5 (5.6%) were less than 50 years. Furthermore, 43 (47.8%) of the patients were married, 8 (8.9%) were widowed whereas 34 (37.8%) have attained tertiary education and 16 (17.8%) attained only primary education. Regarding the monthly income earnings, 40 (44.4%) of the patients earned between ₦10,000 to ₦19,999 while 4 (4.4%) earned less than ₦40,000 which indicates that most of the patients were low income earners (Table 1).

3.2. Concentration and percentage daily value intakes of micronutrients and carrot-ginger blend

The concentration of Selenium, Zinc and Vitamins C and E from natural sources (carrot and ginger) were significantly ($p < 0.05$) low compared to SelACE®. The result revealed that carrot-ginger blend (75:25) has the highest concentration of Vitamin A (748.44 ± 0.43 μg/100 g) which contributes about 249.48% of daily value intake (DVI) requirement for Vitamin A. This is almost five times higher than the value obtained for SelACE® (150.00 μg and 50% DVI). However, the levels of Vitamin C, Vitamin E, Selenium and Zinc were 3.87 ± 0.04 mg/100 g, 6.07 ± 0.06 μg/100 g, 3.56 ± 0.09 μg/100 g and 11.58 ± 0.74

Table 1
Socio-demographic characteristics of HIV-infected-patients attending Kafanchan general hospital, Kaduna state.

Characteristics of Respondents	Frequency (n = 90)	Percentage (%)
Gender		
Female	62	68.9
Male	28	31.1
Age (years)		
18-28	39	43.3
29-39	17	18.9
40-50	29	32.2
>50	5	5.6
Marital Status		
Single	18	20
Married	43	47.8
Widow	8	8.9
Divorced	21	23.3
Level of Education		
No Formal Education	16	17.8
Primary Education	18	20
Secondary Education	22	24.4
Tertiary Education	34	37.8
Monthly Income		
<₦5000	29	32.2
₦10,000 - ₦19,999	40	44.4
₦20,000 - ₦29,000	7	7.8
₦30,000 - ₦39,000	10	11.1
>₦40,000	4	4.4

mg/100 g respectively for Carrot-Ginger blend, which were significantly lower compared to standard (SelACE®) which had higher micronutrient concentrations as indicated in Table 2.

3.3. Body mass index of HIV-infected-patients on ART before and after supplementation

The BMI of the patients was evaluated before and after micronutrients supplementation. The result showed no significant ($p > 0.05$) difference in the BMI between the control group (22.03 ± 1.87 kg/m^2), Carrot-Ginger group (22.52 ± 1.83 kg/m^2) and standard SelACE® group (22.43 ± 1.92 kg/m^2) at baseline day 0 (Table 3). However, there was a significant ($p < 0.05$) increase in BMI of HIV-infected-patient for carrot-ginger group (25.17 ± 0.64 kg/m^2) and standard SelACE® group (26.65 ± 0.68 kg/m^2) after 90 days supplementation compared to HIV-infected-patient on ART alone (22.96 ± 0.95 kg/m^2) as demonstrated in Table 3.

3.4. Serum concentration of vitamins and micronutrient supplementation of HIV-infected-patients

The present data showed significant increase ($p < 0.05$) in serum vitamin A, C and E among the HIV-infected-patients after 90 days supplementation with standard SelACE® supplement (Group 2), and carrot-ginger blend (Group 3) as shown in Table 4. Vitamin A recorded highest concentration of 41.89 ± 0.61 μg/dl for supplement group and 38.02 ± 0.62 μg/dl for standard group. Vitamin C concentration was lower 2.88 ± 0.05 mg/dl for supplement group and 8.94 ± 0.03 mg/dl for the standard group. In addition, lower concentration of Vitamin E was obtained for supplement group 7.26 ± 0.16 and 14.71 ± 0.06 μg/dl for the standard group. Consequently, there was no significant ($p > 0.05$) increase in serum vitamin A with mean value of 32.09 ± 0.19 μg/dl, vitamin C (0.99 ± 0.02 mg/dl) and Vitamin E (2.03 ± 0.04 μg/dl) for

Table 3
Body mass index of HIV-infected-patients on ART before and after supplementation.

Groups	Day 0	Day 30	Day 60	Day 90
Group 1	22.33 ± 1.87^a	22.24 ± 2.02^a	22.87 ± 1.89^a	22.96 ± 0.95^a
Group 2	22.43 ± 1.92^a	24.75 ± 1.76^b	25.23 ± 1.77^c	26.65 ± 0.68^c
Group 3	22.52 ± 1.83^a	22.22 ± 1.69^a	24.77 ± 1.88^b	25.17 ± 0.64^b

Results are expressed as Mean ± S.Dof 30 patients per group and values with different superscript alphabets (a-c) along respective horizontal axis are statistically different. (Tukey's multiple range post-hoc test, $p < 0.05$). Group 1: HIV-Infected-Patients on ART alone; Group 2: HIV-Infected-Patients on ART and supplemented with Standard (SelACE®) supplement; Group 3: HIV-Infected-Patients on ART and supplemented with Carrot/Ginger Formulation.

Table 4
Serum Micronutrient Concentrations of HIV-infected-patients on ART and supplementation.

Micronutrients/Groups	0 Day	30 Days	60 Days	90 Days
Vitamin A (β-Carotene: μg/dl)	21.18 ± 0.25^a	26.18 ± 0.25^a	29.12 ± 0.21^a	32.09 ± 0.19^a
Group 1	21.31 ± 0.36^a	31.32 ± 0.52^b	33.14 ± 0.64^b	38.02 ± 0.62^b
Group 2	21.19 ± 0.36^a	33.15 ± 0.51^c	36.00 ± 0.66^c	41.89 ± 0.61^c
Group 3				
Vitamin C (Ascorbic Acid: mg/dl)	0.44 ± 0.02^a	0.84 ± 0.02^a	0.92 ± 0.02^a	0.99 ± 0.02^a
Group 1	0.46 ± 0.01^a	7.25 ± 0.02^c	8.15 ± 0.02^c	8.94 ± 0.03^c
Group 2	0.43 ± 0.02^a	1.14 ± 0.05^b	1.64 ± 0.05^b	2.88 ± 0.05^b
Group 3				
Vitamin E (μg/dl)	1.09 ± 0.03^a	1.30 ± 0.36^a	1.83 ± 0.04^a	2.03 ± 0.04^a
Group 1	1.11 ± 0.02^a	10.68 ± 0.03^c	12.60 ± 0.04^c	14.71 ± 0.06^c
Group 2	1.09 ± 0.03^a	4.90 ± 0.24^b	6.19 ± 0.21^b	7.26 ± 0.16^b
Group 3				
Selenium (μg/dl)	26.07 ± 0.02^a	26.37 ± 0.03^a	26.58 ± 0.08^a	26.68 ± 0.08^a
Group 1	26.13 ± 0.02^a	29.34 ± 0.03^b	29.79 ± 0.08^b	34.20 ± 0.16^c
Group 2	26.04 ± 0.03^a	26.32 ± 0.27^a	26.87 ± 0.19^a	27.01 ± 0.15^b
Group 3				
Zinc (μg/dl)	61.19 ± 0.14^a	62.71 ± 0.13^a	61.59 ± 0.13^a	61.31 ± 0.13^a
Group 1	61.54 ± 0.14^a	66.54 ± 0.27^b	67.38 ± 0.24^b	70.15 ± 0.27^c
Group 2	61.11 ± 0.11^a	63.12 ± 0.29^b	64.53 ± 0.34^b	65.21 ± 0.28^b
Group 3				

Results are expressed as Mean ± S.D of 30 patients per group and values with different superscript alphabets (a-c) along respective horizontal axis for each micronutrient are statistically different. (Tukey's multiple range post-hoc test, $p < 0.05$). Group 1: HIV-Infected-Patients on ART alone; Group 2: HIV-Infected-Patients on ART and supplemented with Standard (SelACE®) supplement; Group 3: HIV-Infected-Patients on ART and supplemented with Carrot/Ginger Formulation.

Table 2
Micronutrients and Vitamins concentration of Carrot-Ginger Blend (75:25) and their Percentage Daily Value Intakes.

Micronutrients	Carrot	DVI	Ginger	DVI	Carrot-Ginger	DVI	SelACE®	DVI	RDA
Vitamin A (μg/100 g)	1013.15 ± 0.49	337.66	2.29 ± 0.42	0.76	748.44 ± 0.43	249.48	150.00	50.00	300.00
Vitamin C (mg/100 g)	4.19 ± 0.03	6.98	2.07 ± 0.02	3.45	3.87 ± 0.04	6.45	500.00	833.30	60.00
Vitamin E (μg/100 g)	6.66 ± 0.02	33.13	4.12 ± 0.01	20.50	6.07 ± 0.06	30.20	12.06	60.00	20.10
Selenium (μg/100 g)	1.42 ± 0.13	2.03	10.02 ± 0.06	14.31	3.56 ± 0.09	5.09	250.00	357.34	70.00
Zinc (mg/100 g)	15.32 ± 0.05	102.13	1.84 ± 0.01	12.27	11.58 ± 0.74	77.20	50.00	333.13	15.00

Results are expressed as mean ± S.D of duplicate determinations. DVI = Daily Value Intake; RDA = Recommended Daily Allowance; SelACE® = Standard Micronutrient Supplements.

HIV-infected-patient on ART alone (Control group) after 90 days experimental period (Table 4).

Furthermore, the serum concentrations of selenium and zinc among HIV-infected-patients on ART were also determined before and after micronutrient supplementation. The data indicated that there was significant ($p < 0.05$) increase in serum selenium and zinc concentrations (27.01 ± 0.15 μg/dl and 65.21 ± 0.28 μg/dl respectively) in HIV-infected-patients supplemented with carrot-ginger formulation (Group 3) as indicated in Table 4. In addition, SelACE® micronutrient supplements also significantly ($p < 0.05$) increase serum selenium (34.20 ± 0.16 μg/dl) and zinc concentrations (70.15 ± 0.27 μg/dl). However, there was no significant ($p > 0.05$) difference in serum selenium and zinc status after 90 days of supplementation in HIV-infected-patients on ART alone.

3.5. Effects of carrot-ginger supplementation on CD4$^+$ T-cell counts among HIV-infected-patients

The CD4$^+$T-cell counts of HIV positive patients were measured for all the groups at baseline and after 90 days of supplementation. The data revealed that there was significant increase ($p < 0.05$) in CD4$^+$T-cell counts among the supplemented HIV-infected-patients. Interestingly, Patients on carrot-ginger formulation and SelACE® micronutrients supplement recorded highest CD4$^+$T-cell counts (401.86 ± 9.03 Cells/μland 477.23 ± 8.29 Cells/μl respectively) after 90 days when compared to patients on ART alone (380.47 ± 11.02 Cells/μl) as shown in Table 5.

3.6. Relationship between serum CD4$^+$ T-cell counts and body mass index among SHIV-infected-patients on ART

The relationship between BMI and serum CD4$^+$ T-cell counts in HIV-infected-patients on ARTwas demonstrated in Fig. 2. There was a linear positive and significant relationship between patients serum CD4$^+$T-cell counts and BMI ($r = 0.771$, $p = 0.00$). This implies that 77.1% positive relationship between CD4$^+$T-cell counts and BMI of patients. In other words, as CD4$^+$ T-cell counts value increase or decrease, the values of BMI also increases or decrease.

3.7. Correlations between CD4$^+$ T-cell counts and serum micronutrients status of HIV-infected-patients on ART

The Pearson moment correlation coefficients between patient's serum CD4$^+$ T-cell counts and serum levels of micronutrients among HIV-infected-patients on ART was presented in Table 6. The results revealed that serum CD4$^+$ T-cell counts have significant ($p < 0.05$) positive correlation with the serum levels of micronutrients among the patients ($p < 0.05$) as shown in (Table 6). This implies that an increase or decrease in serum CD4$^+$ T-cell counts of the patients could result to an increase or decrease in serum Vitamins A, C and E, selenium and zinc.

Table 5
Effects of Carrot-Ginger Supplementation on CD4+T-cell counts of HIV-Infected-Patients.

Groups	0 Day	30 Days	60 Days	90 Days
Group 1 (Cells/μl)	375.97 ± 16.04^b	378.30 ± 9.59^a	381.23 ± 9.92^a	380.47 ± 11.02^a
Group 2 (Cells/μl)	373.23 ± 16.34^a	419.87 ± 6.52^c	455.57 ± 8.53^c	477.23 ± 8.291^c
Group 3 (Cells/μl)	372.74 ± 16.42^a	389.27 ± 6.56^b	391.85 ± 8.45^b	401.86 ± 9.03^b

Results are expressed as Mean ± S.D of 30 patients per group and values with different superscript alphabets (a-c) along respective horizontal axis are statistically different. (Tukey's multiple range post-hoc test, $p < 0.05$). Group 1: HIV-Infected-Patients on ART alone; Group 2: HIV-Infected-Patients on ART and supplemented with Standard (SelACE®) supplement; Group 3: HIV-Infected-Patients on ART and supplemented with Carrot/Ginger Formulation.

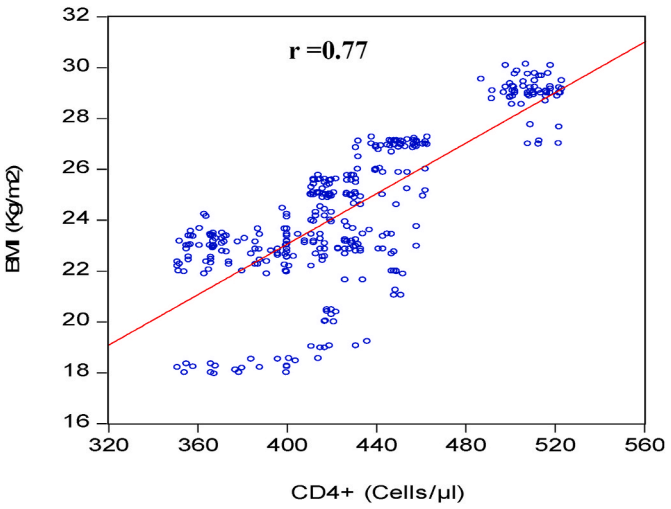

Fig. 2. Relationship between mean serum CD4$^+$ T-cell counts and body mass index of HIV-infected-patients on ART.

Table 6
Correlations between CD4$^+$ T-cell counts and serum micronutrients status of HIV-infected-patients on ART.

Micronutrients	CD4$^+$ T-cell Counts	p-value
Vitamin A	0.961**	0.01
Vitamin C	0.890**	0.01
Vitamin E	0.856**	0.01
Selenium	0.862**	0.01
Zinc	0.863**	0.01

Values are correlation coefficient (r) of two continuous variables. Values with asterisks (**) are statistically significant at p = 0.01 (Pearson Moment Correlation).

Thus, there was significant and strong positive correlation between serum CD4$^+$ T-cell counts and micronutrients status of HIV-infected-patients in this study (Table 6).

4. Discussion

This study revealed that socio-economic factors such as level of education, income, gender, number of partners and sexual habit are some of the major predisposing factors likely to increase the prevalence of HIV/AIDS in low income communities. The present findings indicated that HIV/AIDS prevalence was higher in female than their male counterparts. This could likely be due to the facts that more females present themselves for medical checkup HIV status than the males. While males due to fear of stigmatization and consequents do not frequently visit hospital for check up until they plunge into sickness [3]. These findings are in agreement with previous study [3,25]. In addition, low level of education and low income were high among females than male counterpart and could likely be one of the reasons women are more affected with HIV than men. This finding was also in conformity with the report of UNAIDS in 2017.

Moreover, patients within the age groups of 18–28 years as well as 29–39 years were mostly infected with the virus. Also patients that are singles are predisposed to the infection than married patients. Furthermore, the demographic data revealed that females are mostly infected with the virus. The current findings agree with previously reported data [26]. Therefore, it is highly important for Government and its relevant agencies to put more emphasis on creating awareness on HIV infection, HIV services and also provide financial support especially among women and adult within the age range of 18–39 years as a means of livelihood and self-dependent which could help to reduce the spread of

HIV infection [3].

The study also demonstrated that carrot-ginger formulation could be a good source of micronutrients (Vitamin A, Vitamin C, Vitamin E, Selenium and Zinc), even though the amounts are low when compared to the SelACE® micronutrients supplement. The result revealed that 75:25 carrot-ginger formulations were able to provide certain amount of micronutrients to meet up with the recommended dietary allowance (RDA). Besides, the composition of Vitamin A, Vitamin C, Vitamin E, Selenium and Zinc was in agreement with the findings of [27]; who reported that carrot powder provide good amounts of vitamins A, C, E, Selenium and Zinc [27]. Similarly, previous study on chemical analysis in ginger powder reported the presence of significant amount of Vitamin A, Vitamin C, Vitamin E, Selenium and Zinc [28]. Therefore, carrot-ginger formulation could be a cost effective supplement for improving immunity and management of HIV-infected-patients on ART since majority of people in the communities were low income earners and cannot afford or have access to standard micronutrients supplement.

Malnutrition, which is often accompanied by low level of micronutrients was common in HIV infection prior to introduction of ART and is still common especially in low income communities where there is limited access to ART [25]. Chronic diarrhea, malabsorption, impaired nutrient storage, increase energy demand and altered metabolism are the primary contributors to these nutritional deficiencies [29]. The present study indicated increased BMI in the supplemented (Carrot--ginger) group compared with its corresponding baseline as previously reported by Ref. [30]. In ART era, wasting has become less common and patients are increasingly overweight and obese [30]. The current study also demonstrated the importance of micronutrients supplementation among HIV-infected patients on ART which showed increased BMI with duration of supplementation [25]. [26] reported a significant increase in BMI among HIV-infected-patients on ART and micronutrients supplement in Sokoto and Kano States, Nigeria. This study indicated that patients on standard micronutrients supplements (SelACE®) and on natural micronutrient supplements from carrot-ginger formulation demonstrated a remarkable increase in their BMI compared to their corresponding baseline and patients on ART alone. This showed the role of natural micronutrients supplements in improving the nutritional status of HIV-infected-patients on ART. The present findings agree with previous study by Ref. [31] that reported significant increase in weight of HIV-infected patients on ART supplemented with nutritional supplements from plant sources (Whey and Soy) in Ethiopia compared to their non-supplemented group (ART alone). Increase in BMI observed in this study could be due to increase appetite caused by the intake of micronutrients from the carrot-ginger formulation which could be responsible for improving their BMI, immune reconstitution and recovery.

Micronutrients supplementation has beneficial effects on the micronutrients status of HIV-infected-patients on ART. This study has shown that micronutrients supplementation improves the nutritional status of HIV-infected-patients on ART compared to the control group on ART alone and their corresponding baseline as reflected in the mean serum Vitamins A, Cand E, Selenium and Zinc levels. This was in agreement with the findings by Ref. [29] who reported significant increase in the micronutrients status among HIV-infected-adults on ART and micronutrients supplement in Kaduna, Nigeria. Thus, the present study indicated that the HIV-infected-patients supplemented with carrot-ginger formulation and SelACE® micronutrients supplement had increased levels of serum Vitamins A, C and E, Selenium and Zinc compared to the baseline at day zero and patients on ART alone. The significant increase observed in the supplemented groups was similar to previous findings which reported increase in serum vitamins and minerals of HIV-infected-patients on micronutrients supplementation compared to HIV-infected-patients not on the supplementation [25,30]. This increase may be linked with intake and utilization of micronutrients from carrot-ginger supplements by the patients which further demonstrated the role of the carrot-ginger formulation in enhancing the nutritional status of the HIV-infected-patients as revealed by SelACE® and carrot-ginger supplemented groups.

In a prospective study among 108 HIV-infected-patients in Kano State, Nigeria and on HAART and micronutrients supplements reported an increase in the levels of serum Vitamin A, Vitamin C, Vitamin E, Selenium and Zinc compared to the baseline. This might also be associated with the physiological role of the micronutrients which might have caused increase appetite of the patients [25]. Vitamin A increases lymphocyte response [32] while vitamin C has been linked with increased cell mediated immune response and reduces reverse transcriptase activity [33]; [43]. Vitamin E increases T cell-mediated function and lymphocyte proliferation, reduces nuclear transcription factor XB (NF-XB) concentrations and decreased oxidative stress [34]. Selenium is needed for the proper functioning of neutrophils and T-lymphocytes [43]. Zinc plays specific roles as antioxidant, immune modulator [35] and possible direct antiviral activity [36,40]. Zinc has also been found to inhibit cell death by tumour necrosis factor (TNF), a cytokine linked to cellular apoptosis and wasting syndrome in HIV [37].

Several other studies have shown the relationships between micronutrients and oxidative stress. One randomized place bo-controlled trial shows that a daily supplementation with vitamins A, C and E for a period of three months reduces oxidative stress [41]. A six month placebo controlled trial study of daily supplementation with vitamins A, C and E also recorded a reduced oxidative stress in the supplemented HIV subjects [38]. This study demonstrated that micronutrients supplementation in HIV-infected-patients augment immune-reconstitution, reduced oxidative stress, and increased $CD4^+$ T-cell count in HIV disease condition as reported earlier [25,43]. This finding agrees with that reported by Ref. [26] which reported increase in the $CD4^+$ T-cell count of SelACE® supplemented HIV-infected-patients. The effect of the carrot-ginger formulation observed in this study could be linked to the antioxidant defense properties of the micronutrients such as Vitamins A, C and E, Selenium and Zinc through suppression and elimination of free radicals that are generated due to the activities of the virus itself and from ART medication.

5. Conclusion

In conclusion, the study demonstrated the beneficial effect of micronutrients supplementation in reconstituting the immune system through increasing $CD4^+$T-cell counts thereby decrease mortality and morbidity among HIV-infected-patients on ART. The study revealed a strong positive correlation between serum $CD4^+$ T-cell count and BMI as well as with Vitamins A, Cand E, Selenium and Zinc. The study further demonstrated the role of micronutrients supplement as antioxidant defense properties during ART therapy. Carrot-ginger blend beneficial role in boosting and recovering the immune system, it is available, affordable, and sustainable which could substitute the commercial micronutrients supplements which are expensive.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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