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Iron, Vitamins A and E Assessment on HIV/AIDS Adult Patients in Centre Region of Cameroon: A Pilot Study

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Abstract Hidden hunger is common amongst people living with HIV/AIDS. Good nutrition, with adequate micronutrients intake, is fundamental to improve their quality of life. This study was done to assess iron, vitamins A and E levels on HIV/AIDS adult patients in the Centre Region of Cameroon. The aim is the importance to ameliorate nutritional resources for people living with HIV (PLWH), using the locally available diet. This cross-sectional analysis of 82 adults from 21 to 39 years old was done at Saint Martin de Porres Hospital in Yaounde, Cameroon. Anthropometric variables were body weight and height. Iron, vitamins A and E levels, and CD4 T cells count were determined in blood samples. Seven-day food records were used for nutrient intakes. Iron and vitamin A intakes values range respectively from 5.34 to 70.99 mg/day (with an average of such 15.18 mg/day) and 40.31 to 963.93 mg/day (with an average of such 305.98 mg/day). Iron intake was insufficient for 52.3%. Vitamin A consumption was high for all participants. Iron, vitamin A and vitamin E levels values range respectively from: 0.13 to 3.81 mg/dL (with a mean of 1.28 ± 0.76 mg/dL); 0.14 to 0.86 mg/L (with an average of 0.45 mg/L); and 4.41 to 41.05 mg/L (with an average of 11.32 mg/L). Prevalence of iron and vitamin A deficiencies was 15.9%, and 13% for vitamin E. Only women were affected by iron deficiency. These results suggest that although iron, vitamins A and E levels were normal for most of PLWH, their deficiencies remain present. Hence, their morbidity and mortality could be affected. It is urgent to implement effective strategies for nutritional education on local iron and vitamin C rich foods, and for screening of signs of vitamin A toxicity in routine follow-up of people with HIV/AIDS in Cameroon.

Keywords: *micronutrients, nutritional education, immunodeficiency, Yaounde*

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1. Introduction

Human immunodeficiency virus (HIV) infection remains a public health issue in Cameroon, although the availability of antiretroviral therapy (ART) has improved the quality of life of people living with HIV and acquired immune deficiency syndrome (AIDS), and level of HIV/AIDS transmission [1]. HIV is known as a chronic disease that weakens nutritional status by compromising the immune system as well as the consumption, absorption and enhancement of nutrients [2,3]. The availability of food and good nutrition are therefore crucial to keep PLWH healthy [3,4]. Good nutrition implies an adequate and good quality intake of macronutrients and micronutrients [2,6,7]. Micronutrient's deficiency is also called "hidden hunger" [8]. It is known that during infections, like HIV/AIDS infection, iron is not used for hemoglobin synthesis, because it is sequestered from plasma into storage depots, leading to anemia [9]. Iron is essential for the development of antioxidants which fight against reactive oxygen species [10]. The main cause of iron deficiency is due to low dietary intakes of iron [11,12]. A healthy and balanced diet is necessary to prevent hidden hungry. Besides, many clinical studies have noted low levels of vitamin A in PLWH [13,14]. Vitamin A is known to play an important role on monocyte differentiation and function; it also increases lymphocyte count, particularly CD4 T cells subset, and improve epithelia integrity [2,15]. So, vitamin A is associated with HIV progression and mortality [9,16]. Concerning vitamin E, it plays a key role on the function of immune system and it is a good antioxidant [2,17]. Hence, it was found relevant to determine the micronutrients status of PLWH, because it is a fundamental component of HIV care.

This study was carried out to evaluate iron, vitamins A and E levels on HIV/AIDS adult patients in the Centre Region of Cameroon. The aim would be the importance to improve nutrition counseling resources for PLWH using the locally available diet.

2. Materials and Methods

2.1. Study Site

A cross-sectional study was conducted during 4 months at Saint Martin de Porres Dominican Hospital located in Mvog Betsi, Yaounde, Cameroon. This hospital was created in 2007 by the religious Dominican Sisters. It is one of reference health facilities in Yaounde, which is granted by national and international organizations. The high-quality of medical care and staff team is the particularity of this health center. It offers many medical services and programs, with a HIV testing, care and support unit.

2.2. Ethical Clearance and Consent to Participate

Prior to the study, ethical clearance was got from the Regional Ethics Committee for Human Health Research of the Cameroon Centre Region (Reference Number 0519/CRERSHC/2018). An administrative authorization was obtained from the administrative team of the hospital. A key note about the study was given every day to all the patients coming for follow-up. They were informed to feel free to participate in the study, voluntary, with a possibility to deny or discontinue at any time. Volunteers who were eligible to the study have signed an informed consent form before their enrollment. Anonymity and confidentiality of participants were preserved by assigning a code.

2.3. Study Participants

HIV-positive patients receiving care at Saint Martin de Porres Hospital were randomly selected. Eligibility criteria were: (1) to be aged between 21 to 39 years old; (2) to be taking Tenofovir-Lamivudine-Efavirenz antiretroviral treatment since at least 24 weeks; (3) to have an undetectable viral load (that is less than 40 copies per milliliter of blood) and be no symptomatic; (4) to be free of any nutrient-related diseases and conditions with special diets (e.g., diabetes mellitus, hypertension and other cardiovascular diseases, cancer, etc.) and (5) to inhabit in the Centre Region of Cameroon during the last six months prior to the study.

2.4. Data Collection

2.4.1. Questionnaires

An in-person interview questionnaire was designed for sociodemographic characteristics (age, gender, residence, religion, marital status, employment status, estimated monthly income) and HIV clinical history data.

During this interview, height and body weight were measured to determine body mass index (BMI), using the formula body weight (kg) divided by the square of height (m^2) [18].

2.4.2. Dietary Intakes

Food consumption score (FCS) questionnaire and food diversity score (FDS) questionnaire were used to assess dietary frequency and diversity of participants [19]. To calculate the FCS, the frequency of consumption of seven food groups (cereals; legumes; vegetables and fruits; meat and fish; milk; sugar; and oil), during the last 7 days, was used [19]. Each of these nine food groups (starches; dark green leafy vegetables; fruits and other vegetables rich in vitamin A; other fruits and vegetables; organ meats; meat and fish; eggs; legumes, nuts and seeds; milk and dairy products) reported on a 24-hour dietary recall, were counted to calculate the FDS [20]. Participants filled in 7-day food diaries with food eaten in real-time, time and location, special occasions or no. Food diaries were used for assessment of average nutrient intakes [21]. Food composition tables were used to evaluate nutrients composition of each food [22,23,24].

2.5. Laboratory Analysis

Blood specimens were collected on-site for biochemical markers of nutrient status. Blood samples were taken into a 5 mL plain tube to determine serum iron and 1.8 mg/mL ethylene-diamine-tetra-acetic acid (EDTA) tube for determination of vitamins A and E levels, and CD4 T cells count. Then, they were transferred in insulated bags to laboratories. Iron levels (mg/dL) were measured by colorimetric method [25].

Vitamins A and E (mg/L) were measured by high performance liquid chromatography (HPLC) [26]. The chromatographic separations and quantitative determination were performed on a Walters 2690 HPLC with ultraviolet-detector. Mobile phase, standard, internal standard, precipitation reagent, dilution solution, IC1600ko controls (from human plasma) and HPLC column Vitamin AE (IC1600rp; Nucleosil® C18, 10 µm particle size, 125 mm x 4 mm) were provided from Eagle Biosciences, USA. The flow rate was 0.8 ml/min. The separation program was isocratic, at 30°C, using a “reversed phase” column. Every run was lasting 15 minutes. Detection was monitored at two different wavelengths: l=300 nm for vitamin E and l=325 nm for vitamin A determination. The equipment included: various pipettes, 1.5 mL reaction tubes (Eppendorf), TH-3S TechnoCartel mixer (Vortex), C5.6R centrifuge (Beckman). After extraction, the blood was left to spontaneous coagulation. Then the samples were centrifuged at 300 r.p.m. for 15 minutes. The plasma samples obtained were covered to avoid light, frozen and stored at -30°C. Standard solution was prepared with 250 µL of standard and 50 µL of internal standard. Then, 250 µL of precipitation reagent were added into 250 µL of dilution solution. Samples or control were prepared with 250 µL of samples or control, 50 µL of internal standard and 500 µL of precipitation reagent. Then, the mixture was shaken by a vortex mixer during 2 minutes. The tubes were left for 30 minutes at 2-8°C in the dark and centrifuged at 10.000 g for 2 minutes. One hundred µL of the supernatant were injected into the HPLC-system. The calibration samples were prepared immediately before the analysis.

CD4 T cells count was done by flow cytometry [27].

2.6. Statistical Analysis

Data were recorded on Microsoft® Office Excel 2013 software. Statistical software IBM SPSS Statistics version

21.0 (SPSS Inc., Chicago, IL, USA) was used for analysis. The graphs were constructed using Microsoft® Office Excel 2013 software. Means and standard deviations were used for the description of continuous variables; and percentage, for categorical variables. To compare the means of two quantitative variables between two groups, Student's t-test after checking the hypothesis of normality of the sample was used. Qualitative variables were compared by Fisher's exact test. Differences were considered significant if p≤0.05.

3. Results

3.1. Dietary Survey

After validation, 44 food diaries were analysed. Table 1 shows the median of micronutrients daily intake of volunteers. The median of iron was acceptable. Vitamin A consumption was too higher than recommended. The median of vitamin C and calcium were lower than the recommended ranges.

Table 1. Median of micronutrients intake

Parameters	Median [Q25-Q75]	Normal range
Iron (mg)	15.18 [10.09 – 32.64]	Male: 9 mg/day; Female: 16 mg/day
Vitamin A (mg)	305.98 [161.21 – 595.67]	Male: 0.8 mg/day; Female: 0.6 mg/day
Vitamin C (mg)	58.03 [35.78 – 94.37]	110 mg/day
Calcium (mg)	396.06 [271.33 – 483.94]	900 mg/day

3.1.1. Distribution of Micronutrients Intake

Iron (52.3%), vitamin C (88.6%) and calcium (97.7%) intakes were not sufficient for the majority of participants as presented in Figure 1. But vitamin A intake was higher than the recommended dietary allowance (RDA) for all participants (100%).

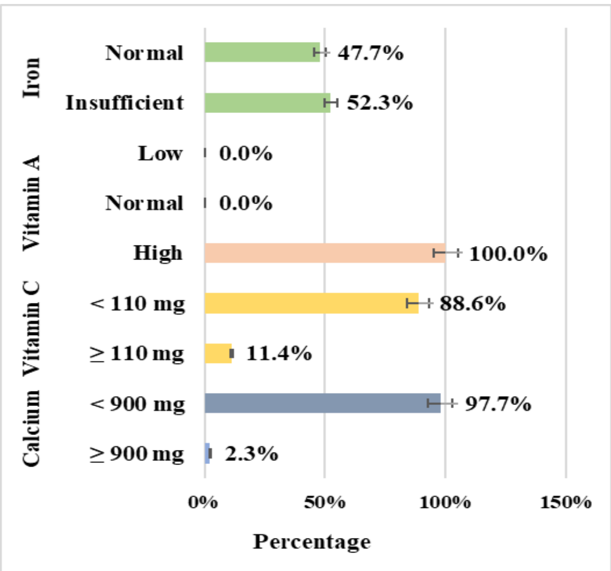

Figure 1. Micronutrients intake

3.1.2. Micronutrients Intakes by Age

Figure 2 shows the distribution of micronutrients intake by age groups. Vitamin C intake was low than RDA for all participants aged between 21 to 30 years old, while calcium consumption was low for all respondents from 31 to 39 years old.

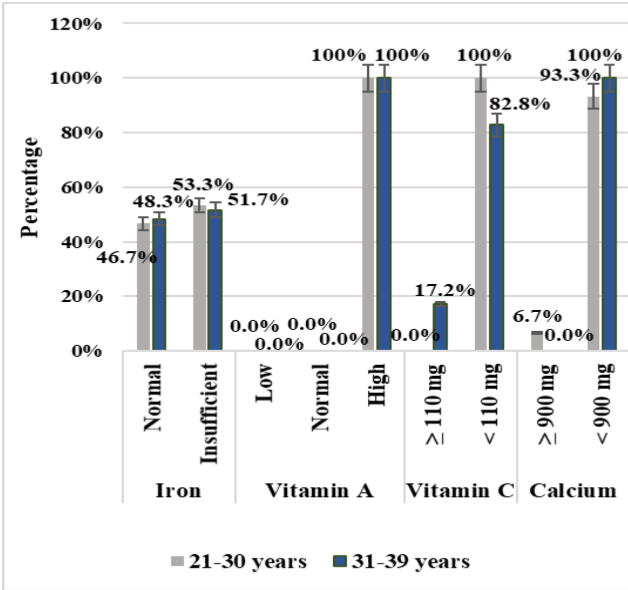

Figure 2. Micronutrients intake by age groups

3.1.3. Micronutrients Intake by Gender

Concerning distribution of micronutrients by gender, iron intake was normal for 7 men (63.6%), while insufficient for more than 50 % of women, as presented in Figure 3. Calcium intake was insufficient than RDA for all men.

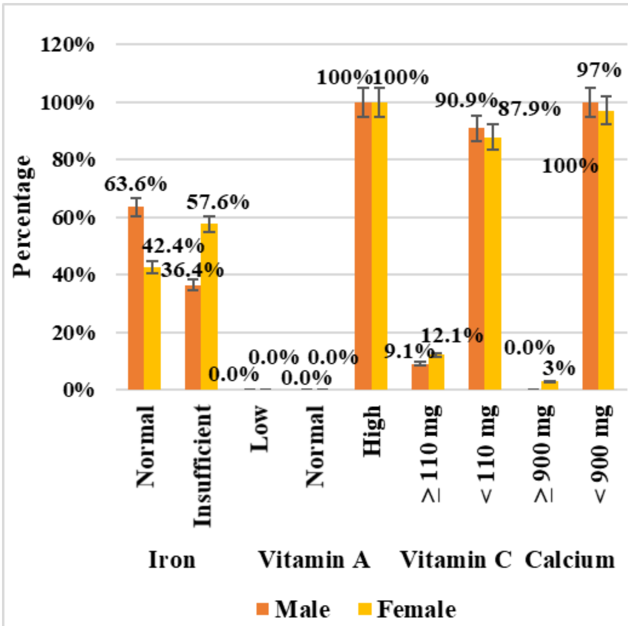

Figure 3. Micronutrients intake by gender

3.1.4. Micronutrients Intake by BMI

Table 2 resumes the apportionment of micronutrients by BMI classes. Iron intake was normal for all underweight participants. Calcium consumption was low for all participants of underweight, overweight and obesity groups. Besides, 16 participants (94.1%) with normal weight had low intake of calcium.

Table 2. Micronutrients intake by BMI groups

	Underweight		Normal		Overweight		Obesity	
	n[a]	%[b]	n	%	n	%	n	%
Iron								
Normal	**3**	**100**	7	41.2	8	50	3	37.5
Insufficient	0	0	**10**	**58.8**	8	50	**5**	**62.5**
Vitamin A								
Low	0	0	0	0	0	0	0	0
Normal	0	0	0	0	0	0	0	0
High	**3**	**100**	**17**	**100**	**16**	**100**	**8**	**100**
Vitamin C								
< 110 mg	**2**	**66.7**	**16**	**94.1**	**14**	**87.5**	**7**	**87.5**
≥ 110 mg	1	33.3	1	5.9	2	12.5	1	12.5
Calcium								
< 900 mg	**3**	**100**	**16**	**94.1**	**16**	**100**	**8**	**100**
≥ 900 mg	0	0	1	5.9	0	0	0	0

[a] n = Frequency, [b] %= Percentage.

3.1.5. Micronutrients Intake by CD4 T Cells Levels

Micronutrients were also presented by levels of CD4 T cells as shown in Figure 4. Iron intake was normal for more than 50% of participants with normal CD4 T cells levels (>350/µL), versus 4 (33.3%) of participants with low level of CD4 T cells (≤350/µL), with a statistical difference (p=0.01). Vitamin A intake was high for all participants. Vitamin C and calcium consumptions were low for more than 80% of patients, independently of CD4 T cells levels.

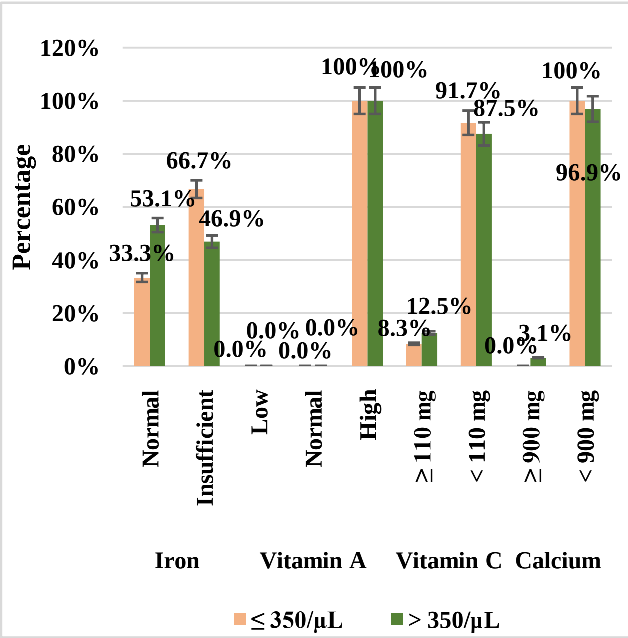

Figure 4. Micronutrients intake by CD4 T cells levels

3.2. Biochemical and Immunological Markers

Eighty-two blood samples were analyzed for iron, and 69 for vitamins A and E. Mean of iron and medians of vitamins A and E levels in blood of participants are presented in Table 3. All markers were in normal range: 1.28 mg/dL for iron, 0.45 mg/L for vitamin A, 11.32 mg/L for vitamin E.

Table 3. Mean/medians of iron, vitamins A and E levels

Parameters	Mean ± SD/Median [Q25-Q75]	Normal range
Iron (mg/dL)	1.28 ± 0.76	Male: 0.65-1.75 mg/dL Female: 0.5-1.70mg/dL
Vitamin A (mg/L)	0.45 [0.33 - 0.57]	0.31-0.82 mg/L
Vitamin E (mg/L)	11.32 [9.12 – 18.19]	6.6-14.3 mg/L

3.2.1. Distribution of Micronutrients Blood Levels

Figure 5 resumes iron, vitamins A and E levels of participants. More than half of participants had normal levels of iron [37 participants (58.7%)], vitamin A [56 patients (81.2%)] and vitamin E [39 participants (56.5%)].

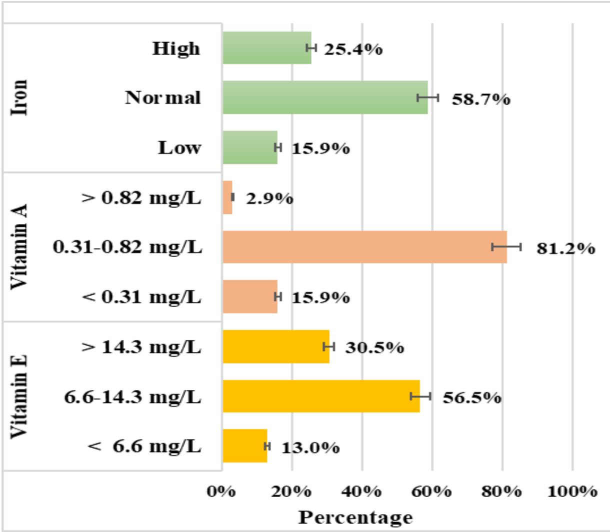

Figure 5. Iron, vitamins A and E levels

3.2.2. Micronutrients Blood Levels by Age

Concerning distribution by age, iron level was normal for 28 participants aged between 31 to 39 years old (65.1%), while less than half (9 participants) aged from 21 to 30 years old had normal iron level. Vitamin E level was normal for 17 patients (68%) aged between 21 to 30 years old. Figure 6 presents this result.

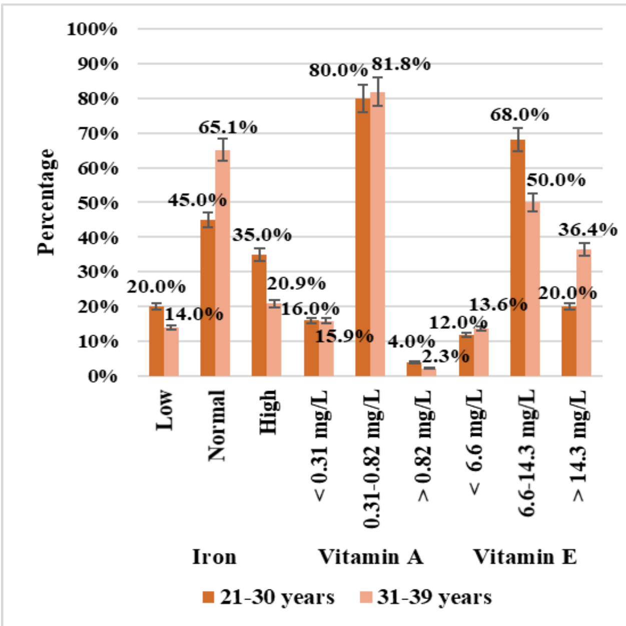

Figure 6. Iron, vitamins A and E levels by age groups

3.2.3. Micronutrients Blood Levels by Gender

There was no low iron level on male participants. Vitamin A level was normal for all men. We found 11 women (20.8%) with low vitamin A level. Vitamin E level was normal for 11 men (68.8%) and 28 women (52.8%). Eighteen women (34%) had high level of vitamin E. Figure 7 shows the distribution of iron, vitamins A and E levels by gender.

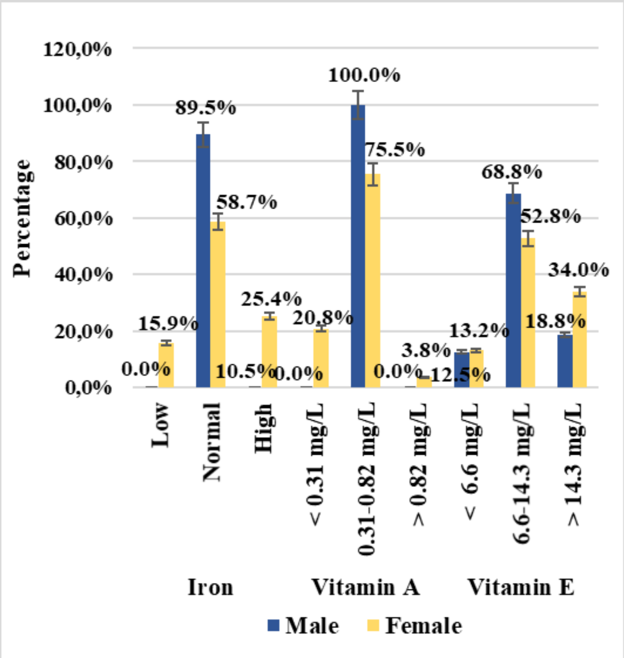

Figure 7. Iron, vitamins A and E levels by gender

3.2.4. Micronutrients Blood Levels by BMI Groups

Table 4 presents micronutrients levels by BMI. Iron was normal for the majority of participants, and for all obese patients. That is 3 (60%) underweighted; 14 (50%) patients with normal BMI; 11 (52.4%) overweighted. All underweighted participants had normal level of vitamin A as more than 60% of other groups. Concerning vitamin E, it was found that less than two-thirds of participants were in normal range: 3 (60%) of underweighted; 18 (60%) of normal BMI; 14 (53.8%) of overweighted; and 4 (50%) of obese participants.

Table 4. Iron, vitamins A and E levels by BMI groups

	Underweight		Normal		Overweight		Obesity	
	n	%	n	%	n	%	n	%
Iron								
Low	2	40	6	21.4	2	9.5	0	0
Normal	**3**	**60**	**14**	**50**	**11**	**52.4**	**9**	**100**
High	0	0	8	28.6	8	38.1	0	0
Vitamin A (mg/L)								
< 0.31	0	0	5	16.7	3	11.5	3	37.5
0.31-0.82	**5**	**100**	**25**	**83.3**	**21**	**80.8**	**5**	**62.5**
> 0.82	0	0	0	0	2	7.7	0	0
Vitamin E (mg/L)								
< 6.6	0	0	5	16.7	2	7.7	2	25
6.6-14.3	**3**	**60**	**18**	**60**	**14**	**53.8**	**4**	**50**
> 14.3	2	40	7	23.3	10	38.5	2	25

3.2.5. Micronutrients Blood Levels by CD4 T Cells Levels

Concerning micronutrients levels by CD4 T cells levels, Figure 8 shows that more than a half of participants of the 2 groups had normal micronutrients levels on their blood samples.

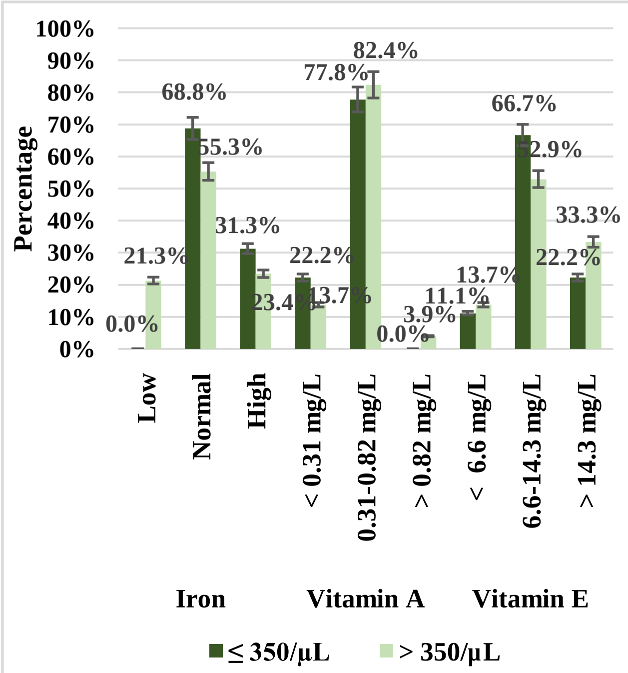

Figure 8. Iron, vitamins A and E levels by CD4 T cells levels

4. Discussion

The effect of nutrition on immunity is well known [5,7]. But hidden hunger is not systematically checked in patients daily routine care, especially for PLWH in Cameroon. This study was conducted to assess iron, vitamins A and E levels on HIV/AIDS adult patients in the Centre Region of Cameroon. Several studies have shown that despite the fact that many PLWH maintained a normal weight, achievement of required nutrients intake is not sufficient; hence it worsens the evolution of HIV/AIDS [28]. So, it is important to implement effective strategies to better manage PLWH nutritional health, using locally food [28].

The results of this study showed that micronutrients intake unmet RDA for the majority of participants. The higher intake of vitamin A may be due to the consumption of palm oil, fishes and dairy products which are some usual components of food in Centre Region of Cameroon [22,23,29]. It means that it would be relevant to look for signs of vitamin A toxicity on PLWH. Deficiencies noted in this study about iron, vitamin A and calcium intake are similar to those of Isabirye *et al.* [30] in Uganda, but they differ for vitamin C intake. This dissimilarity could be due to the food rich in vitamin C diversity between these two African countries. Women of the study are more affected by iron deficiency. It is known that women are at risk of iron deficiency, specially HIV infected women in Africa [9,31,32]. Results of the present survey suggested that there still a big nutritional challenge to fight against iron deficiency in PLWH, particularly for women in reproductive age. The need to improve nutritional counseling about iron food intake in Cameroon is fundamental, knowing that inadequate iron food intake is the major cause of anemia [9,11]. It is known that vitamin C enhances iron absorption [11,33]. However, in the present study, vitamin C intake was low. So, they should be a double cause of iron deficiency. This suggests that it would be suitable to educate PLWH on the importance to eat vitamin C rich food, like fruits and vegetables [34], associated with rich iron food.

Globally, prevalence of 15.9% for iron and vitamin A deficiency, and 13% for vitamin E deficiency was found in this cohort. Itinoseki Kaio *et al.* [35] reported deficient (<11.6 µmol/L) and low (11.6 to 16.2 µmol/L) vitamin E concentrations in 18.7% of their study population. Kpewou *et al.* [17] observed vitamin E deficiency (<12 µmol/L) in 82.5% of patients on ART in Ghana, with the higher value (91.7%) in underweighted and obese patients. These findings contrast with those of the study. It might be due to the use of different cut-off values and the gap of age of participants. The divergence in food habits might also explain these reports.

For Kharb *et al*. [36], at low CD4 T cells levels, serum iron levels were higher. In their study, they included only women, aged between 18 to 25 years old. These parameters might explain the discordance observed with the cohort of Centre Region of Cameroon. In regards to vitamins A and E, Shivakoti *et al*. [37], in 2016 in nine countries, found 8.1% of vitamin A deficiency and no vitamin E deficiency on PLWH after 48 weeks of ART. These findings are lower than those of Cameroon study cohort. This might be due to the different cut-off values used in these two studies, and food intakes qualities, compared to the Centre Region of Cameroon.

These purposes revealed that micronutrients intake is not meeting RDA amongst people living with HIV/AIDS in the Centre Region of Cameroon, with a high vitamin A intake. Despite the fact that blood levels of micronutrients are normal for the majority of participants. Women living with HIV are more concerned by iron deficiency. Therefore, caregivers should be sensitized about assessment of signs of vitamin A toxicity and anemia, especially on women. This evaluation should be done at every visit of HIV/AIDS patients to the health facilities. The aim will be to reduce morbidity and mortality of PLWH, particularly in Cameroon.

The present research has some limits. The first one is his cross-sectional nature. Hence, further analytical studies should be carried out for better results and conclusions. Besides, results could be refined by a more representative study of the Centre Region of Cameroon. As key limitations, we have some systematic errors like measurement errors and recall bias for self-reported components.

5. Conclusion

This study showed that micronutrients intake amongst HIV population of Centre Region of Cameroon is far from RDA. Iron, vitamins A and E levels are normal for the most of respondents of the study. Iron deficiency remains a problem for HIV population, specifically for women living with HIV/AIDS, despites their ART since at least 24 weeks, their high levels of CD4 T cells and their undetectable viral load. Nutritional advices about iron and vitamin C rich foods locally available, and nutritional screening of signs of vitamin A toxicity should be introduce in routine follow-up of people with HIV/AIDS in Cameroon.

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Conflicts of Interest

We, authors, declare no conflicts of interest.

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