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Serum level of two antioxidant vitamins (A and E) in Ivorian (Côte d'Ivoire) people living with human immunodeficiency virus

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ABSTRACT

Côte d'Ivoire is the West African's country most affected by HIV/AIDS. Unfortunately HIV infection has the effect of increasing the production of free radicals in the body. To counter the damaging effects of free radicals, the body increases the production of antioxidant molecules (vitamins A, C and E). The micronutrients play an important role in the immune system, in the protection and renewal of cells. In Côte d'Ivoire, very few studies have been devoted to the binomial micronutrient/HIV infection. The main objective of this study is to assess the micronutrient status of people living with HIV (PLHIV). The study involved 346 subjects including 173 adults with positive HIV, and 173 control population (negative HIV). After confirming the HIV status of the included subjects, the whole blood of PLHIV was used for counting CD4 in flow cytometry (FacsCalibur), while Liquid Chromatography (waters®-type) was used to determine serum vitamins A and E concentrations. The results showed a mean serum vitamin A, 0.08±0.01mg/L in PLHIV against 0.14±0.01mg/L in con trol population (p <0.0001). However, for vitamin E, concentration was 5.48 ± 0.30 mg/L in control popul ation against 1.27±0.19mg/L in PLHIV. Reduction lev els of vitamins (A, E) in PLHIV were 42.86% and 76.82%, respectively. In general, the results showed a significant deficiency of vitamins A (89/173, 51.44%) and E (128/173, 74%) in PLHIV compared to control population (P <0.0001). The vitamins deficiency may be due to the increased use of their antioxidant on oxidative stress caused by the overproduction of free radicals during HIV infection.

Key words: Côte d'Ivoire, Micronutrients, People living with HIV, Vitamin A, Vitamin E

INTRODUCTION

The HIV/AIDS is a real public health problem worldwide. In fact, 33 million people are living with HIV/AIDS worldwide, of which 22 million are in sub-Saharan Africa, with more than 1.5 million deaths recorded [1]. The AIDS epidemic in Africa leaves an unprecedented morbidity and mortality among young adults, decreasing life expectancy and drawing on the limited resources devoted to other pressing development problems (Piwoz, 2001). Côte d'Ivoire is among the most affected countries in West Africa with a prevalence rate of 3.4% in 2011. In that same year, 450,000 people living with HIV (PLHIV) were on antiretroviral (ARV) treatment [2]. Unfortunately, presently, ARVs do not cure HIV/AIDS infection, but result in slowing the spread of the virus in the absence of vaccine. However, the life expectancy of people with HIV is almost normal with treatment initiated around 350 CD4 lymphocytes [3].

Majority of free radicals play a major role in the production of cellular mediators and removal of toxic products. These free radicals also provide defense against invading microbes and viruses and against tumor cells.

Unfortunately, some may be involved in disease pathogenesis and production of changes that may eventually lead to the disruption of the body and its accelerated aging. Free radicals also affect the production of certain cells involved in the cellular immunity, such as cytokines (mediators allowing certain cells to communicate with each other), and are also involved in cell division [4].

Fortunately, free radicals can be neutralized easily through the body immunity system provided it is strongly protected by providing enough antioxidants (such as anti -oxidant molecules). Some anti -oxidant molecules such as fat-soluble vitamins are nutrients whose role is limited to combat the emergence of free radicals, or even eliminate the effects of those already present. Thus, a deficiency in antioxidant compounds or an increase the production of reactive oxygen species (ROS) may be the cause of oxidative stress. In the context of HIV infection, these ROS can be associated with the installation of structural and functional abnormalities of the cells of the immune response or participate in the increase of viral replication [5].

Present in very small quantities in the body without energy value, vitamins and minerals are essential for the proper functioning of cells. They play a crucial role in the immune system, protection and cell renewal [6]. A number of these antioxidant molecules such as beta carotene (provitamin A), ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) prevent oxidation caused by free radicals *in vitro* and *in vivo* [7]. They increase the humoral and cellular immune responses in some patients, indicating that the processes induced by endogenous free radicals have adverse effects on the immune system.

In sub-Saharan Africa, the relationship between nutrition and infection is complicated by an adverse nutritional environment. For decades, malnutrition was a problem among the African population, particularly in children. It has been amplified in recent years by the emergence of HIV/AIDS [8,9].

In Côte d'Ivoire, very few studies have focused on the binomial micronutrient/infectious disease [10]. The existing studies, especially in the case of HIV infection obscure the biological and biochemical aspects such as the determination of micronutrients. Indeed, micronutrient supplementation in HIV infection to enhance the therapeutic effect of antiretroviral and immunity of PLHIV must be based on real biological basis.

The main objective of this study is to assess the micronutrient status of people living with HIV, biologically monitored at the Institute Pasteur in Côte d'Ivoire.

MATERIAL AND METHODS

Population

The study took place from August 2011 to December 2012 at the Department of fundamental and Medical Biochemistry of "Institut Pasteur of Côte d'Ivoire" (IPCI). The Institute is responsible, in accordance with the National Program Support for People Living with HIV/AIDS (PN-PEC PLHIV/AIDS) and AIDS Global Fund, for biological monitoring of (Lab. tests) people living with HIV. To this end, all blood samples from health centers in charge of the clinical monitoring of subjects are received and analyzed in different laboratories of IPCI.

This is a prospective experimental study a cohort of adult subjects (men and women) HIV positive. The control population consisted of HIV-negative subjects; however pregnant HIV positive women were excluded from this study.

Biological materials and reagents used

Sera from different blood samples from HIV positive and negative subjects [11] were used to perform the assay of micronutrients (vitamins A and E).

Blood were collected in EDTA tubes necessary for the counting of CD4 T cells.

Standard reference solutions (1g/L of vitamin A and 10 g/L of vitamin E) and an internal standard (retinyl acetate, 1g/L) were used to prepare different diluted concentrations from which calibration curves were plotted.

Methodology

The CD4 count was carried out in flow cytometry (FacsCalibur) as follows: to 20µL of TriTEST (CD3, CD4, CD45) distributed to each trucount tube, 50µL of whole blood are added. Five hundred microliters of the Lysing solution (lyse cells) 1/10 diluted were added to each tube that had been homogenized and incubated for 15 min in the dark. This (homogenization and incubation of tubes in the dark) was repeated a second time, then trucount tubes are placed on the rack of the device FacsCalibur after a third homogenization for lymphocytes CD4 counting.

Normal reference values for CD4 according to WHO are: 600-1750 cells/mm^3 (31-60 %).

The dosage of vitamins A and E was performed using UV detection in high performance liquid chromatography (HPLC) gradient mode with a Waters® device after soluble vitamins extraction in hexane done away from light. The extraction and determination of vitamin concentrations were achieved in the following manner: to 300 µL of serum was added an equal volume of retinyl acetate (1mg /L), internal standard of the two vitamins [12,13]. The mixture obtained after adding 300 µL of absolute ethanol was centrifuged at 3500 rpm for 15 min. After evaporation in nitrogen (pressure: 0.5 bar), 300 µL of methanol are added to the residue and the mixture 20 µL (residue - methanol) was injected from the injection loop of the C18 column for the qualitative and quantitative determination of vitamins [14].

The parameters recorded for the simultaneous analysis of these vitamins are the following: stationary phase (reverse phase) bonded silica (C18), mobile phase methanol/water (98/2, v/v), a flow 1.5 ml/min, column temperature 30°C, detecting ultraviolet (UV) at 290 nm. Moreover, the detection limits for vitamins A and E are 0.003 mg/L and 0.2 mg/L, respectively.

Reference serum values are 0.1-0.5 mg /L for vitamin A and 7.8 -12 mg/L for vitamin E [15].

Statistical Analysis

The analyses were realized by using graph pad prism 5 demo software. The paired t-test was used to compare means. A p-value < 0.05 was considered as statistically significant. Percentages of vitamins reduction in infected population have been calculated by the ratio (difference between mean values of HIV subjects and control subjects divided by mean values of control subjects) multiplied by 100

Ethical considerations

Informed consent and ethical approval was obtained from participants and ethical committee respectively.

RESULTS AND DISCUSSION

Results

The serum mean value for retinol (vitamin A) was 0.08 ± 0.01 mg/L in population living with HIV (PLHIV) against 0.14 ± 0.01 mg /L in control populations ($p < 0.0001$). For vitamin E, it was 5.48 ± 0.30 mg /L in control population against 1.27 ± 0.19 mg /L in PLHIV. Reduction levels of vitamins A and E in PLHIV were 42.86 % and 76.82 %, respectively (Figure 1).

Whatever the age, the average value of vitamin A in PLHIV was 0.08 mg /L. However, the level of reduction is 52.9% (the highest) in the age group of 26-49 years. Contrary to vitamin E, where the average value varies with age ($P < 0.05$) and the level of reduction was 88.84% in the age group 16 - 25 years (Table 1, Figure 2).

The results indicate a deficiency of vitamin A and E in control subjects as well as in PLHIV ($P< 0.05$). Thus, 89 of the 173 PLHIV have vitamin A deficiency against 59 in control subjects, for vitamin E, 128 (74%) of PLHIV have Vitamin E deficiency against 111 in control subjects (64.16%) (Table 2).

By Sex, 84.85% (28) of male PLHIV are deficient in vitamin A against 43.57 % (61) HIV+ female subjects. The same goes for vitamin E that has touched 87.88% (29/33) of HIV^+ male against 70.71% (99 /140) of PLHIV females ($P = 0.0001$).

Considering the concentrations of vitamin A, the average value (0.08 mg /L) did not vary with age. However, the reduction rate of 52.9% (the highest) in the age group 26 - 49 years. With vitamin E, there is a significant variation ($P < 0.05$) according to age. The rate of reduction was 88.84 % in the age group of 16-25 years.

Based on sex, the average values are significant ($P < 0.05$). The reduction levels of vitamins A and E are 82.17% and 75.4% in HIV positive female against 55.55% and 40% in HIV+ males, respectively.

Based on the level of CD4 lymphocytes, the values of vitamins A and E have all been lowered compared to reference values. They are not significantly different (Pearson $\chi 2$ test = 0.01111 for vitamin E and 0.06 for Vitamin A) (Table 3). Similarly, the values of average concentrations of micronutrients are not significant, whether the subjects are on ARV on not $p > 0.05$.

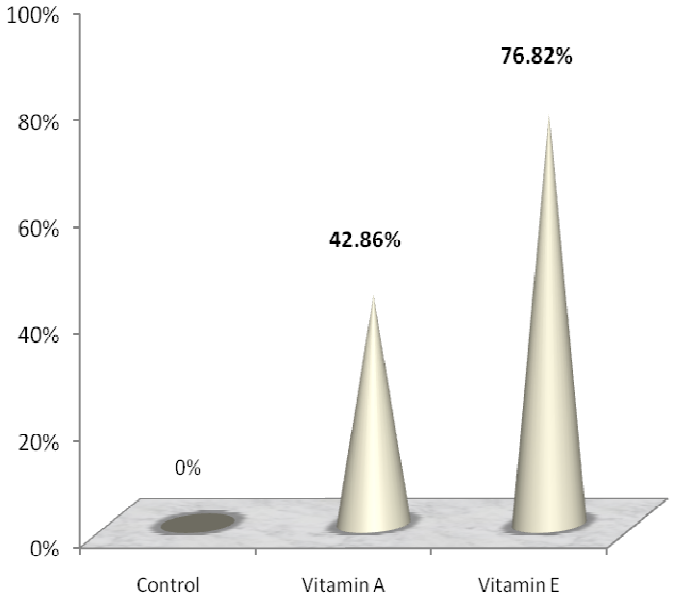

Figure 1: Level of reduction in concentration of vitamins A and E in HIV+ subjects

Table 1: Average concentrations level in vitamins A and E according to age of HIV+ subjects

Ages	Vitamin A (mg/L)		P*	Vitamin E (mg/L)		P*
	Control	PLHIV		Control	PLHIV	
16 - 25 ans	0.12 ± 0.01	0.08 ± 0.02	0.2121	4.50 ± 0.36	0.52 ± 0.36	< 0.0001
26 -49 ans	0.17 ± 0.02	0.08 ± 0.01	< 0.0001	6.39 ± 0.48	1.37 ± 0.21	< 0.0001
≥ 50	0.13 ± 0.02	0.08 ± 0.02	0.1214	6.94 ± 1.64	1.27 ± 0.60	0.0005

** The difference is significant for p < 0. 05.*

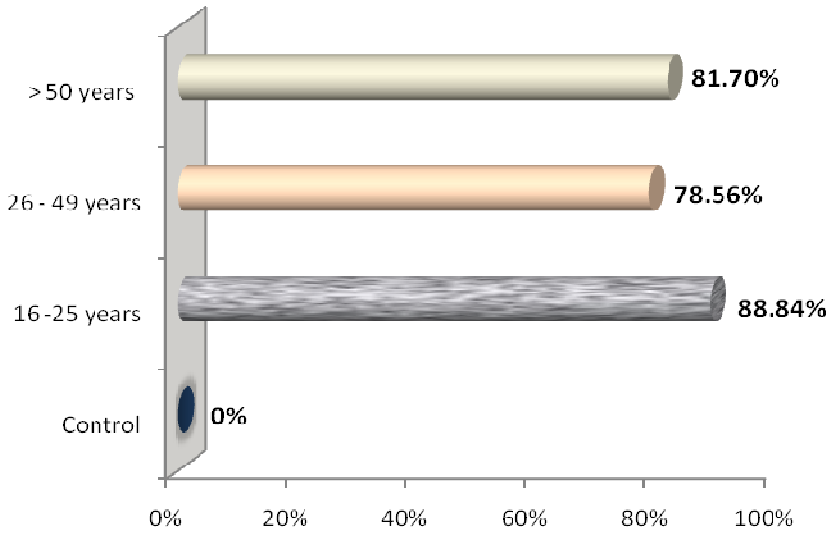

Figure 2: Level of reduction in concentration of vitamin E according to age of subjects

Table 2: Distribution of people living with HIV based on their vitamins A and E status

Vitamins	Type of populations	
	PLHIV*(n = 173)	Control (n = 173)
Vitamin A		
Deficiecy (< 0.35 mg/L)	89 (51.45%)	59 (34.10%)
Normal (0.1 - 0.5 mg/L)	84 (48.55%)	114 (65.90%)
Vitamin E		
Deficiency (< 7 mg/L)	128 (74%)	111 (64.16%)
Normal (7.8 -12 mg/L)	45 (26%)	62 (35.84%)

** PLHIV = people living with HIV*

Table 3: Vitamins A and E concentrations based on lymphocytes CD4

Lymphocytes CD4	PLHIV* (n = 173)	Vitamins concentrations	
		Vitamin A (mg/L)	Vitamin E (mg/L)
> 500 cellules/mL	38 (21.97%)	0.06 ± 0.01	0.47 ± 0.18
350 - 499 cellules/mL	25 (14.45%)	0.1 ± 0.01	2.7 ± 0.6
200 - 349 cellules/mL	59 (34.10%)	0.09 ± 0.01	0.90 ± 0.31
< 200 cellules/mL	51 (29.48%)	0.08 ± 0.01	1.4 ± 0.32

** PLHIV = People living with HIV*

DISCUSSION

The results of this study showed that approximately 51% of HIV+ individuals have a deficiency of vitamin A against 74% for vitamin E. Moreover, the reduction rate of 53% for vitamin A and 89% in the vitamin E were obtained with the age group of 26-49 years and 16-25 years, respectively.

Regarding vitamin A, the average concentrations are lowered and significant compared to control subjects. These results are consistent with those obtained by other researchers in various regions. They also showed a link between the reduction of vitamin A and HIV infection [16,17]. Indeed, among all the micronutrients, the role of vitamin A in HIV infection receives the most attention in Africa, given its involvement in the morbidity and especially infants' mortality. Indeed, its deficiency causes anemia, because retinol and retinoic acid are required for the synthesis of transferrin (protein transporting iron to the liver). In addition, vitamin A deficiency increases the incidence and / or severity of many infections [8]. In general, vitamin A deficiency and its reduction can be explained by an inadequate intake of food rich in this vitamin [18], poor absorption [19], an inability to stockpile (due to liver disease) and use or increased urinary loss of the vitamin during acute and chronic infection [20,21] or a lack of transport of this vitamin in plasma by retinol binding protein (RBP) [22].

Average concentrations of vitamin A in 66% of control subjects were normal (0.14mg/mL). This observation ruled out the hypothesis of a decrease in dietary vitamin A in controls and HIV^+ patients included in this study, because in fact, according to our investigations, these people live in the same area and have the same eating habits.

In addition, there is no significant difference between the values of vitamin A concentrations of patients on ARV therapy with CD4 ≤ 200 and those of patients without antiretroviral treatment having the same CD4 count. We can deduce that vitamin A deficiency cannot be linked to taking antiretroviral therapy.

In terms of vitamin E, in addition to the significant reduction observed, the average concentrations of tocopherol in PLHIV are lowered significantly. These results are consistent with those of some authors who showed a total decrease in serum α-tocopherol in patients infected with HIV [16,17,23]. However, studies in the U.S. have shown that high serum levels of vitamin E in the beginning are associated with a slower progression of HIV [24].

The average value of vitamin E in the control subjects who have almost the same eating habits and living in the same study area as those of PLHIV is low (5.48 mg/L). In addition, a high percentage of control subjects with vitamin E deficiency (64.16%) were obtained. Physiologically, the main metabolic function of vitamin E as an antioxidant is its ability to prevent the oxidation of lipoproteins and oppose the development of atherosclerotic

plaque. Vitamin E may play an important role in the regulation of heme synthesis [25]. It works by preventing the chain reactions that generate free radicals. Vitamin E is necessary for the proper functioning of the immune system and enhances the humoral immune and cell-mediated response, including the production of antibodies, and phagocytic cell responses and resistance to viral infectious diseases [26]. Oxidative stress created by HIV and opportunistic infections, increase the utilization of vitamin E and can lead to its deficiency. The vitamin E deficiency weakens the immune system due to its role in stimulating immune function, and therefore people living with HIV/AIDS become more susceptible to opportunistic infections [8]. Considering the CD4 lymphocytes, the results show that there is no relationship between vitamin E deficiency and low CD4 count. The same observation was made in the study of Skurnick *et al* and those of Jones *et al*, made in 1996 and 2006, respectively [16,27]. Deficiency of vitamin E cannot be attributed to taking of antiretroviral therapy.

CONCLUSION

The decrease in concentrations of vitamins A and E may be due to the increased utilization of their antioxidant properties on oxidative stress caused by the overproduction of free radicals in HIV infection. Thus, in addition to triple therapy, which since January 2013 is recommended to patients with a CD4 cell count ≤ 350 cells /mL in Côte d'Ivoire, it could also makes sense to integrate micronutrients supplementation to PLHIV once the HIV seropositivity is declared. This will allow a better biological balance and for the purpose of improving the functioning of the immune system and prolong the survival of these people. For indeed, vitamin E is one of the few micronutrients of which its supplementation at higher doses than recommended daily levels increases the immune response and disease resistance [28].

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REFERENCES

[1]- Anonyme 1, Le point sur l'épidémie de sida. Rapport spécial sur la prévention du VIH. **2007**, ONUSIDA /07.27F /JC1322F.
[2]- Anonyme 2, Suivi de la déclaration de politique sur le sida de juin 2011: Rapport National GARP (Côte d'Ivoire), **2012**, 43p.
[3]- O. Adolfsson, B. T. Huber, S. N. Meydani, *J Immunol* **2001**, *167*, 3809.
[4]- M. May, M. Gompels, V. Delpech, K. Porter, F. Post, M. Johnson, D. Dunn, A. Palfreeman, R. Gilson, B. Gazzard, T. Hill, J. Walsh, M. Fisher, C. Orkin, J. Ainsworth, L. Bansi, A. Phillips, C. Leen, M. Nelson, J. Anderson, C. Sabin, *BMJ*, **2011**, *343*, d6016.
[5]- A. M. Tang, N. M. Graham, A. J. Kirby, L. D. McCall, W. C. Willett, A. J. Saah, *Am J Epidemiol* **1993**, *138*, 937.
[6]- P. Gelas, M. C. Tronel, Nutrition & VIH : Vitamines & minéraux. Cahier pratique Nutrition & VIH. http://www.sidaweb.com/information/vitamines.htm, **2010**, consulté 16 juil. 2012, 1.
[7]- V. Lobo, A. Patil, A. Phatak, N. Chandra, *Pharmacogn Rev*, **2010**, *4*, 118.
[8]- E. G. Piwoz, E. A. Preble; HIV/AIDS and Nutrition: A Review of the Literature and. Recommendations for Nutritional**.** Care and Support in Sub-Saharan Africa**.** Support for Analysis and Research in Africa (SARA) Project. U.S. Agency for International Development Document PDF, **2001**, 66p.
[9]- R. Becquet, V. Leroy, D. K. Ekouevi, I. Viho, K. Castetbon, P. Fassinou, F. Dabis, M. Timite-Konan, *Pediatrics* **2006**, *117*, e701.
[10]- M. G. M'boh, M. Aké, F. H. Yapi, Y. Soko, A. Yapo, J. Djaman, *Eur. J. Sci. Res.*, **2010**, **44**,159-166.
[11]- S. Koblavi-Deme, C. Maurice, D. Yavo, T. S. Sibailly, K. N'Guessan, Y. Kamelan-Tano, S. Z. Wiktor, T. H. Roels, T. Chorba, J. N. Nkengasong, *J Clin Microbiol* **2001**, *39*, 1808.
[12]- G. L. Catignani, J. G. Bieri, *Clin Chem* **1983**, *29*, 708.
[13]- Z. Zaman, P. Fielden, P. G. Frost, *Clin Chem* **1993**, *39*, 2229.
[14]- M. Ake, A. G. Poby, K. A. Malan, A. Tebi, D. Monnet, *Ann Biol Clin (Paris)* **2001**, *59*, 417.
[15]-Ch. Beglinger, F. Seibold, G. Rogler, Valeurs standard en laboratoire http://www.gastro.medline.ch/Services_et_outils/Valeurs_standard_en_laboratoire/Valeurs_standard_en_laboratoire_Biochimie.php, **2010**, consulté le 02 Septembre 2013, 1
[16]- J. H. Skurnick, J. D. Bogden, H. Baker, F. W. Kemp, A. Sheffet, G. Quattrone, D. B. Louria, *J Acquir Immune Defic Syndr Hum Retrovirol* **1996**, *12*, 75.
[17]- L. S. Bilbis, D. B. Idowu, Y. Saidu, M. Lawal, C. H. Njoku, *Ann Afr Med*, *9*, 235.
[18]- D. I. Thurnham, R. Singkamani, *Trans R Soc Trop Med Hyg* **1991**, *85*, 194.

[19]- S. Brooker, N. Peshu, P. A. Warn, M. Mosobo, H. L. Guyatt, K. Marsh, R. W. Snow, *Trans R Soc Trop Med Hyg* **1999**, *93*, 240.
[20]- R. D. Semba, *Acta Paediatr Suppl* **1997**, *421*, 107.
[21]- A. Mitra, T. D. Bailey, A. L. Auerbach, *Structure* **2004**, *12*, 1909.
[22]- F. J. Rosales, A. C. Ross, *J Nutr* **1998**, *128*, 1681.
[23]- M. K. Baum, G. Shor-Posner, Y. Lu, B. Rosner, H. E. Sauberlich, M. A. Fletcher, J. Szapocznik, C. Eisdorfer, J. E. Buring, C. H. Hennekens, *AIDS* **1995**, *9*, 1051.
[24]- A. M. Tang, N. M. Graham, R. D. Semba, A. J. Saah, *AIDS* **1997**, *11*, 613.
[25]-Anonyme 3, Dosage: Vitamine A (rétinol) et vitamine E (tocophérol), http://www.admedne.ch/files/flhn/flhninfo/065_2013_01_AdmedInfo_VitA_E_rev1.pdf **2013**, 3p.
[26]- O. E. Odeleye, R. R. Watson, *Prog Food Nutr Sci* **1991**, *15*, 1.
[27]- C. Y. Jones, A. M. Tang, J. E. Forrester, J. Huang, K. M. Hendricks, T. A. Knox, D. Spiegelman, R. D. Semba, M. N. Woods, *J Acquir Immune Defic Syndr* **2006**, *43*, 475
[28]- S. N. Meydani, M. Hayek, L. Coleman, *Ann N Y Acad Sci* **1992**, *669*, 125.