INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 4, p. 58-67, 2013

RESEARCH PAPER OPEN ACCESS

Frequency of deficiencies in trace elements and correlations with the degree of immunosuppression in people living with HIV in Cotonou (Benin)

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Key words: Trace element deficiency, malnutrition, HIV / AIDS, micronutrient.

doi: http://dx.doi.org/10.12692/ijb/3.4.58-67 Article published on April 22, 2013

Abstract

The micronutrient deficiencies are a form of malnutrition detrimental to the success of antiretroviral therapy. In order to assess the frequency of deficiencies in some trace elements among People Living with HIV (PLHIV) in Cotonou and their correlation with the degree of immunosuppression, a cross-sectional study was conducted from January to April 2012. 93 patients aged of 37.56 years were interviewed and subjected to laboratory analysis. Chi² test allowed the statistical comparison of frequencies at significance level of 5%. The results showed 31.87% of low zinc content, 26.37% of hypo plasma copper and 46.43% deficit in serum iron. Zinc deficiency has been accompanied in 65.55% of cases of copper deficiency. Unlike iron, zinc and copper deficiency have not varied by gender. HIV positive with a CD4 count ≤ 200 cells / µL (group G4) are significantly affected by zinc deficiency (p = 0.008) and copper (p = 0.043) compared to those with CD4> 200 cells / uL (G3). Indeed, 51.85% of low zinc content in G4 against 23.44% in G3 was noted while 40.74% in plasma copper (G4) was noted against 20.31% in G3. But these correlations proved with a threshold of 200 CD4/µL were not observed when considering the reference of 350 CD4/µL. For iron, the differences observed between G4 (53.84%) and G3 (41.37%), and between G2 (51.11%) and G1 (38.46%) were not statistically significant (p = 0.245 and 0.289 respectively). These results suggest that it is imperative to improve the nutritional status of PLHIV including food supplements rich in trace elements.

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Introduction

In recent years, the World Health Organization (WHO) recommends that nutrition is an integral part of overall strategies of response against the HIV / AIDS (Kelem, 2008., Djibril and Diene, 2009). Indeed, without a healthy and balanced diet, the immune system is unable to perform its role of defense (Montagnier, 2005; Ndangurura, 2008). Thus, during HIV infection particularly, malnutrition increases the risk of opportunistic infections and accelerate the progression to the critical stages of the disease (WHO and FAO, 2002). Conversely, the only presence of HIV and / or prolonged use of antiretroviral drugs causes or aggravates malnutrition in many PLHIV (WHO and FAO, 2002 ; Montagnier 2005) with consequences significant organic impact such as metabolic disorders, decreasing of lean mass, fluid and electrolyte imbalances, hormonal dysfunctions, etc.. (Nvondo, 2000.; Taverne *et al.,* 2012).

Currently, in sub-Saharan Africa characterized by the world record in terms of high prevalence of HIV infection and alarming levels of food insecurity (PNLS, 2010a) with high rates of chronic malnutrition (Ake-Tano et al, 2006. Diouf et al, 2006), some countries already successful realized integration of the component into nutritional support for PLHIV (Kelem, 2008). In Benin, despite the political will (CNLS, 2011), patients of all sites do not yet benefit from nutritional support. The food support is summarized with distributions of primarily energetic food and that only partially fill the needs of micronutrients (vitamins and trace elements). Beside, lack of appropriate methods to assess the nutritional status of patients from comprehensive clinical and biological assessments (Bach et al., 2004), the diagnosis of malnutrition is based only on anthropometric and clinical criteria. This occult deficiencies in vitamin and trace elements generally observed in PLHIV (Montagnier, 2005., Djinhi et al., 2009). However, some micronutrients such as zinc, copper, selenium, iron plays a crucial role in the immune system and would influence the rate of CD4 (Baum, 2000., Hurwitz et al., 2007) which is the main biological marker used to characterize the degree of immunosuppression among PLHIV (Urussa et al, 2003., Diaw, 2003).

Currently, there are no published studies on the prevalence of certain deficiencies in trace elements in Benin, because medical laboratories do not realize these tests routinely and clinicians do not care so much in their diagnoses. Thus, the guidelines of the nutritional policy based only on statistics available which indicate 38% of chronic malnutrition in the general population (EDS, 2007) and levels of food insecurity beyond 30% in concordance with situations observed throughout in sub-Saharan Africa (Kelem, 2008). In the new National Strategic Plan against HIV / AIDS, the ambition is to see 90% of PHAs with a good nutritional status in 2016 (CNLS, 2011). And this, with priority actions such as distributing food to poor patients, nutrition education and promotion of local foods rich in micronutrients. The justification of this study include the need to have some basic indicators on the size of deficits in trace elements. The general objective is to assess in the country's economic capital, the frequency of deficiencies in zinc, copper and iron and the correlation with the degree of immunosuppression defined by the number of CD4 cells

Materials and methods

Framework and study population

This analytical cross-sectional study was conducted from January 12 to April 24, 2012 in Cotonou. Indeed, Benin is an African country of about 9,067,076 inhabitants occupying an area of 114,763 km² and is located in the Gulf of Guinea (SNIGS-MS, 2011). Patients were selected from one of the largest sites of care namely Bethesda Hospital where 10.76% of PLHIV receiving antiretroviral treatment were treated in Cotonou according to the current statistics of the National Program for the Fight against AIDS.

Sampling

Sampling was done by convenience. The inclusion criteria for patients were: HIV status confirmed after

testing algorithm in force in Benin (PNLS, 2010b), possession of a medical file and strictly greater than 15 years. In addition, the free and informed consent of each patient was obtained before inclusion in the study. Sample size was calculated using the Epi-info 3.3.1.

Data collection

Medico-clinical, sociodemographic and nutritional informations were collected using a standardized questionnaire with one hand patients themselves and other hand staff in charge their monitoring medical or psychosocial. Biological parameters were measured from blood samples conducted in patients. Measures have been taken to maintain confidentiality according to the recommendations of the National Committee of Ethics and Health Research (CNERS, 2010).

Table 1. Distribution of HIV patients by gender and status in blood zinc, copper and iron at Bethesda Hospital, Cotonou.

		Male		Female		Total	
Trace element content		**N**	**%**	**N**	**%**	**N**	**%**
Zinc content	< 0.8 mg/L (*)	9	33.33%	20	31.25%	29	31.87%
	> 0.8 mg/L	18	66.67%	44	68.75%	62	68.13%
Cuprémie	< 0.8 mg/L (*) *	7	25.93%	17	26.56%	24	26.37%
	> 0.8 mg/L	20	74.07%	47	73.44%	67	73.63%
Ferémie	< 0.6 mg/L (*)	8	28.57%	30	53.57%	39	46.43%
	> 0.8 mg/L	20	71.43%	26	46.43%	45	53.57%

N = absolute frequency *%* = relative frequency *(*) = deficiency*

Techniques of sampling and laboratory analysis

Blood samples were performed in fasting by Vacutainer method (Ibeagha-Awemu et al., 2012) in two tubes containing EDTA anticoagulant and a dry tube (without anticoagulant). All information sheets and tubes were labeled by the same anonymous code assigned by patient. The specimens were processed according to the rules of good performances analysis. The first tube of blood collected in EDTA was used for complete blood count and CD4 lymphocyte count within 4 hours of time. The second EDTA tube and the dry tube was centrifuged at low speed (1500 rpm) for 15 minutes to avoid hemolysis. Serum and plasma aliquots were collected, properly labeled and stored at 4°C or - 20°C depending on the type of analysis. The hemogram was realized using a hematology analyzer Sysmex KX-21N (Briggs et al., 2003). The system FASCount BD (Becton Dickinson) as described by Pattanapanyasat et al (2008) was used to enumerate CD4 T lymphocytes. Zinc and copper were determined from an Atomic Absorption Spectrophotometry brand SpectrAA110 after acid digestion of the blood plasma according to the method described by Arnaud et al. (1986). The determination of serum iron was made by the ferrozine colorimetric method (Ogbe et al., 2012) with the Elitech reagent and a Molecular Absorption spectrophotometer (Erba Chem 7).

Statistical treatment

Data were entered in Excel 2007 and then exported into SPSS 16.0. Statistical analysis consisted of calculating proportions and means and their confidence intervals. The chi-square test of Pearson allowed the comparison of the frequencies of deficiency between groups with a significance level of 5%. Indeed, the comparison groups were formed by considering two baselines for the values of CD4 (350 cells / uL and 200 cells / uL). The first (350 cells / uL) is the value recommended since 2006 for the ARV treatment for HIV patients (PNLS, 2010a WHO, 2010a). The second (200 cells / uL) is the threshold admitted before 2006 for ARV treatment for HIV patients. G1 is the group of patients with a CD4 count > 350 cells / uL and G2, that of patients with a CD4 count ≤ 350 cells / microL. G3 includes

patients with a CD4 count> 200 cells / uL and G4 includes patients with a CD4 count ≤ 200 cells / microL. The threshold for each deficiency of trace elements were selected from references proposed by Blague-Belar et al. (1991) and is 0.80 mg / L for copper, 0.80 mg / L for zinc and 0.60 mg / L for serum iron.

Results and discussion

Frequency of plasma zinc, serum iron and plasma copper deficiency in PLHIV

A total of 93 HIV-positive patients aged of 37.56 ± equal to 2.35 years (median 37 years and mode between 35 and 40 years) were surveyed. The average concentration of plasma zinc found among the latter is 1.10 ± 0.11 mg / L. Those of plasma copper and serum iron levels were respectively 1.08 ± 0.06 mg / L and 0.75 ± 0.12 mg / L. Comparing blood levels of each of these trace elements in relation to the normal values reported by blague-Belar et al. (1991) revealed a frequency of 31.87% of low zinc content, 26.37% of hypo-plasma copper and 46.43% of serum iron deficiency among all PLHIV. These frequencies of trace elements deficiencies are high and reflect the extent of chronic malnutrition detected in Benin (38%) (INSAE *et al.*, 2007).

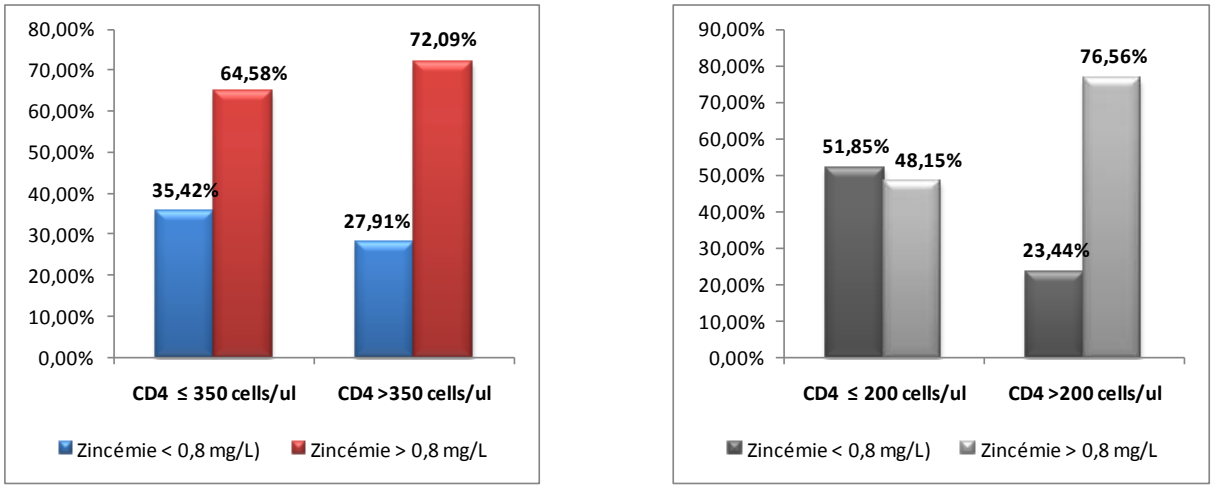


Fig. 1. Comparison of frequency of zinc deficiency based on the immune status set from two reference values of CD4 T lymphocytes.

1 A (left): comparison of groups G1 (CD4> 350 cells / ul) and G2 (CD4 ≤ 350 cells / ul)
$X^2 = 0.589$, $p = 0.443$

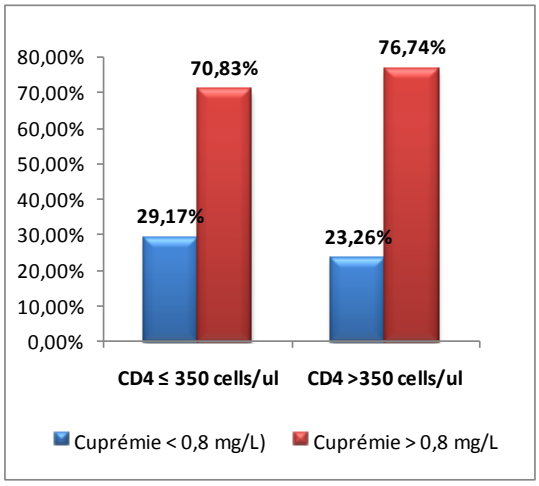
1 B (right): comparison of groups G3 (CD4> 200 cells / ul) and G4 (CD4 ≤ 200 cells / ul)
$X^2 = 7.061$, $p = 0.008$

The frequency of zinc deficiency exceeds relatively the 25% found by Rousseau et al. among HIV-positive patients in France (Maurisson, 2010), but it is less than the rate of 56% of HIV positive drug users detected in the United State (Baum, 2000., Lai, 2001). These are accompanied by zinc deficiency in 65.55% of cases of copper deficiency demonstrating the correlation observed (Abi et al., 2001) between these two trace elements ($p = 0.008$ and average ratios Zinc/Copper ∈ [0 , 97 - 1.21]). In addition, deficiencies in zinc and / or copper are not varied by sex ($p = 0.848$ and $p = 0.950$ respectively) unlike iron which significant differences were observed between the frequency of deficiencies in men (28, 57%) and women (69.64%). The fact that women with HIV are twice as iron-deficient than men (Table 1) is linked to monthly losses (due to menstruation) in 50% of women, 12.5 to 15 mg of iron outside the ordinary losses (through sweat, urine or feces) known among all adults (Hercberg, 1988).

Correlation between the frequency of deficiencies in trace elements and degree of immunosuppression of PLHIV.

Considering the threshold value of 350 T lymphocytes CD4/µL, immunocompromised

patients with a CD4 count ≤ 350 cells / microL (group G2) are affected by zinc deficiency at 35.42% while those with CD4> 350 cells / uL (Group G1) are achieved in an amount of 27.91% (Figure 1A). However, this difference was not significant (p = 0.443). However, when the reference threshold brings back to 200 cells / µL, we observe that very immunocompromised HIV patients with a CD4 count ≤ 200 cells / microL (group G4) were significantly affected (p = 0.008) by deficiencies zinc compared to those with CD4> 200 cells / uL (Group G3) is 51.85% and 23.44%, respectively (Fig. 1B). This positive correlation between zinc deficiency and immune status was observed in a longitudinal study in the United States (Baum, 2000., Lai, 2001., Maurisson, 2010). They had shown that zinc deficiency is correlated (p <0.002) in advanced stages of HIV infection (CD4 ≤ 200/µL) and inadequate dietary intake of zinc (less than 9.34 mg / day) are significantly associated with an increased risk of mortality (p<0.03) among PLHIV (Maurisson, 2010; Baum, 2000). Indeed, zinc play a role in the immune system (Nvondo, 2000., WHO and FAO, 2002., Claeyssen, 2009., Bunupuradah et al., 2012). The decrease of its concentration in the blood promotes the sensitivity of the body to several pathogens responsible for opportunistic infections and other immunological disorders (Claeyssen, 2009), most of which are corrected by supplementation zinc (Baum, 2000., Claeyssen, 2009., Kaiser et al., 2006). That is why this type of nutritional intake is recommended for HIV-infected children especially if chronic diarrhea occurred (WHO, 2010b).



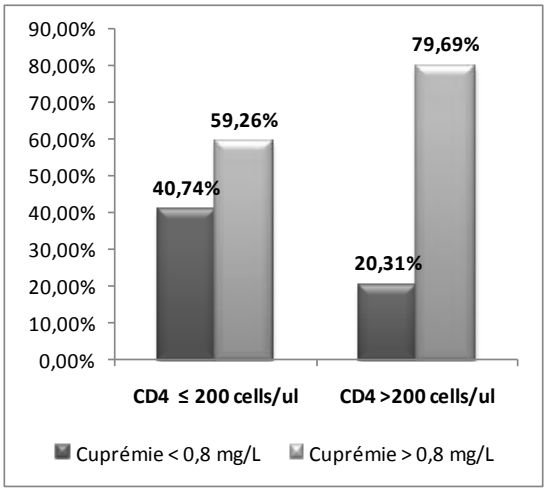


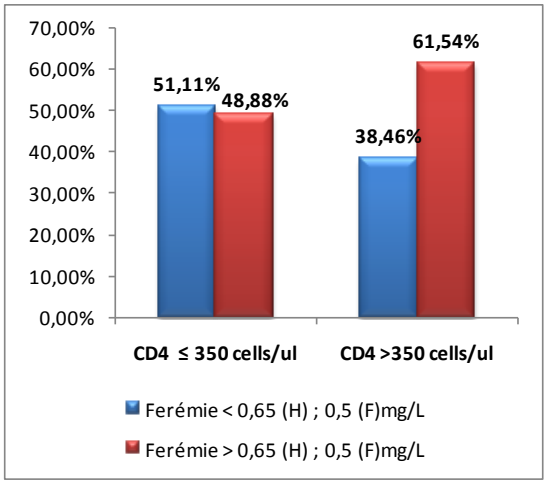
Fig. 2. Comparison of frequency of copper deficiency based on the immune status set from two reference values of CD4 T lymphocytes.

2 A (left): comparison of groups G1 (CD4> 350 cells / ul) and G2 (CD4 ≤ 350 cells / ul)
$X^2 = 0.408$, p = 0.523

2 B (right): comparison of groups G3 (CD4> 200 cells / ul) and G4 (CD4 ≤ 200 cells / ul)
$X^2 = 4.081$, p = 0.043

Regarding the copper deficiency, they are like zinc, twice as frequent (Fig. 2B) in HIV-positive group G4 (with CD4 ≤ 200/µL) compared with that in group G3 whose frequency is no less important (20.31%). This correlation proved by considering the limit of 200 CD4/µL was not observed with the threshold of 350 CD4/µL (Fig. 1B). Rousseau et al., comparing two groups classified positive sides of the limit of 250 CD4/µL (admitted in their study for the implementation the patients under HAART), found that the difference between the frequencies of low plasma copper is not statistically significant (Maurisson, 2010). Indeed, the use of a single value (350, 250 or 200 cells / uL) as reference limit does not always allow to classify PLHIV because in reality the influences of age (Centers for Disease Control and Prevention, 1994., Wananukul et al., 2003), sex (Urassa et al., 2003), schedule (Ritchie et al., 1983

Paglieroni *et al.,* 1994) ethnic group or geographic location (Kassu et al., 2002; Wolday, 2002) on this marker of immunity are not negligible. this leads by example, that patients whose CD4 measured values are slightly above 350 cells / uL for example, can be treated among non-immunocompromised (having no need of ARVs) when in reality their system already weakened immune as well as that of patients with less than 350 cells / uL (which are treated systematically with immunocompromised needing ARVs). In principle, it is recommended that, like many other biological markers used in explorations paramedical each country to define the laboratory reference intervals for CD4 in its target populations (Clinical and Laboratory Standards Institute, 2000; Jones and Barker, 2008; CTB, 2010; Sagnia *et al.,* 2011).



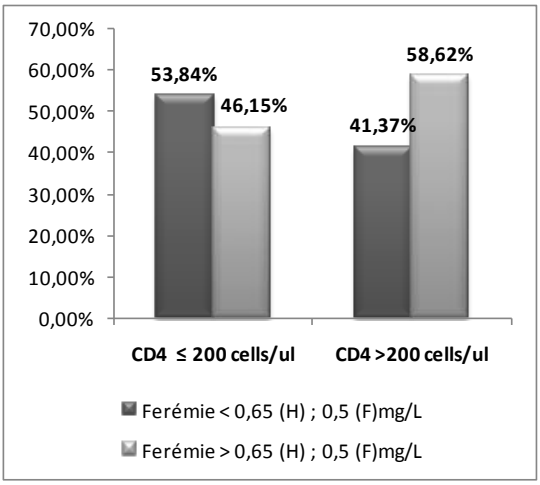


Fig. 3. Comparison of frequency of iron deficiency based on the immune status set from two reference values of CD4 T lymphocytes.

3A (left): comparison of groups G1 (CD4> 350 cells / ul) and G2 (CD4 ≤ 350 cells / ul)
$X^2 = 1.126$, p = 0.289

3B (right): comparison of groups G3 (CD4> 200 cells / ul) and G4 (CD4 ≤ 200 cells / ul)
$X^2 = 1.349$, p = 0.245

The frequency of iron deficiency did not change significantly regardless of the comparison groups (Fig. 3A and 3B) classified according to levels of 200 and 350 CD4/µL (p = 0.289 and 0.245 respectively). Rousseau et al have also made the same observation (Maurisson, 2010). However, the high proportion of PLHIV with iron deficiency (about one in two) corroborates the assertion of Roussel et al. (2009) that state"infections cause a rapid decrease in serum iron" and back iron deficiency promotes immune decline. According to Besson et al. (2006), deficits in iron increase the risk of infections and decrease the body's ability to resist attacks against pro-oxidative, such as free radicals (Perron et al, 2009). This reduction of antioxidant power of the body can also be observed in case of deficiency of many micronutrients (Richard et al., 1997., Perron et al., 2009). Indeed, trace elements such as copper and zinc, manganese, selenium serve as cofactors (Pincemail, 2000, Mistry HD, and Williams, 2011) to antioxidant enzymes (glutathione peroxidase, superoxide dismutase, catalase, etc.).

Conclusion

This study revealed high frequencies of deficiencies in trace elements among PLHIV population at Bethesda Hospital in Cotonou ie respectively 31.87% for plasma zinc, 26.37% for plasma copper and 46.43% for serum iron. The comparisons have confirmed that people with a CD4 count ≤ 200 cells / uL had greatest risk of being deficient in zinc and / or copper. The existence of a statistical relationship between the frequency of deficits in trace elements and the number of lymphocyte T CD4 could not be demonstrated when comparing groups either side of the limit of 350 CD4/µL. Sex significantly influenced

iron deficiency and serum but not those of zinc and copper. These data are the starting point of an epidemiological investigation leading to the identification of major risk factors contributing to micronutrient deficiencies among PLHIV in Cotonou and the proposition of solutions to overcome this food phenomenon while strengthening the immune system of body.

Acknowledgements

We thank the authorities of the National Program to Fight against AIDS and those of the institution GIP ESTHER especially Dr. Lise H. POURTEAU ADJAHI for their guidance in this work. We also thank the staffs of Bethesda Hospital and patients who have freely consented to participate in this study. We can't forget Mr Pierre ALAKPATO of the Company OMEGA Diagnostics for their support in laboratory reagent and Mr Elias POGNON, Chemical Engineer at the Laboratory of National Directorate of Public Health in Benin.

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