

Original Research Article

25-OH-Vitamin D level and calcium/phosphorus metabolism disorders in patients living with HIV in Abidjan

ABSTRACT

Aims: The present study was to evaluate the serum level of calcium, phosphorus and 25 (OH) vitamin D during HIV infection; precisely in patients living with HIV (PLHIV).

Study Design: Prospective and experimental study, involving a cohort of blood samples from HIV-negative and positive subjects.

Place and duration: The study was carried out in the clinical and fundamental biochemistry Department of the Institut Pasteur of Côte d'Ivoire from June 2014 to December 2014.

Methodology: A cohort of 220 samples from adult blood samples including 110 HI-negative controls and 110 samples from subjects PLHIV were analyzed. After confirmation of HIV status (through HIV rapid test) of subjects, CD4 lymphocytes were measured through flow cytometry analysis using an automated FacsCalibur™. Analysis of calcium and phosphorus electrolytes was performed on the Cobas Integra 400 Plus and 25 (OH) vitamin D on the automated Mini VIDAS, from the sera obtained from different blood samples from HIV positive and negative subjects.

Results: The identified disorders of the PLHIV were hypocalcemia ($p = 0.0009$) and hyperphosphoremia ($p < 0.0001$). 57% of PLHIV against 67% of the controls subjects showed normal levels of 25 (OH) vitamin D ($P > 0.05$).

Conclusion: We noticed a good level of 25 (OH) Vitamin D reserve in PLHIV among more than 50% of PLHIV. Calcium and Phosphorous metabolism disorders observed in people living with HIV in Côte d'Ivoire are likely due to a deficiency of the active form 1,25 (OH)₂ vitamin D.

Keywords: 25-OH-Vitamin D, Calcium, Côte d'Ivoire, HIV, Phosphorus

1. INTRODUCTION

HIV use nutrients especially micronutrients (vitamins and minerals) in the body for its replication [1]. This causes long-term metabolic disorder [2]. Calcium and phosphorus metabolism is involved in homeostasis of the human organism [3]. Its disorders are responsible for metabolic disorders (vitamin D deficiency, Parathyroid hormone (PTH) [4]. Vitamin D, fat-soluble vitamin very essential to the body organism, involve in the maintenance of calcium and phosphorus homeostasis [5]. 25 (OH) Vitamin D is the bulk storage form of vitamin D in the body and is hydrolyzed in the kidney to 1-25-dihydroxyvitamin D, the active form of Vitamin D [6]. Indeed, many National and International Medical Societies recommend to measure 25OH vitamin D at least in any situation of bone fragility, in malabsorptions, in chronic kidney disease, in any phosphocalcic pathology, etc. [7].

In Côte d'Ivoire, HIV prevalence in the population is 3.7% [8]. Very few studies have been conducted concerning calcium phosphate metabolism and vitamin D during HIV infection. Evaluation of calcium and phosphorus levels during HIV infection is very important in order to assess the vitamin D status and to examine its concentration level in PLHIV. The main objective was to evaluate the calcium, phosphorus and 25 (OH) vitamin D serum concentration levels in patients living with HIV compared to HIV negative (HIV-).

2. MATERIAL AND METHODS

The prospective and experimental study, case-control, involving a cohort 220 adult subjects (males and females) HIV positive was conducted from June 2014 to December 2014. The blood samples used in the study came from the medical center of the Institut Pasteur of Cote d'Ivoire (IPCI) and the Community Health Center of Yopougon Toit Rouge (Abidjan). The controls consisted of HIV-negative subjects. However, HIV-positive pregnant women, elderly women 50 years and over were excluded from this study. Vitamins supplements are excluded in the therapeutic management of people living with HIV. The whole blood samples from HIV positive and negative subjects were collected into tubes without anticoagulant (dry tubes) and tube with ethylene diamine tetra acetic (EDTA) for measuring CD4+ T cells. Sera

obtained after centrifugation at 3000 rev / min for 5 min from dry tubes were used for HIV serological tests and for the determination of calcium, phosphorus electrolytes and 25 (OH) vitamin D.

2.1 Biochemical Analysis

After confirmation of HIV status of subjects involved by rapid HIV test DETERMINE [9] and GENIE II [10]. The CD4 cell count was carried out using flow cytometry technology (FacsCalibur) which the principle is based on the rapid analysis of particles (cells) in a movement that scroll to an inside of a sheath liquid, in front of a light beam to the laser beam [11]. Electrolyte parameters of calcium and phosphorus were performed on the analyzer Cobas 400 Plus. The principle is based on the reaction of TRINDER = enzymatic method + colorimetric using a chromogen [12]. The deepness of the color is directly proportional to the concentration of the substances measured. The assay of the 25 (OH) vitamin D was performed by combining the competitive enzyme linked immunosorbent assay to a final fluorescence detection (enzyme linked fluorescent Assay ELFA) on an automated "mini VIDAS" of Biomerieux brand. The value of the fluorescence signal is inversely proportional to the concentration of antigen present in the blood sample. The reference values of 25 OH Vitamin D are: Deficient (< 20 ng/mL); Insufficient (20-29 ng/mL); Sufficient (30-100 ng/mL); Potential toxicity (> 100 ng/mL) [13].

2.2 Statistical Analysis

The statistical analysis was performed using the Excel spreadsheet for calculating means and standard deviations, Student's t test for comparison of means and the Chi2 test for independence or dependence between two characters (significance threshold $p < 0.05$; CI 95%).

3. RESULTS

3.1 Epidemiology of HIV Infection in the study population

Of the 220 samples from PLHIV analyzed for the study of the 25 OH VITD, Women are more infected (130; 59.09 %) than men (40; 40.91%). The average age of the study population was 37 ± 1.59 years for PLHIV and 26 ± 8.39 years for HIV-controls ($P < 0.0001$) with a predominance of HIV-1 serotype (98/100; 98%).

Results should be clearly described in a concise manner. Results for different parameters should be described under subheadings or in separate paragraph. Table or figure numbers should be mentioned in parentheses for better understanding.

3.2 Phosphorus, calcium and 25 OH Vitamin D profiles

The mean calcium values, in PLHIV were significantly lowered (2.08 ± 2.27 mmol/L) than in controls subjects (2.30 ± 0.97 mmol/L) $p = 0.0009$. In contrast phosphorus mean values were significantly higher in people living with HIV (2.13 ± 4.69 mmol/L) than in controls subjects (1.10 ± 1.26 mmol/L) $p < 0.0001$.

Deficiency of 25 OH Vitamin D was observed in 43% of PLHIV and 33% of controls. While 57% of PLHIV and 67% of control showed normal levels of 25 OH Vitamin D (Fig 1).

The average calcium values in PLHIV were lowered and were almost the same value in sexes, 2.08 ± 2.7 mg/L in men and 2.10 ± 3.7 mg/L in women respectively. However, with phosphorus (reference value $0.90 - 1.45$ mmol/L), the mean values were higher 2.42 ± 6.2 mg/L in women with HIV than in men 1.84 ± 6.8 mg/L. This increased value in women with HIV is highly significant compared to controls subjects ($p < 0.0001$) (Table 2). The Mean values of 25 OH Vitamin D in PLHIV is not significantly different from those of witnesses ($P > 0.05$) (Table 1)

The mean concentrations values of calcium and phosphorus vary insignificantly according to WHO classification of CD4 in PLHIV. However, the average values of 25 OH Vitamin D in PLHIV vary not significantly ($P = 0.77$) according to WHO classification of CD4 count (Table 2).

4. DISCUSSION

Hypocalcemia and hyperphosphatemia observed in patients living with HIV showed a disorder of mineral metabolism. Indeed, hypocalcemia is due to a deficiency of 1,25-dihydroxy-vitamin-D3 caused by decreases in secretion of parathyroid hormone and also inactivation of extracellular calcium receptor (CaR) [14]. Therefore, there will be no distribution of calcium from the cell into the blood [15]. The inflammatory process due to virus replication causes hypocalcemia [16]. Hyperphosphoremia is due to the low serum parathyroid hormone (PTH) level and the inhibitory action of the FGF-23 on the α -1-hydroxylase. There will be no phosphate reabsorption in the proximal tubule [15]. However, certain antiviral molecules involving nucleotide analogs to nucleoside analogues can cause damage and kidney

dysfunction which leads to the reduction of the phosphorus filtration rate in the kidney thus raising its level in the blood serum [17].

Sufficiency in 25 (OH) vitamin D reserves observed in our study could be explained due to the fact that Cote d'Ivoire is a tropical country with heavy sunshine, the major intakes pathway of vitamin D to the body is the way of skin synthesis [18]. The deficiency cases observed in control subjects may be due to consumption of diets poor in vitamin D, since its supply has two origins (food and synthesis). In PLHIV, this study showed calcium and phosphorus disorders and an important quantity of 25 (OH) vitamin D reserves. This is due to abnormality in the nuclear Vitamin D receptor (VDR). The second hydroxylation of 25 (OH) vitamin D into the active form 1, 25-(OH) 2 vitamin D3 via VDR will not be performed. This would cause disorders in the calcium and phosphorus metabolism [19]. Furthermore, there is no relationship between the level of calcium and phosphorus metabolism disorders, 25 (OH) vitamin D and the degree of immune suppression [20].

Table 1. Average concentrations of calcium, phosphorus and 25-OH- vitamin D in patients HIV and control subjects according to gender

	PLHIV N= 100		Control N = 100		P < 0.05
	Male (n=50)	Female (n=50)	Male (n=50)	Female (n=50)	
Calcium (mmol/L)	2.08 ± 2.7 [*]	2.10 ± 3.7 [#]	2.25 ± 1.3 [*]	2.35 ± 1.4 [#]	[*] 0.0225 [#] 0.0165
Phosphorus (mmol/L)	1.84 ± 6.8 ^{**}	2.42 ± 6.2 ^{##}	1.03±0.92 ^{**}	1.19 ± 2.3 ^{##}	^{**} 0.0004 ^{##} < 0.0001
	PLHIV N= 120		Control N = 120		P < 0.05
	Male (n=40)	Female (n=80)	Male (n=40)	Female (n=80)	
25-OH-VITD (ng/mL)	36 ± 2.83 [*]	31 ± 1.91 [#]	37 ±2.58 [*]	33 ± 2.11 [#]	[*] 0.75 (NS)

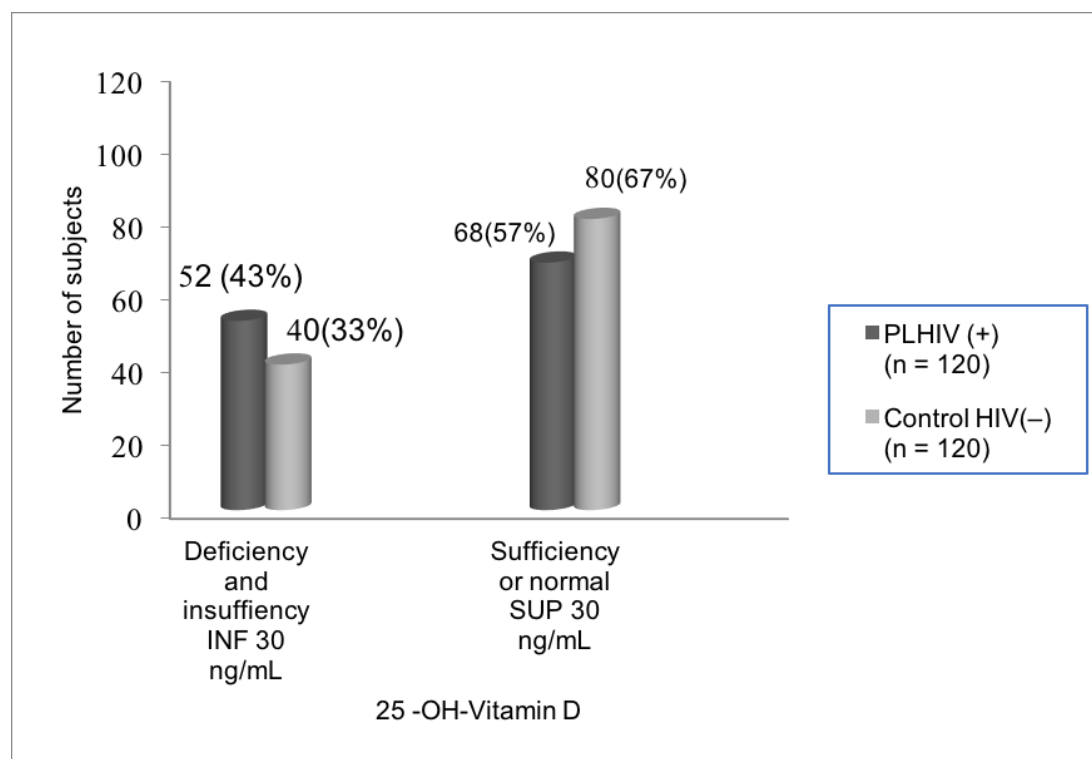
0.62 (NS)

Reference values of vitamin D: 30-100 ng/mL

Table 2. Average concentrations level of calcium, phosphorus and 25(OH) Vitamin D in PLHIV according to CD4⁺ count

CD4 ⁺ Range	PLHIV (N = 100)	Calcium		Phosphorus	
		Mean concentration (mg/L)	Chi2 Pearson P*	Mean concentration (mg/L)	Chi2 Pearson P*
> 500	17	1.98 ± 5.39	0.39 (NS)	1.87 ± 4.41	0.17 (NS)
499 – 350	28	2.10 ± 4.71	0.30 (NS)	1.71 ± 4.86	0.25 (NS)
349 – 200	42	1.98 ± 4.18	0.45 (NS)	2.48 ± 7.49	0.35 (NS)
< 200	13	2.33 ± 2.27	0.32 (NS)	2.90 ± 14.46	0.17 (NS)

25 OH Vitamin D				
CD4 ⁺ Range	PLHIV (N = 120)	Mean concentration (mg/L)	Chi2 Pearson P*	Chi2 Pearson P*
> 500	28	31 ± 5.5	0.64 (NS)	0.77(NS)
499 – 350	32	33 ± 7.0	0.38 (NS)	
349 – 200	36	32 ± 11	0.61 (NS)	
< 200	24	37 ± 11	0.41 (NS)	



5. CONCLUSION

Calcium and phosphorous metabolism disorders observed during HIV infection are likely due to a deficiency of 1,25 (OH) 2 vitamin D therefore, further study of parathyroid hormone, calcitonin and on the mechanism of action of vitamin D and its nuclear receptors is required. The serum calcium and phosphorus level monitoring is very important before and during antiretroviral treatment to prevent bone complications.

ETHICAL SATATEMENT

The study was conducted in accordance with the Helsinki Declaration 2000 on HIV and AIDS research conducted in poor countries and to the local legislation of the national program on treatment management for People Living with HIV / AIDS (decree No. 411 of December 23, 2001).

Informed consent was obtained from patients for the use of their blood for research purpose after completing the standard analyzes prescribed by their doctor.

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ABBREVIATIONS

AIDS: Acquired Immune Deficiency Syndrome

CaR: Calcium Receptor

EDTA: Ethylene diamine tetra acetic

188 FGF-23: *Fibroblast growth factor 23*
189 IPCI: Institut Pasteur of Cote d'Ivoire
190 PLHIV: Patients Living with HIV
191 PN PEC PLHIV/ AIDS: National Program on treatment management for People Living with
192 HIV / AIDS
193 PTH: Parathyroid hormone
194 VDR: Vitamin D receptor
195 VIT D: Vitamin D
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