

Prevalence of hypovitaminosis D and its association with oral lesions in HIV-infected Brazilian adults

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ABSTRACT

Introduction: This study assessed the prevalence of hypovitaminosis D and its association with oral candidiasis and clinical parameters of periodontitis (CPP) in HIV-infected patients. **Methods:** Periodontal examinations for the 113 HIV-infected patients were recorded using the Community Periodontal Index. A cytological smear from the lateral borders of the tongue was performed to evaluate candidiasis. **Results:** The frequency of hypovitaminosis D was 23.9%. In multivariate analysis, only the duration of exposure to HIV was associated with CPP [OR 4.72 (95% CI: 0.97-23.00)]. **Conclusions:** The prevalence of hypovitaminosis D was 23.9% and was not related with oral candidiasis or CPP.

Keywords: Hypovitaminosis D. HIV infection. Chronic periodontitis.

A high prevalence of low vitamin D (cholecalciferol/vitamin D3; ergocalciferol/vitamin D2) levels has been reported in human immunodeficiency virus (HIV)-infected patients⁽¹⁾⁽²⁾⁽³⁾⁽⁴⁾. In particular, vitamin D supplementation has been recommended for HIV-infected patients undergoing non-nucleoside reverse transcriptase inhibitor-based (NNRTI) treatment^{(5) (6) (7) (8)}. Furthermore, vitamin D deficiency has been related with disease progression and complications⁽⁷⁾.

Vitamin D is a steroid hormone; the endogenous form (cholecalciferol/vitamin D3) is synthesized in the skin by the action of ultraviolet rays, and the exogenous form (ergocalciferol/vitamin D2) is acquired through dietary consumption or supplements^{(6) (9)}. The active form of vitamin D (1,25-dihydroxyvitamin D)^{(6) (9)} is a potent immunomodulator with receptors in macrophages and monocytes as well as T and B lymphocytes. Therefore, vitamin D deficiency is associated with the risk of chronic diseases such as osteoporosis, cancer, obesity, and cardiovascular diseases⁽⁶⁾.

Because vitamin D plays an important role in bone maintenance and immunity, deficiency might adversely affect the periodontium, and patients with vitamin D deficiency reportedly receive minimal benefits from periodontal surgery⁽¹⁰⁾.

Furthermore, patients supplemented with calcium and vitamin D have better periodontal health than those who are not⁽¹¹⁾⁽¹²⁾.

Oral candidiasis, which is an infection produced by fungi of the genus *Candida* (*Candida spp.*), is also linked to vitamin D deficiency⁽¹³⁾. Oral candidiasis occurs by the combination of host, fungal, and oral microenvironment-modifying factors. A relationship between vitamin D deficiency and oral candidiasis has been observed in HIV-infected patients; however, this relationship was not dependent on the number of CD4⁺ T lymphocyte cells⁽¹³⁾.

To date, the relationship between oral candidiasis, periodontal disease, and vitamin D deficiency in HIV-infected individuals in Brazil has not been studied. Thus, the aim of this study was to estimate the prevalence of hypovitaminosis D and its association with oral candidiasis and clinical parameters of periodontitis (CPP) in a group of HIV-infected patients in the City of Maceió, Alagoas, Brazil.

Participants for this study were recruited from April to October 2014 at the Hospital University Professor Alberto Antunes (HUPAA) of the Federal University of Alagoas. All patients had a clinical and laboratorial diagnosis of HIV infection, were ≥ 20 years of age, and had ≥ 15 teeth. The exclusion criteria included all conditions that could possibly be associated with deficiency/insufficiency of vitamin D. This research was conducted ethically in accordance with the Declaration of Helsinki, and the study protocol was approved by the Review Committee for Human Subjects of HUPAA.

The demographic data included name, age, sex, level of education, oral hygiene practices, and adverse oral habits.

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Laboratory data (absolute CD4+ T lymphocyte levels and plasma HIV viral load), clinical medical information, and antiretroviral use were collected from the patients' medical records.

The main variable of interest was hypovitaminosis D (deficiency/insufficiency of vitamin D), which was assessed using plasma 25(OH)D levels that were determined using a chemiluminescence immunoassay technique (ARCHITECT Reagent Kit, Abbott Laboratories of Brazil Ltda). Hypovitaminosis D was defined as 25(OH)D levels <30ng/mL (<74.9nmol/L), and plasma vitamin D levels were considered sufficient at ≥ 30 ng/mL.

After a clinical medical examination, a clinical oral assessment, including a visual inspection of the oral mucosa and periodontal evaluation, was performed for all participants. Before the oral examination, a cytological smear was obtained from the lateral borders of the tongue to identify the presence of spores, yeast cells, and pseudohyphae suggestive of *Candida* species.

Periodontal measurements were recorded using the Community Periodontal Index (CPI) as an estimate of periodontitis. The oral cavity was divided into six sextants, with tooth indexing in each: first (17/16), second (11), third (26/27), fourth (36/37), fifth (31), and sixth (47/46) sextants. The teeth were examined using a periodontal probe with two marks located at 8.5 and 11.5mm. The following CPI codes were assigned: 0, healthy cases; 1, bleeding on probe; 2, calculus; 3, periodontal pocket depth (PPD) of 4-5mm; and 4, PPD of 6mm. Clinical attachment level (CAL) was recorded for each of the tooth indices. CPPs were defined as the presence of at least one sextant with CPI = 3 and CAL ≥ 4 .

To standardize the sampled data, the results from three examiners were calibrated during theoretical (4 hours) and practical (12 hours) clinical courses. The Kappa index for intra-examiner agreement of the PPD and CAL findings ranged from 0.79 to 0.89 and 0.78 to 0.88, respectively. The Kappa index for inter-examiner agreement varied from 0.77 to 0.87 and 0.75 to 0.88 for PPD and CAL, respectively. Kappa index values <0.40 indicate poor agreement, 0.40-0.75 indicate good agreement, and >0.75 indicate excellent agreement.

All statistical tests were performed using SPSS software, version 19.0 (IBM, São Paulo, SP, Brazil). Bivariate analyses to compare socio-demographic characteristics, duration of exposure to HIV, highly active antiretroviral therapy (HAART) use, efavirenz use, smoking, absolute CD4+ T lymphocyte levels, plasma HIV viral load, and CPP based on hypovitaminosis D were conducted using Chi-square tests, Fisher's exact tests, or Mann-Whitney tests, as appropriate. Unconditional univariate and multivariate logistic regression analyses were performed to assess the association between hypovitaminosis and CPP; the latter was adjusted for all of the variables in the univariate analyses as potential confounders. As only one case had a clinical diagnosis of oral candidiasis, this variable was not included. Significance was established at 5% for all tests.

Table 1 presents the characteristics of the sample (n = 113). The majority of the participants reported sexual transmission

(85.8%), >5 years of exposure to HIV (64.6%), had CD4+ T lymphocyte levels >200 cells/mm³ (70.8%), and were undergoing HAART (94.8%).

Oral lesions were observed in 24 (21.3%) of the 113 patients, and prosthetic stomatitis (9.7%) and unspecific ulcers (6.2%) were the most frequent oral lesions (**Table 2**). Hypovitaminosis D was present in 27 (23.9%) patients (**Table 2**). Cytological smears from the lateral borders of the tongue were positive in 11 (15%) patients, suggesting an oral *Candida* species infection; however, only one (0.9%) case had a clinical diagnosis (**Table 2**).

Hypovitaminosis D and CPP were not significantly associated in the unadjusted or adjusted logistic regression analyses (**Table 3**). However, crude statistical associations were observed between CPP and age (odds ratio [OR] 1.04; 95% confidence interval [CI]: 1.01-1.09) and duration of exposure to HIV (OR 6.69; 95% CI: 1.47-30.41). The results of the multivariate analysis showed that only the duration of exposure to HIV was associated with the outcome; individuals with >5-year exposure to HIV were 4.72 (95% CI: 0.97-23.00) times more likely to have CPP than individuals with a duration ≤ 5 years.

The prevalence of hypovitaminosis D was low (23.9%) and differs from the high prevalences of hypovitaminosis D reported in the literature for HIV-infected individuals⁽¹⁾⁽²⁾⁽³⁾⁽⁴⁾⁽¹⁴⁾. The synthesis of vitamin D is influenced by several factors; of these, latitude is important. High levels of UVB exposure on the skin are of great importance for vitamin D synthesis, thereby increasing serum levels⁽³⁾⁽⁵⁾. The latitude of Maceio in the State of Alagoas, Brazil, is 9°39'57"; consequently, days with sunshine are predominant throughout the year.

CPP and vitamin D levels were not related in the present sample of HIV-infected patients, similar to the findings in healthy individuals of Garcia et al.⁽¹²⁾, who did not find any statistically significant relationship between vitamin D levels and periodontal health.

HAART involves the administration of three or more associated drugs: two nucleotide reverse transcriptase inhibitors and a NNRTI or protease inhibitor⁽¹⁵⁾. In the present study, 94.8% of the participants were undergoing HAART, and 36.5% of the patients were taking efavirenz. Efavirenz, an NNRTI antiretroviral, has been associated with decreased serum vitamin D levels and can be a risk factor for reduced serum vitamin D levels⁽³⁾⁽¹⁴⁾. This could also help explain the possible relationship between HAART and vitamin D status. However, the low prevalence of hypovitaminosis D in the present study might be the result of the low proportions of efavirenz use.

The use of antiretroviral drugs has also been associated with the low frequency of oral lesions in HIV-infected patients⁽¹⁵⁾. In this study, relatively few (21.2%) oral mucosal changes were detected, and cytology for *Candida* species infection was positive in only 11 (15%) patients. Furthermore, only 10 HIV-infected individuals had a CD4+ T lymphocyte count ≤ 200 mm³, contributing to the low frequency of oral lesions. Prosthetic stomatitis was the most (9.7%) frequently diagnosed lesion. Comparatively, the most common oral manifestation reported in HIV-infected patients and especially

TABLE 1 - Characteristics of patients infected with human immunodeficiency virus (n = 113).

Variable	Frequency	
	n	%
Transmission		
sexual	97	85.8
drugs	2	1.8
occupational accident	1	0.9
other	13	11.5
Exposure to HIV (months)		
<1	5	4.4
1-12	8	7.1
12-60	27	23.9
>60	73	64.6
TCD4+ lymphocytes (cells/mm ³ of blood)		
>200	80	70.8
≤200	10	8.8
No registration	23	20.4
HIV plasma viral load		
detectable	40	35.4
undetectable	50	44.2
no registration	23	20.4
HAART*		
yes	91	94.8
no	5	5.2
Efavirenz use*		
yes	35	36.5
no	61	63.5

HIV: human immunodeficiency virus; **HAART:** highly active antiretroviral therapy. *Data available for 96 patients.

TABLE 2 - Frequency of oral manifestations and hypovitaminosis D in 113 patients infected with human immunodeficiency virus.

Manifestation	Frequency	
	n	%
No lesion	89	78.8
Pyogenic granuloma	1	0.9
Actinic cheilitis	1	0.9
Focal fibrous hyperplasia	1	0.9
Traumatic keratosis	1	0.9
Ulcers	7	6.2
Prosthetic stomatitis	11	9.7
Dental abscess	1	0.9
Candidiasis	1	0.9
Clinical parameters of periodontitis	21	18.6
Hypovitaminosis D	27	23.9

TABLE 3 - Univariate and multivariate logistic regression analyses to assess the association between hypovitaminosis D and clinical parameters of periodontitis.

Patient characteristics	Clinical parameters of periodontitis		P*	Crude OR [95%CI]	P	Adjusted OR [95%CI]†	P
	no	yes					
Age (years)	37 (20–70)	46 (33–57)	0.012	1.04 [1.01–1.09]	0.047		
Hypovitaminosis D			0.396				
yes	23 (25)	4 (19.0)		1			
no	69 (75)	17 (81.0)		1.42 [0.43–4.64]	0.565		
Sex			0.178				
female	48 (52.2)	8 (38.1)		1		1	
male	44 (47.8)	13 (61.9)		1.77 [0.67–5.68]	0.248	3.15 [0.88 – 11.20]	0.077
Smoking			0.295				
no	71 (77.2)	18 (85.7)		1			
yes	21 (22.8)	3 (14.3)		0.56 [0.15–2.10]	0.393		
TCD4+ lymphocyte levels¶							
≤200 cells/mm ³	9 (12.5)	1 (5.9)	0.390	1			
>200 cells/mm ³	64 (87.5)	16 (94.1)		2.25 [0.27–19.07]	0.457		
Plasma viral load¶			0.072				
detectable	36 (48.6)	4 (25.0)		1			
undetectable	38 (51.4)	12 (75.0)		2.84 [0.84–9.63]	0.093		
Duration of exposure to HIV infection			0.004				
≤5 years	38 (41.3)	2 (9.5)		1		1	
>5 years	54 (58.7)	19 (90.5)		6.69 [1.47–30.41]	0.014	4.72 [0.97–23.00]	0.048
HAART			0.605				
no	18 (19.8)	4 (19.0)		1			
yes	73 (80.2)	17 (81.0)		1.05 [0.31–3.50]	0.939		
Efavirenz			0.171				
no medication	16 (17.4)	4 (19.0)		1			
no	44 (47.8)	14 (66.7)		0.38 [0.08–1.88]	0.233		
yes	32 (34.8)	3 (14.3)		1.27 [0.36–4.44]	0.705		

OR: odds ratio; **CI:** confidence interval. Values are reported as median (range) or n (%). *P values were determined using Fisher's exact tests or, for age, the Mann-Whitney tests. †Adjusted for age, sex, smoking, TCD4+ lymphocyte levels, plasma viral load, highly active antiretroviral therapy (HAART), duration of exposure to human immunodeficiency virus (HIV), and efavirenz use. ¶Data available for 90 subjects.

those with vitamin D deficiency is oral candidiasis, given that vitamin D is an immunomodulator of T lymphocytes⁽¹³⁾. However, the association between cytology suggestive of *Candida* species infection and hypovitaminosis D was not significant in this study ($p = 0.369$) (data not shown). Moreover, only one lesion was clinically diagnosed as oral candidiasis, but this patient had normal vitamin D levels.

The current study has some limitations; therefore, the findings should be considered with caution. The cross-sectional design using a convenience sample limits the generalizability of the results. In addition, it is virtually impossible to control

for the effects of the predominant sunshine throughout the year in Alagoas; thus, there is no guarantee that our results were not influenced by exposure to sunlight.

Although the current study did not demonstrate a significant association between hypovitaminosis D and the presence of oral candidiasis or CPP, the results showed that these diseases are frequent and therefore the general practitioner needs to know how to solve these problems and thus offer a treatment suitable for HIV-infected patients. Another relevant point of this study is that so far no study was performed with similar methodology in Brazilian individuals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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