# Risk factors associated with hypovitaminosis D in HIV/aids-infected adults

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## ABSTRACT

**Objective:** To investigate risk factors associated with hypovitaminosis D in adult patients infected with HIV/aids, at a referral hospital in Maceió, Brazil. **Subjects and methods:** This cross-sectional study involved 125 patients evaluated from April to September 2013 by means of interviews, review of medical records, physical examination, and laboratory tests. The data were analyzed using the SPSS® software, version 17.0; the prevalence of hypovitaminosis D and mean levels of vitamin D were determined. The association between hypovitaminosis D and the independent variables was assessed using the Chi-square or the Fisher's exact tests; mean vitamin D concentrations were analyzed using Kolmogorov-Smirnov, Mann-Whitney, and Kruskal-Wallis tests. The level of significance was set at 5% across tests. **Results:** The prevalence of hypovitaminosis D was 24%, with a significant association with higher household income ($p < 0.05$). Higher vitamin D levels were associated with female gender ($p < 0.001$), no use of sunscreen ($p < 0.05$), and previous opportunistic infections ($p < 0.01$). Lower values were associated with the use of antiretroviral medication ($p < 0.05$), overweight and obesity ($p < 0.01$). **Conclusion:** Lower vitamin D concentrations were significantly associated with well-known risk factors for hypovitaminosis D: use of sunscreen, antiretroviral medication, overweight, and obesity. The prevalence of hypovitaminosis D in this study, considering values > 20 ng/mL or > 30 ng/mL as vitamin D sufficiency, was lower to that of previous studies with HIV-infected patients, a fact that might be related to the low latitude and high intensity of solar radiation of the location of the present study. Arch Endocrinol Metab. 2015;59(1):34-41

**Keywords**
Vitamin D; vitamin D deficiency; HIV

## INTRODUCTION

Vitamin D is as a steroid hormone that is essential for calcium homeostasis and bone metabolism. The main endogenous source of vitamin D in humans is the irradiation of ultraviolet rays (UVB) on the skin, which convert 7-dehydrocholesterol in vitamin $D_3$ (cholecalciferol) (1). From the skin, vitamin $D_3$ gets into the circulation and undergoes a first hydroxylation in the liver, yielding 25(OH)D (calcidiol), the most abundant and stable vitamin D metabolite, which is considered the best indicator of the status of this vitamin in the body (2). After that, circulating calcidiol reaches the kidneys, where it undergoes new hydroxylation and is converted to $1,25(OH)_2D$ (calcitriol), an active form of the hormone that acts on several types of cells by means of its nuclear receptor (3), stimulating, for example, the intestinal absorption of calcium and bone mineralization, and regulating the synthesis and secretion of parathormone (PTH) (4).

Besides its known effects on the musculoskeletal system, vitamin D deficiency and/or insufficiency (hypovitaminosis D) has been associated to several disorders that include autoimmune diseases, such as diabetes mellitus type 1 (2); cardiovascular diseases, such as arterial hypertension and atherosclerosis (5); neoplasms; obesity (6); insulin resistance, and glucose intolerance (1).

The most important causes of hypovitaminosis D are related to low exposure to sunlight and a poor diet, once its natural occurrence in foods is low, and vitamin D supplementation is not routine everywhere (7). Vitamin D synthesis is proportional to the surface of the body exposed to sunlight, and depends not only on environmental factors, such as latitude, season of the year, time of the day, but also to factors related to the individual and his/her habits, such as type of skin, age, use of sunscreen (8), wearing clothes that cover a large surface of the body, and influence the cutaneous synthesis of vitamin D (7).

In Brazil, studies carried out in the city of São Paulo show the high prevalence of hypovitaminosis D, both in healthy adolescents (9) and in elderly populations, either institutionalized or not (4). In Recife, a city of tropical humid climate, there was a high prevalence of vitamin D deficiency associated with low bone density in a study carried out with menopausal women (10).

Studies reinforce that hypovitaminosis D remains common in several populations all over the world (6,7,11), reaching a 20 to 70% prevalence in the USA, for example (3). This finding is not different in immunocompromised individuals, such as HIV/aids carriers, in whom studies have shown prevalence of vitamin D deficiency reaching 10 to 73% (3,12).

Survival increased substantially in patients with HIV/aids after the introduction and advances of antiretroviral therapy (ART) (13), which is related to a more common development of chronic diseases, such as osteopenia and osteoporosis (14). Besides, HIV infection is also associated with high rates of fractures, including of vertebrae and hips (15).

In HIV carriers, studies relate the association between inadequate levels of vitamin D and several complications, such as hyperparathyroidism (16), systemic arterial hypertension (12), increased weight (17), deterioration of immune function, progression of the disease, and mortality (18).

Besides, the population of HIV-positive patients face several other factors that contribute to increasing the prevalence of hypovitaminosis D, such as the use of ART and its potential effects on the metabolism of this vitamin (19), as well as infection with the HIV virus, which increases inflammatory cytokines, such as tumor necrosis factor α (TNFα), that inhibits renal hydroxylation of vitamin D and decreases its concentrations (13).

In spite of the recent studies showing the high rates of hypovitaminosis D and the consequences in several body functions, it is still necessary to better characterize the hypovitaminosis D profile in HIV-infected patients, once this population is susceptible to the inadequate levels of this vitamin and to the complications related to this condition.

Therefore, the objective of this study was to determine the risk factors associated with low levels of vitamin D, in a population of adult patients with HIV/aids of a referral outpatient facility in the city of Maceió, where sunlight irradiation is high during the whole year (Latitude 09°37'57'1 South).

## MATERIALS AND METHODS

### Selection of the patients

A convenience sample was formed by patients who were HIV carriers older than 18 years of age, and were seen from April to September 2013 at a Specialized Attention Service (SAE) /Day Hospital that belonged to Hospital Universitário Prof. Alberto Antunes at Universidade Federal de Alagoas (HUPAA/UFAL). All participants singes and Informed Consent Form (ICF).

The following inclusion criteria were used: participants should be enrolled in the SAE; should be 18 or older; and should accept to take part in the study by signing the ICF. Patients were excluded from the study if they were under treatment with vitamin D or for osteoporosis; had complications that could be associated with vitamin D deficiency/insufficiency, such as recent hospitalization, chronic renal failure, nephrotic syndrome, pancreatic failure, chronic hepatopathy, active hepatitis or cirrhosis, malabsorptive disorders, active malignancy; or were under active or recent use (previous 3 months) of medications that changed vitamin D status, such as carbamazepine, systemic glucocorticoids, hormones, isoniazid, phenobarbital, phenytoin, or rifampicin.

Interview, analysis of the medical records, physical and laboratory examination were all carried out. During the interview, data were collected on demographic characteristics (gender, age, and type of skin), socioeconomic features (education level and household income), social habits (current alcohol intake or smoking habit), time from diagnosis of the infection and route of transmission; occurrence of opportunistic diseases, chronic diseases not related to HIV (arterial hypertension and/or diabetes); use or antiretroviral therapy (ART) in the previous three months; and sunscreen use. Household income was described as a qualitative variable represented by the number of minimum wages divided into two categories: up to one and two or more minimum wages. Skin type was stratified in six phototypes, according to the Fitzpatrick classification (20). In Brazil, there is a wide variety of skin types due to the high level of miscegenation. Therefore, for the inferential analysis of this study, six skin phototypes were dichotomized into non-black (phototypes I to IV) and black (phototypes V and VI), similar to the study by Saraiva and cols. (21).

The medical record was analyzed for exclusion criteria, and for CD4 lymphocyte T counts and viral load from the four months before the interview period. If these recent results were not available, a new sample collection was requested to the patient.

### Physical examination

Blood pressure was measured, and weight and height were recorded for the body mass index (BMI) to be calculated. BMI was classified according to the guidelines of the World Health Organization (WHO) for adults in low weight (BMI < 18.5 kg/m$^2$), eutrophic (BMI between 18.5 and 24.9 kg/m$^2$), overweight (BMI between 25 and 29.9 kg/m$^2$) and obesity (BMI ≥ 30 kg/m$^2$) (22).

Weight and height were measured with a Welmy anthropometric digital scale. Blood pressure was determined by means of a Unitec mercury column sphygmomanometer, according to VI Brazilian Guidelines for Arterial Hypertension (23).

### Laboratory analysis

For the evaluation of the vitamin D levels, blood samples were collected in tubes with a serum separator gel without additives, and immediately placed on reusable ice at 4 to 8°C, until the moment of the biochemical analysis. The levels of vitamin D were determined using the chemiluminescent microparticle immunoassay (CMIA) ARCHITECT reagent kit (Abbott Laboratórios do Brasil Ltda.), in a laboratory certified by the DEQAS (Vitamin D External Quality Assessment Scheme), an international program for the quality control of vitamin D assays. According to the protocol of the Endocrine Society (11), vitamin D profile is defined as deficient (≤ 20 ng/mL); insufficient (de 21 a 29 ng/mL), and sufficient (≥ 30 ng/mL), and according to the Institute of Medicine (IOM) 20 ng/mL of 25(OH)D is sufficient to maintain bone homeostasis in practically all healthy people (24). Although the adequate levels of vitamin D are debatable, in the presente study, values of 20(OH)D greater than 30 ng/mL were considered sufficient, because they presented better correlations with parameters of evaluation of the bone metabolism, such as calcium absorption, bone mineral density, and PTH levels (11). Besides this is the cutoff value adopted by several studies in HIV-infected patients (12,19,25,26).

### Statistical analysis

Data were stored and analyzed by the SPSS® software (Statistical Package for Social Sciences), version 17.0. Simple and percent frequencies of the variables were determined, as well as the prevalence of hypovitaminosis D, and mean levels of vitamin D.

The comparison between groups with hypovitaminosis D and qualitative variables was carried out using the Pearson's Chi-square test, and when indicated, Fisher's exact test. The Kolmogorov-Smirnov test followed by Mann-Whitney test the was used in the comparison of hypovitaminosis D with the quantitative variable age.

The comparison of the distribution of vitamin D mean concentrations with the qualitative variables was initially carried out the Kolmogorov-Smirnov test. As the levels of vitamin D did not show normal distribution, the Mann-Whitney test was used in the comparison between two groups (gender, use of sunscreen, etc.), and the Kruskall-Wallis test was used when more than two groups were involved (BMI, routes of transmission, and education level). In order to assess if there was a correlation between the quantitative variable age and the levels of vitamin D, Spearmen's correlation coefficient was determined.

The significance level adopted for all the tests as 5%.

This study was approved by the Research Ethics Committee (REC) of the Centro Universitário Cesmac.

## RESULTS

From April to September 2013, a total of 224 patients were seen at the SAE and, among these, 144 were carriers of HIV/aids. From these, 125 fulfilled the inclusion criteria for the study, and made up a sample of 64 women (51.2%) and 61 men (48.8%), with minimum age of 19 and maximum age of 70 years old (mean of 40.29 ± 11 years).

The basal characteristics of the patients are presented in table 1. In relation to the skin phototype, the most frequent one was IV (35.2%) and, after this variable was dichotomized, there was a predominance of the non-black group (69.6%). Most had low education level and low household income, did not use sunscreen

(82.4%), did not smoke (76%) and did not report alcohol intake (68%)

Hypovitaminosis D was observed in 24% of the total population of the study, including 22.4% with insufficient vitamin D and 1.6% with vitamin D deficiency.

Mean level of vitamin D was 39.3 ± 12.8 ng/mL (minimum of 18.2 and maximum of 93.2 ng/mL).

As for the clinical and treatment characteristics (Table 2), the most prevalent route of transmission was sexual contact, with more than 5 years since diagnosis. Most patients (56.8%) never presented opportunistic infections, and used ART (83.2%). Mean LT CD4 count was 573.59 cells/mm³. This variable was later on dichotomized in CD4 > 200 and CD4 ≤ 200, and 76.8% of the patients presented CD4 > 200 cell/mm³. As for viral load, this variable was dichotomized in detectable (> 50 copies/mL) or undetectable (≤ 50 copies/mL), with 48% of the participant presenting undetectable levels, and 44% presenting detectable levels. Most patients did

**Table 1.** Frequency distribution according to socio-demographic and behavioral characteristics

| | Frequency | % |
|---|---|---|
| Gender | | |
| Female | 64 | 51.2 |
| Male | 61 | 48.8 |
| Household income | | |
| Up to one minimum wage | 70 | 56 |
| Two or more minimum wages | 55 | 44 |
| Use of sunscreen | | |
| Yes | 22 | 17.6 |
| No | 103 | 82.4 |
| Skin phototype | | |
| Type I – very white | 1 | 0.8 |
| Type II – white | 15 | 12 |
| Type III – slightly brown | 27 | 21.6 |
| Type IV – brown | 44 | 35.2 |
| Type V – brown to black | 29 | 23.2 |
| Type VI – black | 9 | 7.2 |
| Phototype stratified in two categories | | |
| Black | 38 | 30.4 |
| Non-black | 87 | 69.6 |
| Education level | | |
| Did not finish primary education | 55 | 44 |
| Finished primary school | 33 | 26.4 |
| Finished middle school | 30 | 24 |
| Finished higher education course | 7 | 5.6 |
| Smoking | | |
| Yes | 30 | 24 |
| No | 95 | 76 |
| Alcohol intake | | |
| Yes | 40 | 32 |
| No | 85 | 68 |

Source: Research data.

**Table 2.** Frequency distribution according to clinical and treatment characteristics

| | Frequency | % |
|---|---|---|
| Transmission | | |
| Sexual contact | 108 | 86.4 |
| Drugs | 02 | 1.6 |
| Occupational accidents | 01 | 0.8 |
| Other ways* | 13 | 10.4 |
| Does not know | 01 | 0.8 |
| Time from diagnosis | | |
| < 5 years | 46 | 36.8 |
| ≥ 5 years | 79 | 63.2 |
| CD4 | | |
| > 200 | 96 | 76.8 |
| ≤ 200 | 15 | 12 |
| No record | 14 | 11.2 |
| Viral load | | |
| Undetectable | 60 | 48 |
| Detectable | 55 | 44 |
| No record | 10 | 8 |
| Opportunistic infection | | |
| Yes | 54 | 43.2 |
| No | 71 | 56.8 |
| ART use | | |
| Yes | 104 | 83.2 |
| No | 21 | 16.8 |
| Hypertension | | |
| Yes | 26 | 20.8 |
| No | 99 | 79.2 |
| Diabetes | | |
| Yes | 07 | 5.6 |
| No | 118 | 94.4 |
| BMI | | |
| Low weight | 06 | 4.8 |
| Eutrophic | 62 | 49.6 |
| Overweight | 39 | 31.2 |
| Obesity | 18 | 14.4 |

Source: Research data.
* Vertical transmission, hospital procedures, and blood transfusion.
ART: antiretroviral therapy; BMI: body mass index.

not have hypertension (69.3%), or diabetes (94.4%), and were considered eutrophic (49.6%).

There was no significant association between hypovitaminosis D and the independent variables that were analyzed, except for household income ($p < 0.05$) (data not shown). However, the comparison between mean levels of vitamin D and the independent variables showed statistically significant associations, with higher values for the women ($p < 0.001$); lower values for those subjects that used sunscreen ($p < 0.05$); and for those subjects with higher incomes ($p < 0.05$). The use of ART ($p < 0.05$) and the occurrence of overweight and obesity ($p < 0.01$) were also significantly associated with lower levels of vitamin D. Mean 25(OH)D was also higher in patients with a history of opportunistic infections ($p < 0.01$) (Table 3).

**Table 3.** Mean levels of vitamin D in relation to the independent variables

| | N | Mean (ng/mL) | SD | P value |
|---|---|---|---|---|
| Gender | | | | |
| Female | 64 | 43.91 | ± 15.00 | < 0.001 |
| Male | 61 | 34.51 | ± 7.41 | |
| Sunscreen | | | | |
| Yes | 22 | 34.79 | ± 10.08 | 0.042 |
| No | 103 | 40.31 | ± 13.12 | |
| Household income | | | | |
| Up to one minimum wage | 70 | 42.57 | ± 14.16 | 0.029 |
| Two or more minimum wages | 55 | 35.23 | ± 9.4 | |
| BMI | | | | |
| Low weight | 6 | 40.08 | ± 9.52 | 0.005 |
| Eutrophic | 62 | 42.79 | ± 14.71 | |
| Overweight | 39 | 37.23 | ± 10.24 | |
| Obesity | 18 | 31.77 | ± 6.33 | |
| ART use | | | | |
| Yes | 104 | 37.6 | - | 0.018 |
| No | 21 | 47.6 | - | |
| Opportunistic infection | | | | |
| Yes | 54 | 42.69 | ± 13.49 | 0.008 |
| No | 71 | 36.79 | ± 11.64 | |

Source: Research data. Data on the variables that showed statistical significance.
BMI: body mass index; ART: antiretroviral therapy.

## DISCUSSION

Hypovitaminosis D is a common disorder associated with several clinical conditions, far beyond the maintenance of bone balance. Current literature has shown growing interest in the evaluation of the levels of vitamin D and in factors correlated with HIV/aids carriers, because of the high risk of this vitamin deficiency in these patients. The present study was the first that evaluated the prevalence of hypovitaminosis D and its predictors in a population of patients infected with HIV/aids in the city of Maceió-AL, which is located in a sunny, low latitude (09°37'57"South) region.

As for gender, most of the studies on patients infected with HIV do not show statistically significant differences in relation to hypovitaminosis D (12,16,19). However, in the present study, the mean value of vitamin D was significantly higher in women in relation to men (43.9 ± 15 ng/mL *vs.* 34.5 ± 7.4 ng/mL respectively). In the study by Van Den Bout – Van Den Beukel and cols. (27) female individuals presented higher risk of hypovitaminosis D in an univariate analysis, although this finding was not confirmed by the multivariate analysis of the same study.

Although advanced age is considered a traditional risk factor for hypovitaminosis D (28), this association was not observed in the present study, which is in agreement with previous studies with HIV carriers (12,25). Allavena and cols. (19) did not find significant correlations between age and hypovitaminosis D, either, justifying that their sample was made up of younger patients, with few patients above 60 years of age, similar to what occurred in the present study, in which mean age was 40 years.

The prevalence of hypovitaminosis D was 24% and that of vitamin D deficiency was 1.6%, a result that was considered lower than in other studies of HIV-infected patients (12,19,25,26,29). Although prevalence was not high, it should be emphasized that Maceió is a city located at a low latitude, with high UV index (30), and high sunlight incidence all year long.

Oyedele and cols. (3) observed that the prevalence of vitamin D in studies with patients infected with HIV reached rates ranging from 10 to 73%. The authors reinforced that the variation in the rates of vitamin D deficiency may be related to demographic and site differences, besides other variations related to the population studied. They also state that the methodology employed and the different cutoff points used in the definition of vitamin deficiency/insufficiency are large limitations in cross-sectional studies involving patients infected with HIV.

Most of the individuals in the present study (82.4%) did no use sunscreen, and reported high exposure to sunlight. Although the level of sunlight exposure was not quantified, patients frequently reported walking or depending on public transportation, making their exposure to sunlight longer, and possibly influencing the results of this study in relation to the prevalence of hypovitaminosis D. In Brazil, no foods are supplemented with considerable amounts of vitamin D in a regular diet, and sunlight exposure is the most common source of vitamin D.

In this study, a significant correlation was observed between household income and the dependent variable hypovitaminosis D. Higher levels of 25(OH)D were also observed in lower income subjects. However, Ansemant and cols. (26) cite precarious social conditions as risk factors for hypovitaminosis D in patients infected with HIV. The influence of the socioeconomic levels in the occurrence of hypovitaminosis D is complex and difficult to be determined, once several factors may lead to inadequate concentrations of this vitamin.

Lower levels of 25(OH)D were found in individuals that used sunscreen, corroborating the finding of Matsuoka and cols. (8) who showed a reduction in more than 95% in cutaneous vitamin synthesis due to the use of sunscreen with sun protection factor 30. Studies correlated sunscreen use with vitamin D status in populations of HIV/aids carriers were not found.

As for the education level, no significant correlation was observed with hypovitaminosis, similar to previous studies in patients infected (29) or not with HIV (6). Few studies in the literature make reference to correlations between inadequate levels of vitamin D and the education level of the subjects analyzed.

Most of the studies analyzed did not evaluate the skin phototype, and only classified the patients in relation to race. In this study, there was no statistically significant association between hypovitaminosis D and the non-black (phototypes I to IV) and black (phototypes V and VI) groups. Although the literature shows predominance of low levels of vitamin D in black HIV carriers (5,12,17), the study by Wasserman and cols. (25) did not find an association between hypovitaminosis D and the skin color, either.

In this study, there was no association between alcohol intake and/or smoking with hypovitaminosis D. As for smoking, some studies with HIV carriers (19,25,26) showed a significant association with hypovitaminosis D, while other did not (12,29).

Most of the patients controlled well HIV infection and used ART regularly. It should be emphasized that in Brazil HIV carriers have access to free antiretroviral medication by means of the SUS, the Brazilian healthcare system, making it easier to control the infection. Most patients showed undetectable viral load, and there was no significant association with vitamin D levels, as reported by Crutchley and cols. (17), as well. However, other studies reported a statistically significant relationship between low 25(OH)D concentrations and increased viral load (16,31). A significant association was also observed between the levels of vitamin D and LT CD4 counts. There is no consensus on the relationship between 25(OH)D and LT CD4 levels (32), because some studies showed a significant association (19,32) while others did not (12,17,18,25). Lower mean levels of vitamin D were significantly associated with ART use, corroborating previous findings (12,19,33,34).

In the present study, the time since diagnosis of HIV infection did not show a statistically significant relationship with the levels of 25(OH)D, similar to the findings of Allavena and cols. (19). According to Mueller and cols., patients with more recent diagnosis generally show a more precarious health condition, what favors lower levels of vitamin D (33). As most of the patients had their diagnosis more than five years before, and infection was well controlled, this may have contributed for the low prevalence of hypovitaminosis observed in this study.

Reports on the association between vitamin D and opportunistic infections that define aids are scarce. The levels of vitamin D, in this study, were significantly higher in patients that had already presented opportunistic infections. Mehta and cols. demonstrated low levels of vitamin D associated with the higher incidence of pulmonary infections (18). In the EuroSIDA study, hypovitaminosis D was significantly correlated with a greater risk of aids-related events (35). In the present study, the occurrence of opportunistic diseases was not documented at the time the study was carried out, and was referenced to previous events. It is possible that patients that already presented these diseases took better care of their health, which may reflect higher mean vitamin D levels. However, more studies are necessary to elucidate this issue.

The sample of patients in this study showed low prevalence of hypertension and diabetes mellitus, and there were no significant associations with hypovitaminosis D. However, in the study of Vescini and cols.

(28), it was concluded that hypovitaminosis D was moderately associated with the risk of comorbidities, such as cardiovascular diseases and diabetes. Dao and cols. (12) found a significant association between arterial hypertension and hypovitaminosis D. However, they showed no association with diabetes.

A significant predominance of lower levels of vitamin D was observed in individuals that were overweight or obese. The occurrence of hypovitaminosis D in these individuals may be associated with the sequestration of vitamin D by the adipose tissue, decreasing its bioavailability, as well as to the lower exposure to sunlight in obese people (36). In HIV carriers, there are conflicting data in the literature on the influence of the BMI on the levels of vitamin D. Some studies (5,12,17,29) found a significant correlation between high BMI and vitamin D deficiency, while other did not (19,25).

The present study has as a limitation the use of a convenience sample, making it difficult to extrapolate the results to a larger population. Besides, the cross-sectional design makes it impossible to draw causal relationships. It only suggests associations. The researchers examined all patients that regularly came to the specialized attention service, and may have generated a more homogeneous sample in relation to healthcare and, maybe, a selection bias, once patients who sought medical assistance were those who were examined. However, the relevance of this study is the elucidation of the prevalence of hypovitaminosis D and its associated factors in a population of HIV-infected patients seen at a university hospital located in a city that is sunny all year long. In spite of all the studies on this theme all over the world, few of them have been conducted in Brazil, especially in the northeast region of the country.

The results of this study lead to the conclusion that hypovitaminosis D in HIV infected patients is not rare, corroborating the findings of previous studies that suggested routine administration of this vitamin to these patients. The use of sunscreen, ART, and overweight or obesity, which are traditional risk factors for hypovitaminosis D, were significantly associated with lower levels of this vitamin. The prevalence of hypovitaminosis observed in this study, considering vitamin D sufficiency as both values over 20 ng/mL and 30 ng/mL, was lower than that found in previous studies of HIV-infected patients, a finding that may be related to the characteristics of the location studied, at low latitude and with high sunlight incidence all year long.



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