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FULL PAPER

Factors associated with vitamin D deficiency in HIV-1 infected patients on combination antiretroviral therapy: a case-control study

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SUMMARY

The aim of our study was to assess risk factors associated with vitamin D deficiency among HIV-1-infected patients on combination antiretroviral therapy (cART).
A retrospective, case-control study was conducted to assess risk factors associated with vitamin D deficiency among HIV-1-infected adults on stable cART. Vitamin D deficiency was defined as 25-OH vitamin D concentration <30 ng/mL.
A total of 195 patients (77% males, mean age 49.2 years) were enrolled into the study: 98 subjects with vitamin D deficiency (cases) and 97 with normal vitamin D serum concentration (controls). The mean serum concentration ± standard deviation (SD) of vitamin D was 18.2±6.7 ng/mL among cases and 39.6±13.4 ng/mL among controls. Current cART including tenofovir disoproxil fumarate (TDF) (OR 1.65; 95% CI, 1.31 to 1.94), osteoporosis (OR 1.78; 95% CI, 1.25 to 2.09), males who have sex with males (MSM) risk category (OR 1.59; 95% CI, 1.19 to 2.21), chronic hepatitis C (OR 1.44; 95% CI, 1.17 to 1.86), previous or current cancer (OR 1.47; 95% CI, 1.13 to 1.79), metabolic syndrome (OR 2.57; 95% CI, 1.96 to 2.98), and hepatic steatosis (OR 1.59; 95% CI, 1.17 to 2.05) were significant associated with an increased risk of vitamin D deficiency. On the other hand, current CD4+ lymphocyte count >600 cells/mm^3 and current HIV RNA <20 copies/mL were significantly associated with a lower risk of vitamin D deficiency.
In our case-control study, vitamin D deficiency is associated with TDF exposure, osteoporosis, and metabolic disturbances.

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INTRODUCTION

Vitamin D plays an essential role in calcium and phosphate metabolism and in maintenance of normal bone homeostasis. Vitamin D deficiency has been reported very frequently among HIV-infected persons, and the overall estimated prevalence of hypovitaminosis D in people living with HIV ranges from 70 to 90% (Mueller *et al.*, 2010; Viard *et al.*, 2011; Theodorou *et al.*, 2014). Considering the copious metabolic, anti-inflammatory and anti-neoplastic effects of vitamin D, a possible impact of its deficiency on HIV-related comorbidities has been suggested, so knowledge of risk factors associated with hypovitaminosis D is useful to prevent and properly manage this metabolic abnormality.
A retrospective, case-control study was performed to evaluate significant factors associated with vitamin D deficiency in adult HIV-infected patients on stable combination antiretroviral therapy (cART).

Key words:
HIV, vitamin D, inflammation, risk factor, antiretroviral therapy.

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MATERIALS AND METHODS

We performed a single-center, retrospective, case-control study of HIV-1-infected adult patients followed at our Clinic of Infectious Diseases, receiving a stable cART, and with at least one vitamin D measurement reported between July 1 and December 31, 2017. Stable cART was defined as an unchanged antiretroviral regimen during the 12 months before the vitamin D measurement. Cases were defined as HIV-positive patients with vitamin D deficiency (serum vitamin D concentration <30 ng/mL) and controls as HIV-positive patients with normal vitamin D (serum vitamin D concentration ≥30 ng/mL). Each case was individually matched to one control by age (±5 years), sex and ethnicity. Patients with two or more vitamin D measurements within the enrolment period were included in the study only if all measurements were <30 (cases) or ≥30 (controls) ng/mL, and for these subjects only the latter measurement was considered.
Exclusion criteria were active opportunistic diseases or severe infectious diseases, acute or chronic inflammatory diseases, acute or chronic kidney diseases, acute hepatitis,

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liver cirrhosis, pregnancy, or an underlying treatment with vitamin D. HBV and HCV coinfections were diagnosed by the persistent positivity of HBs antigen or HCV serum antibodies associated with HCV-RNA positivity, respectively. Current alcohol use and intravenous drug dependence were defined as daily alcohol consumption >30 g and ≥1 intravenous drug use within 6 months before starting the vitamin D measurement, respectively.

For each patient the following demographic, clinical, and laboratory data were collected: sex, age, race, mode of acquisition of HIV infection, stage of HIV disease, duration of cART, antiretroviral class use, physical examination, smoking habits, body mass index (BMI), arterial pressure, clinical manifestations, spot urinalysis and serum concentration of 25-hydroxyvitamin D (vitamin D), triglycerides, total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol, glucose, complete liver and kidney function tests, CD4+ and CD8+ T lymphocyte count, HIV RNA, parathyroid hormone (PTH), calcium and phosphorus. All the above-mentioned parameters are routinely collected in our Clinic. The season of vitamin D measurement was registered in association with a low (autumn/winter) or a high (spring/summer) sun exposure.

The serum vitamin D concentration was measured using an Automated Chemiluminescence Immunoassay (CLIA) from the IDS-ISYS System (IDS Immunodiagnostic Systems Holdings, Tine and Wear, United Kingdom), and the inferior detection limit was 2.6 ng/mL. All the plasma samples were analyzed for HIV-RNA level using the automated COBAS AmpliPrep Instrument for specimen processing and the COBAS TaqMan Analyzer for amplification and detection (Roche CobasAmpliPrep/Cobas TaqMan HIV-1 tests version 2.0; Roche Diagnostics, Mannheim, Germany) and the limit of quantification was <20 copies/mL.

Osteoporosis was diagnosed depending on the bone mineral density evaluation by dual-energy X-ray absorptiometry (DXA), performed by the Horizon DXA System (Hologic, Marlborough, Massachusetts, U.S.). Osteoporosis was diagnosed when T-score was <2.5 (Siris *et al.*, 2014). Metabolic syndrome was diagnosed when at least 3 of the following 5 criteria were present: waist circumference >102 cm for men and >88 cm for women; fasting triglycerides ≥150 mg/dL; high-density lipoprotein (HDL) cholesterol <40 mg/dL for men and <50 mg/dL for women; fasting glucose ≥110 mg/dL; blood pressure ≥130/85 mmHg (Huang *et al.*, 2009). Liver steatosis was defined by abdominal ultrasound showing fat level involving >5% of the liver parenchyma (Joseph *et al.*, 1991).

The aim of the study was to compare cases and controls in order to identify the factors significantly associated with vitamin D deficiency among HIV-positive people on cART. Variables were described by using proportions for categorical variables, mean and standard deviation (SD) for continuous variables, while comparisons between groups were performed using a Student *t* test or a Wilcoxon test for normally and non-normally distributed continuous variables, respectively. Groups were compared by using the Chi-square test for categorical variables. Univariate and multivariate analyses were performed to assess variables independently associated with vitamin D deficiency. All variables with a *p* value >0.10 on univariate analysis were included in the maximum model and underwent stepwise selection during multivariate analyses. A *p*-value <0.05 was considered statistically significant.

The study was approved by the Ethics Committee of the S. Orsola-Malpighi Hospital in Bologna and all the enrolled patients provided a signed written consent before participating in the study.

RESULTS

From July to December 2017 a total of 195 patients were enrolled in the study: 98 cases and 97 controls. Overall, mean age of the enrolled patients was 49.2 (8.1) years, 150 patients (76.9%) were men, and 187 (95.9%) were white. Mean current CD4+ lymphocyte count was 598 (214) cells/mm^3, mean duration of HIV infection was 12.2 (5.9) years, and 166 subjects (85.1%) had a plasma HIV RNA <20 copies/mL. The mean vitamin D concentration was 18.2 (6.7) ng/mL among cases and 39.6 (13.4) ng/mL among controls (p=0.023). The patient characteristics according to vitamin D status are summarized in *Table 1*.

Compared with controls, cases showed the following statistically significant differences: cases were more frequently males who have sex with males (MSM) and less frequently heterosexual, less frequently with plasma HIV RNA <20 copies/mL, had a lower mean CD4+ lymphocyte count, and were more frequently affected by chronic HCV infection, osteoporosis, metabolic syndrome, liver steatosis, and cancer. With regard to current cART, cases were more frequently treated with tenofovir disoproxil fumarate (TDF) than controls (*Table 1*).

Cancer diagnosis included a current or previous diagnosis of neoplasm, and was represented mostly by a human papillomavirus (HPV)-associated neoplasm. Particularly, the 29 cases of cancer included 21 (72.4%) cases of HPV-associated anal intraepithelial neoplasia (AIN) and 6 (20.7%) cases of HPV-associated cervical intraepithelial neoplasia (CIN).

The results of univariate analysis regarding factors associated with a significantly increased risk of vitamin D deficiency were: MSM condition, chronic HCV infection, osteoporosis, metabolic syndrome, liver steatosis, cancer diagnosis, and current cART including TDF. On the other hand, factors significantly associated with a lower risk of vitamin D deficiency were heterosexual condition, current CD4+ lymphocyte count >600 cells/mm^3 and current plasma HIV RNA <20 copies/mL (*Table 2*).

Multivariate logistic regression analysis showed that MSM condition (adjusted odds ratio [aOR]: 1.59; 95% confidence intervals [CI]: 1.19 to 2.21), chronic HCV infection (aOR: 1.44; 95% CI: 1.17 to 1.86), osteoporosis (aOR: 1.78; 95% CI: 1.25 to 2.09), metabolic syndrome (aOR: 2.57; 95% CI: 1.96 to 2.98), liver steatosis (aOR: 1.59; 95% CI: 1.17 to 2.05), cancer diagnosis (aOR: 1.47; 95% CI: 1.13 to 1.79), and current cART including TDF (aOR: 1.65; 95% CI: 1.31 to 1.94) were independent risk factors for vitamin D deficiency. On the other hand, current CD4+ lymphocyte count >600 cells/mm^3 (aOR: 0.71; 95% CI: 0.32 to 0.95) and current plasma HIV RNA <20 copies/mL (aOR: 0.57; 95% CI: 0.21 to 0.89) were independent protective factors for vitamin D deficiency.

DISCUSSION

The prevalence of vitamin D deficiency among HIV-positive persons is very high and widespread, but observational studies have led to conflicting data about comparison with the general population, risk factors for hypovitaminosis D and clinical significance of low vitamin D levels in the setting of HIV disease.

In a retrospective analysis of 2044 HIV-infected patients, the prevalence of vitamin D deficiency was 89.2% and risk factors included female sex, heterosexual acquisition of HIV, longer duration of cART, CD4 lymphocyte count <200 cells/mm^3, advanced stage of HIV disease, and current use of efavirenz (Theodorou *et al.*, 2014). Other studies have shown a significant association between efavirenz use and low vitamin D levels, suggesting a potential interference of this non-nucleoside analogue with the vitamin D metabolism (Brown *et al.*, 2010; Welz *et al.*, 2010; Nylèn *et al.*, 2016).

On the other hand, the Multicenter AIDS cohort study showed a lower and comparable prevalence of vitamin D deficiency among HIV-infected men on cART and HIV-uninfected men, ranging from 41 to 44%. In this study black race, obesity, and normal renal function were predictors

Table 1 - *Patient characteristics according to vitamin D status.*

	Patients with vitamin D deficiency (cases)	*Patients with normal vitamin D level (controls)*	*p value*
Total number	98	97	
Males, no. (%)	75 (76.5)	75 (77.3)	0.672
White subjects, no. (%)	94 (95.9)	93 (95.9)	0.852
Age (years), mean (SD)	49.7 (7.7)	48.8 (8.3)	0.394
HIV transmission risk category, no. (%):			
– IDU	13 (13.3)	10 (10.3)	0.613
– MSM	54 (55.1)	42 (43.3)	0.047
– heterosexual	31 (31.6)	45 (46.4)	0.041
Vitamin D concentration (ng/mL), mean (SD)	18.2 (6.7)	39.6 (13.4)	0.023
Vitamin D measurement made in winter/autumn season, no. (%)	48 (52.2)	52 (53.6)	0.809
Nadir CD4+ lymphocyte count (cells/mm^3), mean (SD)	276 (123)	292 (145)	0.296
Current CD4+ lymphocyte count (cells/mm^3), mean (SD)	533 (219)	688 (281)	0.041
Duration of HIV infection (years), mean (SD)	13.4 (6.2)	10.9 (5.4)	0.495
Cumulative exposure to cART (years), mean (SD)	11.9 (5.7)	8.8 (4.6)	0.581
Patients with HIV RNA < 20 copies/mL, no. (%)	74 (75.5)	92 (94.8)	0.038
Patients with AIDS diagnosis, no. (%)	9 (9.2)	11 (11.3)	0.087
BMI (Kg/m^2), mean (SD)	23.9 (3.4)	23.5 (3.1)	0.786
Cigarette smokers, no. (%)	53 (54.1)	57 (58.8)	0.652
Patients with chronic HCV infection, no. (%)	19 (19.4)	6 (6.2)	0.021
Patients with chronic HBV infection, no. (%)	3 (3.1)	5 (5.1)	0.778
Patients with arterial hypertension, no. (%)	15 (15.3)	19 (19.6)	0.067
Patients with type 2 diabetes mellitus, no. (%)	5 (5.1)	6 (6.2)	0.783
Patients with osteoporosis, no. (%)	22 (22.4)	12 (12.4)	0.042
Patients with metabolic syndrome, no. (%)	26 (26.5)	13 (13.4)	0.018
Patients with liver steatosis, no. (%)	31 (31.6)	17 (17.5)	0.029
Patients with cancer diagnosis, no. (%)	19 (19.4)	10 (10.3)	0.048
Calcium (mg/dL), mean (SD)	9.4 (1.9)	9.2 (1.7)	0.556
Phosphorus (mg/dL), mean (SD)	2.8 (1.2)	3.1 (1.4)	0.061
PTH (pg/mL), mean (SD)	48.2 (21.5)	41.7 (19.6)	0.062
Creatinine (mg/dL), mean (SD)	0.91 (0.18)	0.87 (0.21)	0.084
eGFR (mL/min/1.73 m^2), mean (SD)	96.2 (20.1)	98.1 (19.5)	0.097
LDL cholesterol (mg/dL), mean (SD)	144 (31)	148 (36)	0.091
Triglycerides (mg/dL), mean (SD)	211 (89)	205 (96)	0.758
Current cART, no (%):			
– TDF	63 (64.3)	42 (43.3)	0.016
– ABC	32 (32.7)	39 (40.2)	0.052
– EFV	15 (15.3)	18 (18.6)	0.177
– PI/r	29 (29.6)	31 (31.9)	0.421
– INSTI	24 (24.5)	28 (28.9)	0.083

SD, standard deviation; IDU, injection drug users; MSM, men who have sex with men; cART, combination antiretroviral therapy; AIDS, acquired immune deficiency syndrome; BMI, body mass index; HCV, hepatitis C virus; HBV, hepatitis B virus; PTH, parathyroid hormone; eGFR, estimated glomerular filtration rate (calculated by the CKD-EPI formula); LDL, low-density lipoprotein; cART, combination antiretroviral therapy; TDF, tenofovir disoproxil fumarate; ABC, abacavir; EFV, efavirenz; PI/r, ritonavir-boosted protease inhibitor; INSTI, integrase strand transfer inhibitor.

Table 2 - *Univariate and multivariate analyses for risk factors of vitamin D deficiency among 195 HIV-1-infected patients on combination antiretroviral therapy.*

Variables	*Crude OR (95% CI)*	*p value*	*Adjusted OR (95% CI)*	*p value*
HIV transmission risk category,:				
– IDU	0.87 (0.51 to 1.17)	0.058		
– MSM	1.76 (1.29 to 2.13)	<0.001	1.59 (1.19 to 2.21)	0.027
– heterosexual	0.66 (0.31 to 0.92)	0.046	0.79 (0.48 to 1.07)	0.059
Nadir CD4+ lymphocyte count <200 cells/mm^3	1.42 (0.94 to 1.76)	0.061	1.24 (0.81 to 1.67)	0.075
Current CD4+ lymphocyte count >600 cells/mm^3	0.61 (0.29 to 0.84)	0.011	0.71 (0.32 to 0.95)	0.044
AIDS diagnosis	1.34 (0.67 to 1.68)	0.441		
Duration of HIV infection >10 years	1.21 (0.68 to 1.89)	0.338		
Cumulative exposure to cART >10 years	1.09 (0.55 to 1.78)	0.133		
HIV RNA <20 copies/mL	0.51 (0.23 to 0.82)	0.042	0.57 (0.21 to 0.89)	0.036
BMI >24 Kg/m^2	1.07 (0.45 to 1.58)	0.688		
Cigarette smoking	1.11 (0.68 to 1.59)	0.178		
Chronic HCV infection	1.66 (1.24 to 2.05)	0.008	1.44 (1.17 to 1.86)	0.041
Chronic HBV infection	1.04 (0.61 to 1.54)	0.228		
Arterial hypertension	1.24 (0.92 to 1.53)	0.072	1.14 (0.81 to 1.47)	0.502
Type 2 diabetes mellitus	1.33 (0.82 to 1.72)	0.188		
Osteoporosis	1.92 (1.51 to 2.44)	<0.001	1.78 (1.25 to 2.09)	<0.001
Metabolic syndrome	2.88 (2.39 to 3.15)	<0.001	2.57 (1.96 to 2.98)	0.005
Liver steatosis	1.84 (1.35 to 2.22)	0.008	1.59 (1.17 to 2.05)	0.031
Cancer diagnosis	1.49 (1.2 to 1.83)	0.025	1.47 (1.13 to 1.79)	0.039
eGFR <70 mL/min/1.73 m^2	1.33 (0.67 to 1.91)	0.109		
PTH <40 pg/mL	1.15 (0.62 to 1.57)	0.409		
Current cART including:				
– TDF	1.76 (1.42 to 2.14)	0.006	1.65 (1.31 to 1.94)	0.018
– ABC	0.78 (0.34 to 1.08)	0.084	0.87 (0.55 to 1.19)	0.124
– EFV	1.55 (0.82 to 2.13)	0.061		
– PI/r	1.34 (0.78 to 1.81)	0.228		
– INSTI	0.91 (0.61 to 2.39)	0.402		

OR, odds ratio; 95% CI, 95% confidence intervals; IDU, injection drug users; MSM, men who have sex with men; AIDS, acquired immune deficiency syndrome; BMI, body mass index; HCV, hepatitis C virus; HBV, hepatitis B virus; eGFR, estimated glomerular filtration rate (calculated by the CKD-EPI formula); PTH, parathyroid hormone; cART, combination antiretroviral therapy; TDF, tenofovir disoproxil fumarate; ABC, abacavir; EFV, efavirenz; PI/r, ritonavir-boosted protease inhibitor; INSTI, integrase strand transfer inhibitor.

of vitamin D deficiency regardless of HIV serostatus, a low CD4 cell count was associated with hypovitaminosis among HIV-positive men, but no associations between efavirenz or other antiretroviral drugs and low vitamin D concentration were reported (Zhang *et al.*, 2017). TDF has been associated with proximal tubular dysfunction, renal calcium and phosphate wasting, higher PTH levels, and reduced bone mineral density among HIV-positive persons, but there are no data to date suggesting a potential correlation between this drug and the vitamin D deficiency (Eckard *et al.*, 2014).

Several studies have also demonstrated an association of vitamin D deficiency with greater systemic inflammation and immune activation, such a potential correlation between the hypovitaminosis and a faster progression of the HIV disease. Patients with low vitamin D levels have increased plasma concentration of several inflammation markers, such as interleukin-6, tumor necrosis factor-alpha, D-dimer, and markers of lymphocyte and monocyte activation (Manion *et al.*, 2017; Schleithoff *et al.*, 2006; Van Etten *et al.*, 2008). At the same time, low vitamin D status appears to accelerate the HIV disease progression and to impair the CD4 lymphocyte count recovery rate after the beginning of cART (Viard *et al.*, 2011; Ezeamama *et al.*, 2016; Sudfeld *et al.*, 2012; Shepherd *et al.*, 2014).

Finally, vitamin D deficiency affects insulin sensitivity and pancreatic cell function, and therefore can lead to several glucose and lipid metabolism alterations. Some observational, cohort studies have shown that HIV-positive patients with vitamin D deficiency had a significantly higher prevalence of insulin resistance, metabolic syndrome, and diabetes mellitus compared to those with normal vitamin D concentration (Boucher *et al.*, 1995; Reis *et al.*, 2009; Forouhi *et al.*, 2008; Szep *et al.*, 2011). Interference with glucose metabolism and pro-inflammatory effects can also explain the association between hypovitaminosis D and an increased risk of cardiovascular diseases reported both in the general population and among HIV-infected subjects (Choi *et al.*, 2011; Lai *et al.*, 2013).

In our case-control study, vitamin D deficiency (<30 ng/mL) among HIV-infected patients on cART is significantly associated with known general risk factors (osteoporosis, chronic HCV coinfection, neoplasm, metabolic syndrome,

liver steatosis) and HIV-specific risk factors (low CD4 lymphocyte count and detectable plasma HIV RNA) in conformity with the literature. However, our study underlines some risk factors for hypovitaminosis D that are not highlighted in other studies, such as MSM acquisition of HIV infection and current use of TDF. While the latter might be related to possible pharmacokinetic interactions involving absorption or metabolism of vitamin D, the observed relationship between low vitamin D levels and MSM acquisition of HIV remains more elusive and cannot be addressed by the retrospective design of the present study. Additional and larger clinical studies evaluating prevalence and risk factors for vitamin D deficiency among HIV-positive people are needed in order to better understand and properly manage this metabolic complication associated with HIV disease and comorbidities.

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