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# Serum 25-Hydroxyvitamin D Levels and C-Reactive Protein in Persons with Human Immunodeficiency Virus Infection

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## Abstract

Human immunodeficiency virus (HIV) infection has frequently been associated with vitamin D deficiency as well as chronic inflammatory response. We tested the hypothesis of an independent relationship between serum concentrations of 25-hydroxyvitamin D [25(OH)D] and high-sensitivity C-reactive protein (CRP) in a cohort of HIV-positive people. A cross-sectional survey was conducted among 316 HIV-positive people (181 men and 135 women) aged 16 to 60 years residing in the Kathmandu Valley, Nepal. Serum high-sensitivity CRP concentrations and serum 25(OH)D levels were measured by the latex agglutination nephelometry method and the competitive protein-binding assay, respectively. The relationship between serum CRP concentrations and 25(OH)D serum level was assessed using multiple logistic regression analysis with adjustment of potential cardiovascular and HIV-related factors. The proportions of participants with 25(OH)D serum levels <20 ng/ml, 20–30 ng/ml, and ≥30 ng/ml were 83.2%, 15.5%, and 1.3%, respectively. The mean 25(OH)D serum levels in men and women were 15.3 ng/ml and 14.4 ng/ml, respectively. Participants with a 25(OH)D serum level of <20 ng/ml had a 3.2-fold higher odds of high CRP (>3 mg/liter) compared to those with a 25(OH)D serum level of ≥20 ng/ml ( $p=0.005$ ). Men and women with a 25(OH)D serum level of <20 ng/ml had 3.2- and 2.7-fold higher odds of high CRP (>3 mg/liter), respectively, compared to those with a 25(OH)D serum level of ≥20 ng/ml. The relationships remained significant only in men ( $p=0.02$ ) but not in women ( $p=0.28$ ). The risk of having a high level of inflammation (CRP >3 mg/liter) may be high among HIV-positive men and women with a 25(OH)D serum level of <20 ng/ml.

## Introduction

**I**NFLAMMATION IS A KEY PROCESS in human immunodeficiency virus (HIV) infection.<sup>1–4</sup> HIV infection may activate inflammatory pathways through activating endothelium by secreting cytokines in response to mononuclear or adventitial cell activation by the virus or by the direct effect of the secreted HIV-associated proteins gp120 (envelope glycoprotein) and tat (transactivator of viral replication) on endothelium.<sup>1</sup> One of the bioactive molecules that may mediate the systemic effects of HIV infection includes high-sensitivity C-reactive protein (CRP), a proinflammatory biomarker. CRP

has been associated with parameters of disease progression, such as CD4 cell count and HIV viral load, as well as a decreased time to AIDS<sup>5</sup> and it is an independent predictor of survival.<sup>6</sup>

A low vitamin D level has been observed in people infected with HIV. Accordingly, a number of studies have revealed higher rates of vitamin D deficiency among HIV-positive people.<sup>7–9</sup> In recent studies, low levels of vitamin D were related to disease progression of HIV/AIDS and its complications.<sup>10,11</sup> The causes of this deficiency are not known but might include a defect in renal 1 $\alpha$ -hydroxylation of 25-hydroxyvitamin D<sub>3</sub> mediated by proinflammatory cytokines<sup>12</sup> or by the intake of antiretroviral drugs.<sup>13,14</sup> Thus, the

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literature suggests that HIV-positive people have low vitamin D levels as well as chronic inflammation.

Vitamin D deficiency has been reported as a risk factor for inflammation and inflammatory diseases in populations other than HIV-positive people.<sup>15–17</sup> Vitamin D deficiency increases systemic inflammation as documented by elevated levels of CRP.<sup>18,19</sup> The immunomodulating effects of vitamin D may explain the reported epidemiological associations between vitamin D status and inflammation.<sup>15</sup> Mechanistically, vitamin D down-regulates the activation of nuclear factor- $\kappa$ B, an important regulator of genes encoding for several inflammatory cytokines.<sup>20</sup> In addition, vitamin D diminishes the production by macrophages of proinflammatory cytokines and chemokines that may interfere with systemic inflammation.<sup>21</sup>

Nevertheless, the association between vitamin D status and systemic inflammation in HIV-positive people has not been investigated to the best of our knowledge. In this study, we aim to assess the relationship between serum concentrations of 25-hydroxyvitamin D [25(OH)D] levels and CRP in a cohort of HIV-positive people.

## Materials and Methods

### *Study design and setting*

This cross-sectional survey was conducted among HIV-positive people living in the Kathmandu Valley, Nepal during February and March 2010 in the wider context of the baseline survey of a longitudinal healthy living intervention headed by the first and second authors. All HIV-positive people who were in contact with five local nongovernmental organizations (NGOs) working within HIV-positive communities in the Kathmandu Valley were invited to participate in the study.

The Kathmandu Valley comprises three districts (Kathmandu, Lalitpur, and Bhaktapur) with an estimated population of 2.2 million.<sup>22</sup> Of Nepal's estimated 70,000 HIV-positive people aged 15–49 years,<sup>23</sup> 15.7% were living in the Kathmandu Valley at the end of 2006.<sup>24</sup> Nepal has been facing an escalating, concentrated epidemic of HIV/AIDS with prevalence especially high among injecting drug users (IDUs).<sup>23</sup> The HIV prevalence among IDUs living in the capital city of Kathmandu was 34.8% in 2007.<sup>25</sup>

### *Study participants*

The 322 HIV-positive people, who were in contact with five NGOs working within HIV-positive communities, participated in the study. All participants were adults from 16 to 60 years of age with a self-reported diagnosis of HIV-positive status, and gave their written informed consent to participate. Participants who had no information on serum CRP concentration or 25(OH)D serum levels or sociodemographic or anthropometric information were excluded, resulting in a final study population of 316 participants (181 men and 135 women). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of the Nepal Health Research Council, Kathmandu, Nepal, National Center for Global Health and Medicine, Japan, and Waseda University, Tokyo, Japan.

### *Interview data*

Data were collected by means of personal interviews. We used the structured pretested Nepali language questionnaire

to collect the information. Trained interviewers administered the questionnaire face-to-face in a private setting with each interview lasting approximately 45–60 min. We used a prepared information sheet to inform all participants about the study procedures. We requested participants to sign informed consent forms prior to being interviewed. Interviewers reassured participants that numerical codes would be used in place of names in all records to ensure confidentiality.

Trained interviewers collected information from HIV-positive participants on demographics, 24-h dietary intake, HIV-related clinical events, and therapy. The antiretroviral therapy was measured by participants' current use of medicine at the time of the survey along with specified duration and names of medication and adherence. Alcohol consumption and smoking status were categorized as never and ever been drinking and smoking, respectively. From the reported years of formal education, we categorized education into never or ever been to school. Similarly, from the reported specific types of work, we categorized occupation into yes or no work. Physical activity was assessed in average hours spent per day in the past 12 months on common physical activities on the basis of the usual frequency and duration of two different activities (walking and any types of mild, moderate, or hard labor work). The past history of any disease was asked by the question "In the past 12 months, did you suffer from any type of diseases including minor illnesses?" with a response of yes or no. If the response was yes, the signs or symptoms of disease or disease diagnosis with details of health-seeking behavior and treatment of each disease were inquired about.

### *Laboratory methods*

Blood samples were obtained from 322 participants after an overnight fast during the survey period. Ten milliliters of venous blood was drawn into an evacuated tube and centrifuged immediately for 15 min, after which the serum samples were placed in a cooler box and transported to a laboratory. The separated serum was stored in three tubes (2.0 ml, 1.5 ml, and 1.5 ml) and was stored at  $-80^{\circ}\text{C}$  until analysis. One tube of 2.0 ml was sent to an external laboratory (Mitsubishi Chemical Medicine Corporation, Tokyo, Japan) for testing, where high-sensitivity serum CRP concentrations were measured by the latex agglutination nephelometry method and the 25(OH)D serum level was measured by the competitive protein-binding assay. The intraassay coefficient of variation at CRP levels of 0.09 mg/liter and 1.45 mg/liter was 5.0 and 1.7%, respectively. The intraassay coefficient of variation of vitamin D was 10.93 and 7.70%, respectively. The blood sample of all the study participants was drawn from mid-February to mid-March. Therefore the timing of blood sample collection may not have a significant effect on either serum 25(OH)D or serum CRP levels.

Measurements of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were assessed by the enzymatic colorimetric method. The interassay coefficient of variation at total cholesterol levels of 125.48 mg/dl, 166.93 mg/dl, and 238.32 mg/dl was 1.24, 1.09, and 0.97%, respectively. The intraassay coefficient of variation at total cholesterol levels of 127.77 mg/dl, 169.90 mg/dl, and 241.04 mg/dl was 0.67, 0.62, and 0.77%, respectively. The interassay coefficient of variation at LDL cholesterol levels of 75.54 mg/dl, 106.17 mg/dl, and

116.86 mg/dl was 1.32, 1.20, and 1.24%, respectively. The intraassay coefficient of variation at LDL cholesterol levels of 74.47 mg/dl, 104.96 mg/dl, and 118.20 mg/dl was 0.82, 0.58, and 0.66%, respectively. The interassay coefficient of variation at HDL cholesterol levels of 33.97 mg/dl, 44.97 mg/dl, and 57.38 mg/dl was 1.01, 1.25, and 1.04%, respectively. The intraassay coefficient of variation at HDL cholesterol levels of 34.50 mg/dl, 45.98 mg/dl, and 59.52 mg/dl was 0.73, 0.49, and 0.80%, respectively. The interassay coefficient of variation at triglyceride levels of 47.50 mg/dl, 80.63 mg/dl, and 98.98 mg/dl was 1.70, 1.31, and 1.35%, respectively. The intraassay coefficient of variation at triglyceride levels of 47.71 mg/dl, 79.90 mg/dl, and 109.13 mg/dl was 0.92, 0.70, and 0.67%, respectively. CD4 cell counts were determined using a specific monoclonal antibody and fluorescence-activated cell sorter (FACS) analysis. The viral load measurement was not done due to lack of resources.

### Physical examination

Body height and weight were measured while the participants were fasting overnight and wearing light clothing without shoes. The weight was measured in kilograms on a digital scale and the height was measured in centimeters by a stadiometer. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of body height in meters. Waist circumference was taken without outer clothing, using a tape measure in light contact with but not compressing the skin. It was measured at the level of the umbilicus region at the end of normal expiration while participants were standing. Blood pressure was measured with the Omron Automatic Blood Pressure Monitor after the participants had been seated for at least 10 min with his or her feet on the floor and his or her arms supported at heart level. Two independent measurements were done to estimate mean values for all parameters.

### Statistical analyses

The *t* test and the chi-square test were used to assess the demographic, lifestyle, anthropometric, and 25(OH)D serum level difference, between the cutoff value of 25(OH)D serum level of <20 ng/ml and  $\geq$ 20 ng/ml, for continuous variables and categorical variables, respectively. This cutoff value was utilized because the literature defines vitamin D deficiency as a 25(OH)D serum level of <20 ng/ml (50 nmol/liter).<sup>26</sup>

The relationship between serum CRP concentrations and 25(OH)D serum level was assessed using multiple logistic regression analysis. CRP was studied as a categorical variable based on the Center for Disease Control/American Heart Association guidelines of high risk as CRP >3 mg/liter.<sup>27</sup> Therefore the serum CRP concentrations were categorized into high (CRP >3 mg/liter) and not high (CRP  $\leq$ 3 mg/liter). The odds ratios and 95% CIs for high CRP were calculated for each category of 25(OH)D serum level. Major socio-demographic characteristics and other mediators having previously established or theoretically feasible associations with the dependent variable were included as covariates or potential confounders in the analyses. The age (years, continuous), sex (men or women), marital status (married or unmarried/separated), education (never or ever been to school), occupation (yes or no), alcohol intake (never or ever), smoking (never or ever), physical activity ( $\leq$ 3.5 or

>3.5 h/day), body mass index (kg/m<sup>2</sup>, continuous), history of any disease in the past 12 months including minor illnesses (yes or no), systolic blood pressure (mm Hg, continuous), cholesterol (mg/dl, continuous), triglycerides (mg/dl, continuous), CD4<sup>+</sup> T cell count ( $\leq$ 200 or >200; cells/ $\mu$ l), duration of antiretroviral therapy (ART) (no, 0–12, 13–24, 25–36, >36 months), and efavirenz exposure (yes or no) were adjusted for in the multivariate model. The multiple linear regression analysis was performed between serum CRP concentrations and serum 25(OH)D level. To better approximate normal distributions, serum CRP concentrations were log-transformed prior to analysis. All *p* values were two-sided and *p* values less than 0.05 were considered statistically significant. Analyses were performed with SAS statistical software version 9.1 (SAS Institute, Inc., Cary, NC).

### Results

The proportions of participants with a 25(OH)D serum level of <20 ng/ml, 20–30 ng/ml, and >30 ng/ml were 83.2%, 15.5%, and 1.3%, respectively. The means (SD) of 25(OH)D serum levels in men and women were 15.3 (6.24) and 14.4 (4.71), respectively.

Demographic and clinical characteristics of the 316 HIV-positive participants by 25(OH)D serum level of <20 ng/ml and  $\geq$ 20 ng/ml are shown in Table 1. HIV-positive participants with a 25(OH)D serum level of <20 ng/ml were female more than male; a higher proportion was taking antiretroviral therapy, had a lower mean BMI, and had a lower total mean cholesterol than those with a 25(OH)D serum level of  $\geq$ 20 ng/ml. The exposure to antiretroviral therapy (ART) such as nevirapine, efavirenz, tenofovir, and nucleoside reverse transcriptase inhibitors (NRTIs) was not significantly associated with a 25(OH)D serum level of <20 ng/ml/ $\geq$ 20 ng/ml.

The association of the 25(OH)D serum level with high inflammation is shown in Table 2. HIV-positive participants with a 25(OH)D serum level of <20 ng/ml had a higher proportion of high CRP (>3 mg/liter) of 33.5% compared with 17% in those with a 25(OH)D serum level of  $\geq$ 20 ng/ml (Table 2). After adjustment for demographic, anthropometric, lifestyle, and HIV-related factors, those with a 25(OH)D serum level of <20 ng/ml had a 3.2-fold higher odds of high CRP (>3 mg/liter) compared to those with a 25(OH)D serum level of  $\geq$ 20 ng/ml (*p*=0.005). The multiple linear regression analysis also showed an inverse relationship between serum CRP concentrations and 25(OH)D serum level ( $\beta$ =−0.03; *p*=0.06) (data not shown).

In stratified analysis by sex, HIV-positive participants with a 25(OH)D serum level of <20 ng/ml had a higher proportion of high CRP (>3 mg/liter) of 42% and 23% in men and women, respectively, compared with 19% and 12% in those with a 25(OH)D serum level of  $\geq$ 20 ng/ml in men and women, respectively. The overall significant protective effect of a 25(OH)D serum level of  $\geq$ 20 ng/ml against inflammation remained significant in men but not in women. In men, those with a 25(OH)D serum level of <20 ng/ml had a 3.2-fold higher odds of high CRP (>3 mg/liter) compared to those with a 25(OH)D serum level of  $\geq$ 20 ng/ml (*p*=0.02). In women, those with a 25(OH)D serum level of <20 ng/ml had a 2.7-fold higher odds of high CRP (>3 mg/liter) compared to those with a 25(OH)D serum level of  $\geq$ 20 ng/ml (*p*=0.28). Although the difference was not statistically significant in

TABLE 1. CHARACTERISTICS OF HIV-POSITIVE PEOPLE WITH 25-HYDROXYVITAMIN D LEVELS

	25-Hydroxyvitamin D		p-value <sup>a</sup>
	< 20 ng/ml	≥ 20 ng/ml	
Subjects (n)	263	53	
Age (mean ± sd, year)	34.2 ± 6.91	35.0 ± 7.90	0.47
Sex (men, %)	144 (54.75)	37 (69.81)	0.04
Marital status (married, %)	184 (69.96)	32 (60.38)	0.17
Education (never been to school, %)	50 (19.01)	8 (15.09)	0.50
Occupation (no work, %)	79 (30.04)	21 (39.62)	0.17
Smoking (never, %)	143 (54.37)	25 (47.17)	0.33
Alcohol consumption (never, %)	228 (86.69)	48 (90.57)	0.43
Duration of antiretroviral therapy (months, %)			
Not taking antiretroviral medicine			
0–12	63 (23.95)	23 (43.40)	
13–24	62 (23.57)	12 (22.64)	
25–36	47 (17.87)	6 (11.32)	
>36	36 (13.69)	6 (11.32)	
>36	55 (20.91)	6 (11.32)	0.05
History of any disease in past 12 months (yes, %)	165 (62.74)	35 (66.04)	0.64
Efavirenz exposure (yes, %)	57 (21.67)	8 (15.09)	0.27
Nevirapine exposure (yes, %)	126 (47.91)	20 (37.74)	0.17
Tenofovir exposure (yes, %)	5 (1.90)	1 (1.89)	0.99
Nucleoside reverse transcriptase inhibitor exposure (yes, %)	12 (4.56)	1 (1.89)	0.37
History of any disease in past 12 months (yes, %)	165 (62.74)	35 (66.04)	0.64
Body mass index (mean ± sd, kg/m <sup>2</sup> )	21.55 ± 2.97	22.52 ± 2.81	0.03
Physical activity (>3.5, h/day)	132 (50.19)	23 (43.40)	0.37
Systolic blood pressure (mean ± sd, mm Hg)	115.69 (20.34)	116.51 (25.31)	0.82
Cholesterol (mean ± sd, mg/dl)	142.98 (44.18)	153.61 (41.17)	0.09
Triglycerides (median, range; mg/dl)	115 (44–791)	107 (24–1071)	0.62
CD4 <sup>+</sup> T cell count (median, range; cells/μl)	347 (60–1009)	349 (15–1551)	0.82

<sup>a</sup>p values were based on the Student's *t* test for continuous variables and chi-square test for categorical variables.

women, the adjusted odds ratio was increased [2.67 (0.44–15.96)]. The protective effect of a 25(OH)D serum level of ≥20 ng/ml against inflammation did not change in further analysis after excluding participants with any history of disease in the past 12 months including minor illnesses or par-

ticipants without a history of antiretroviral medication (data not shown).

In further stratified analysis by dividing vitamin D deficiency into severe [25(OH)D serum levels of <10 ng/ml] and moderate [25(OH)D serum levels of 10–19.99 ng/ml], the

TABLE 2. ODDS RATIOS AND 95% CIs FOR HIGH HS C-REACTIVE PROTEIN (CRP &gt; 3 mg/liter) ACCORDING TO SERUM 25-HYDROXYVITAMIN D CONCENTRATIONS IN HIV-POSITIVE PEOPLE

	25-Hydroxyvitamin D		p value
	< 20 ng/ml	≥ 20 ng/ml	
Serum 25-hydroxyvitamin D (median, ng/ml)	12.9 (3.48)	23.8 (3.62)	
n with higher CRP	88/263	9/53	
Univariate model OR (95% CI)	2.45 (1.15–5.26)	1.00 (ref)	0.02
Multivariate model <sup>a</sup> OR (95% CI)	3.19 (1.39–7.30)	1.00 (ref)	0.005
Men	12.6 (3.39)	25.0 (3.81)	
n with higher CRP	60/144	7/37	
Univariate model OR (95% CI)	3.06 (1.26–7.43)	1.00 (ref)	0.01
Multivariate model <sup>a</sup> OR (95% CI)	3.22 (1.19–8.67)	1.00 (ref)	0.02
Women	13.4 (3.56)	22.5 (2.47)	
n with higher CRP	28/119	2/16	
Univariate model OR (95% CI)	2.15 (0.46–10.05)	1.00 (ref)	0.33
Multivariate model <sup>a</sup> OR (95% CI)	2.67 (0.44–15.96)	1.00 (ref)	0.28

<sup>a</sup>All multivariate models adjusted for age (years, continuous), sex (men or women), marital status (married or unmarried/separated), education (never or ever been to school), occupation (yes or no), alcohol intake (never or ever), smoking (never or ever), physical activity (≤3.5 or >3.5, h/day), body mass index (kg/m<sup>2</sup>, continuous), history of any disease including minor illnesses in past 12 months (yes or no), systolic blood pressure (mm Hg, continuous), cholesterol (mg/dl, continuous), triglycerides (mg/dl, continuous), CD4<sup>+</sup> T cell count (≤200 or >200; cells/μl), and duration of antiretroviral therapy (no, 0–12, 13–24, 25–36, >36 months), and efavirenz exposure (yes or no).



HIV-positive participants with 25(OH)D serum levels of <10 ng/ml had an increased proportion of high CRP levels (24/61=39.3%) than those HIV-positive participants with 25(OH)D serum levels of 10–19.99 ng/ml (64/202=31.7%) and  $\geq 20$  ng/ml (9/53=16.9%). The risk of having high CRP was also increased in HIV-positive participants with 25(OH)D serum levels of <10 ng/ml. The multivariate-adjusted odds ratios (95% confidence intervals) of having high CRP for HIV-positive participants with 25(OH)D serum levels of <10 ng/ml and 10–19.99 ng/ml as compared to HIV-positive participants with 25(OH)D serum levels of  $\geq 20$  ng/ml were 4.00 (1.52–10.55) and 2.99 (1.29–6.94), respectively ( $p$  for trend=0.007) (data not shown).

## Discussion

In our cohort of HIV-positive participants, we found greater odds of having high inflammation (CRP >3 mg/liter) among HIV-positive men and women with a 25(OH)D serum level of <20 ng/ml, although the relationship was not significant in women. To our knowledge, this is the first study exploring the association between 25(OH)D serum levels and inflammatory markers among HIV-positive people.

We found that the risk of having high inflammation (CRP >3 mg/liter) was greater among HIV-positive people with a 25(OH)D serum level of <20 ng/ml. We are unaware of any similar studies reporting an association between 25(OH)D serum levels and inflammatory markers in HIV-positive people for our comparison. However, our findings are in line with those studies previously highlighting an inverse association between vitamin D and inflammatory markers in different populations. For example, in a European cohort of obese subjects, 25(OH)D serum concentrations were inversely related to significant levels of high CRP, regardless of the total quantity of fat mass.<sup>28</sup> In another study among patients with early inflammatory polyarthritis, each 10 ng/ml increase in 25(OH)D serum level was associated with a 25% decrease in serum CRP.<sup>19</sup> In addition, two clinical trials have suggested that vitamin D supplementation markedly reduces serum levels of CRP and several other inflammation markers in critically ill patients and patients with congestive heart failure, respectively.<sup>29,30</sup>

We could not find any significant difference between the ART exposure such as efavirenz and others and 25(OH)D serum level of <20 ng/ml/ $\geq 20$  ng/ml among our participants. The relationship between serum CRP concentrations and 25(OH)D serum level did not change in further adjustments of efavirenz exposure in the multivariate model in our study. An efavirenz intake has been shown to affect vitamin D metabolism in HIV-positive adults in previous studies.<sup>31–33</sup> The low 25(OH)D serum levels among overall participants may be a reason for the lack of association between vitamin D levels and efavirenz exposure in our study.

In our study, men and women with a 25(OH)D serum level of <20 ng/ml had a higher risk of high CRP (>3 mg/liter). Although the association in women did not reach statistical significance, the odds ratio was increased after taking into account other variables in the multivariate analysis.

The present findings supporting a protective role of 25(OH)D serum level of  $\geq 20$  ng/ml against inflammation can be explained through the immunomodulating effects of vitamin D referring to mechanistic studies.<sup>15</sup> Vitamin D may act

as an immune modulator and interfere with systemic inflammation through the expression of nuclear vitamin D receptors in most cells of the immune system, including activated CD4 and CD8 T lymphocytes, as well as macrophages.<sup>34,35</sup> For example, some experimental studies have suggested that vitamin D can exert regulatory effects on the cytokine production of human peripheral blood lymphocytes<sup>35,36</sup> and can also down-regulate the activation of nuclear factor- $\kappa$ B, an important regulator of genes encoding for several inflammatory cytokines.<sup>20</sup>

Some limitations of our study deserve comment. First, the possibility of reverse causality must be considered, namely that inflammation may influence the 25(OH)D serum level, a possibility that the cross-sectional design of our study prevents us from ruling out. It may be less likely to influence our study result because we adjusted information on past history of any disease including minor illnesses over the past 12 months in the multivariate models and also did further analysis excluding participants with any history of disease including minor illnesses in the past 12 months. The protective effect of a 25(OH)D serum level of  $\geq 20$  ng/ml against inflammation did not change in either analysis. Though the past history of any disease including minor illnesses was self-reported, we asked for even mild illnesses so that it would not affect the study result. In addition, our hypothesis that vitamin D deficiency influences inflammation was based on previous study findings including clinical trials.<sup>29,30</sup> Second, although we adjusted for factors known to influence serum 25(OH)D level and CRP level, the possibility of residual confounding cannot be excluded. Finally, the conclusions drawn from subgroup analysis might be due to chance, and should thus be interpreted with caution.

In conclusion, the present study suggests that the risk of having high inflammation (CRP >3 mg/liter) may be elevated among HIV-positive people with a 25(OH)D serum level of <20 ng/ml. These findings are important in that they may lead to potential new intervention strategies (e.g., supplementation, dietary changes) to increase improved health and quality of life for HIV-positive people. Further prospective studies to confirm the role of vitamin D against inflammation in HIV-positive people are warranted.

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### Author Disclosure Statement

All of the authors read and approved the manuscript. None of the authors had a conflict of interest.

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