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Vitamin D Levels, Natural H1N1 Infection and Response to H1N1 Vaccine among HIV-Infected Individuals

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Abstract

Background: Beyond its role in calcium homeostasis, vitamin D plays a critical role in immunological responses to pathogens. We evaluated the relationship between 25-OH vitamin D levels and susceptibility to natural H1N1 infection and H1N1 vaccine responses in HIV infected individuals.

Methods: This was a sub study of an H1N1 vaccine trial conducted at the University of Pennsylvania in 2009/10. We compared the 25-OH vitamin D levels among individuals with and without baseline evidence of prior H1N1 infection and between vaccine responders and non-responders.

Results: 120 participants enrolled in the trial, 71% male, 68% African American, median age 46 years. The majority had controlled HIV disease. At baseline, 86% had 25-OH vitamin D levels < 30 ng/ml and 54% had levels < 20 ng/ml. Thirty participants (25%) had evidence of prior H1N1 exposure. There was no difference in mean 25-OH vitamin D levels among patients with or without prior natural H1N1 infection (21 ng/ml vs 20 ng/ml, p=0.72). Among participants without previous H1N1 exposure, only 61% developed protective antibody titers following vaccination. 25-OH vitamin D levels were similar between vaccine responders (20 ng/ml) and non-responders (20 ng/ml) (p=0.83).

Conclusion: Although 25-OH vitamin D deficiency was very common among HIV-infected individuals, it was not associated with natural susceptibility to H1N1 or to vaccine responses.

Key words: HIV; H1N1; Influenza; Vitamin D deficiency; Vaccine response

Introduction

Twenty-five hydroxy (25-OH) vitamin D deficiency is highly prevalent among HIV-infected adults [1-3] and has well-established consequences on bone mineralization and calcium homeostasis. Recently, 25-OH vitamin D deficiency has been linked to other clinical problems, including increased susceptibility to heart disease [4], diabetes mellitus [5], metabolic syndrome [6] and dysregulation of the immune system. *In vitro* [7-9] and *in vivo* [10,11] studies have demonstrated the ability of vitamin D to up-regulate immunological responses through activation of human macrophages [7], monocyte chemotaxis [8], oxidative burst [9], and other mechanisms [8,9]. These immunological effects mayexplain the possible links between vitamin D receptor polymorphisms and susceptibility to upper respiratory infections (URIs), as well as the results of multiple controlled and observational studies examining the effect of vitamin D supplementation on reducing the risk of URIs [12-14].

Patients with HIV respond immunologically relatively poorly to the H1N1 vaccine and other influenza vaccines when compared to the general population [15]. This study examined the prevalence of vitamin D deficiency in HIV-infected individuals and its relationship to prior H1N1 exposure and immunologic response to H1N1 vaccination.

Methods

This is a prospective, single-arm, non-randomized substudy of a single-dose H1N1 vaccine trial conducted at the University of Pennsylvania's Center for AIDS Research in the fall/winter 2009-2010. The vaccine consisted of a 15 µg dose of the monovalent, unadjuvanted, inactivated, split H1N1 virus (Novartis, Basel, Switzerland). 120 HIV-infected individuals seeking routine HIV care at the Infectious Diseases clinic were enrolled into the study. Inclusion and exclusion criteria are described elsewhere [15]. All patients signed an informed consent. The study was approved by the University of Pennsylvania institutional review board and registered in clinical trials.gov #NCT01111162.

At time zero, patients had baseline labs drawn which included a CD4 count, HIV viral load (VL), and influenza antibody titers measured using the hemagglutination inhibition assay (HAI). A single intramuscular dose of the H1N1 vaccine was given. Patients with baseline HAI titers greater than 1:40 were categorized as having a prior exposure to H1N1. Serological response, defined as a fourfold increase of influenza titers among patients with prior H1N1 exposure or a titer great than 1:40 among H1N1 naïve patients, was evaluated 3 weeks after vaccination. Patients with such titers were categorized as vaccine responders, and all others were categorized as non-responders. We compared total 25-OH vitamin D levels among individuals with and without baseline natural exposure to the H1N1 virus and between vaccine responders and non-responders.

25-OH vitamin D levels were evaluated at Quest Diagnostics Nichols Institute in appropriately cryopreserved baseline serum specimens. Briefly, this method utilized a liquid chromatograpy, tandem mass spectrometry (LC/MS/MS) process to quantify total 25-

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OH vitamin D by separately quantifying the D2 and D3 forms of this analyte and summing them together to provide the total 25-OH vitamin D value. This testing process involved stepwise: protein precipitation from serum, followed by liquid chromatography separation of the target components, and finally detection and quantitation of the target analytes using a tandem mass spectrometry technique. The resultant 25-OH Vitamin D_2 and 25-OH Vitamin D_3 subcomponents were summed to obtain the total 25-OH vitamin D levels. The analytical sensitivity of the method was 4 ng/mL for each subcomponent of 25-OH vitamin D. The analytical specificity demonstrated no cross-reaction with; 1alpha,25-$(OH)_2D_2$; 1alpha,25-$(OH)_2D_3$, calcitriol; 25,26-$(OH)_2D_3$; and 1alpha $(OH)D_2$, doxercalciferol; and 1 alpha $(OH)D_3$, alfacalcidiol. The average interassay coefficient of variation across the analytical range of the method was 7%.

Statistical analysis

Differences by category of 25-OH vitamin D level (<20, 20-30, >30 ng/ml) and demographic and clinical characteristics were evaluated with the Student's t test for continuous variables and Fisher's exact test for categorical variables. Differences in mean 25-OH vitamin D between patients with and without prior H1N1 natural exposure and between vaccine responders and non-responders were assessed using the student *t*-test.

Results

Patient demographic and clinical characteristics

120 participants enrolled in the trial, all of whom completed the study at the 3-week follow-up. Baseline clinical and demographic characteristics are shown in table 1. Participants had a median age of 46 years, the majority were male (70.8%), and 67.5% were African-American. Overall, study participants had stable HIV disease with 70% exhibiting a current CD4 count > 350 cells/mm^3 and 91.6% having an HIV VL < 400 copies/ml. All patients except one were receiving antiretroviral therapy.

Distribution of 25(OH) vitamin D levels among study participants

The mean 25-OH vitamin D level among the 120 participants was 20 ng/ml. Most (86%) patients had vitamin D deficiency (25-OH vitamin D level < 30 ng/mL) and 54% had severe vitamin D deficiency (25-OH vitamin D < 20 ng/mL). Besides race (p<0.0001) and HIV VL (p=0.01), there was no significant difference in vitamin D levels across other clinical and demographic markers (Table 1). Vitamin D deficiency was more common among black participants than among other races, with 66.7% having severe vitamin D deficiency.

	All (n=120)	25 (OH) Vitamin D Levels			
Patient characteristics		<20	20-30	>30	p-value
Age (median)	120 (46.1)	44.1	48.9	47.6	0.13
Sex, n (% exposed)					0.47
Male	85 (70.8)	43 (50.6)	25 (29.4)	17 (20.0)	
Female	35 (29.2)	22 (62.9)	7 (20.0)	6 (17.1)	
Race, n(% exposed)					<0.001
White	30 (25.0)	8 (26.7)	10 (33.3)	12 (40.0)	
Black	81 (67.5)	54 (66.7)	19 (23.4)	8 (9.9)	
Hispanic	7 (5.8)	3 (42.8)	2 (28.6)	2 (28.6)	
Asian	2 (1.7)	0 (0)	1 (50.0)	1 (50.0)	
CD4 count, n(% exposed)					0.39
≤ 350 cells/mm^3	36 (30.0)	23 (63.9)	7 (19.4)	6 (16.6)	
> 350 cells/mm^3	84 (70.0)	42 (50.0)	25 (29.8)	17 (20.2)	
Viral load, n(% exposed)					0.01
< 400 copies/ml	110 (91.6)	55 (50.0)	32 (29.1)	23 (20.9)	
≥ 400 copies/ml	10 (8.4)	10 (100)	0 (0)	0 (0)	

Table 1: Demographic and clinical characteristics of 120 patients with HIV infection, by vitamin D level.

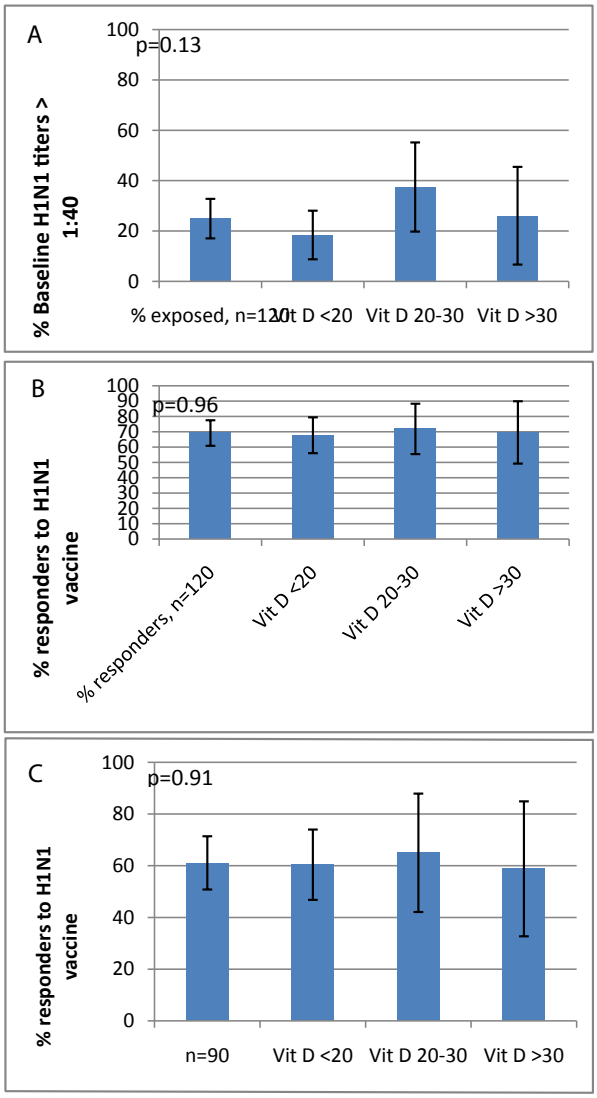

Figure 1: H1N1 immunological response by vitamin D levels.
A) Baseline H1N1 rate of natural infection according to vitamin D level. **B)** Percentage of seroprotection at the end of the study according to baseline vitamin D level. **C)** Rates of seroconversion among H1N1 naïve individuals according to baseline vitamin D level.

Baseline exposure to H1N1 by vitamin D levels

Thirty out of 120 patients (25%) had evidence of prior H1N1 natural exposure with HAI titers greater than 1:40 at baseline (Figure 1A). There was no difference in mean vitamin D levels among patients with or without prior natural H1N1 infection (21 ng/ml vs 20 ng/ml, p=0.72). Baseline evidence of prior H1N1 infection was less common in patients with severe vitamin D deficiency (19%; 95% CI, 8.8-28.1) than in patients with a mild deficiency (38%; 95% CI, 19.7-55.2%) or normal vitamin D levels (26%; 95% CI, 9.3-45.5%); however, the group

sizes were relatively small and the differences did not reach statistical significance.

Serological response to the H1N1 vaccine categorized by vitamin D levels

At week 3, 69.2% (95% CI, 60.8-77.5) of all HIV-infected participants mounted an immunological response to the H1N1 vaccine (Figure 1B). Among the 90 participants without previous exposure to H1N1, only 61.1% (95% CI, 50.8-71.4) developed protective titers by week 3 of the study (Figure 1C). Mean vitamin D levels were similar among vaccine responders (20 ng/ml) and non-responders (20 ng/ml, p=0.83). There were small, non–statistically or clinically significant differences in serologic response among patients with different degrees of vitamin D deficiency. Among all study participants, the rates of seroprotection were similar for patients with severe vitamin D deficiency (67.7%), mild deficiency (71.9%), and no deficiency (69.6%) (Figure 1B). Among patients without previous H1N1 exposure and with severe vitamin D deficiency, 60.4% responded to the vaccine versus 65% and 59% in the mild or no vitamin D deficiency groups (Figure 1C). Non-vaccine responders had lower current CD4 counts than vaccine responders (42.3% versus 24.1% had CD4 count <350 cells/ml, p=0.05) and a higher HIV VL (13.5% versus 6%, p=0.28 had a VL > 400 copies/ml). Patients with severe vitamin D deficiency were more likely to be HIV viremic compared to patients with mild/ no vitamin D deficiency (p=0.01); 100% of patients with severe vitamin D deficiency had active viremia.

Discussion

In this prospective study of patients with HIV receiving the H1N1 vaccine, we found that there was no difference in the mean vitamin D levels between patients with or without prior natural H1N1 infection or between vaccine responders and non-responders. The prevalence of vitamin D deficiency among our study participants was very high: 86% of our sample had 25 (OH) vitamins D < 30 ng/ml and over half had levels < 20 ng/ml. The reported prevalence of vitamin D deficiency ranges widely, from 45% to 87% in studies conducted in developed nations [1,16-21]. In two recent large cohort studies performed in the US, the prevalence of vitamin D deficiency was 60% [21] among HIV infected women and 70% [1] among men and women, which is slightly lower than what we found. These studies were performed retrospectively over a one-year period and did not correct for the seasonal variation of vitamin D levels. It is possible that the prevalence of vitamin D deficiency in our study was higher because blood samples were drawn in a relatively short period of time during the fall/winter of 2009 when vitamin D levels are known to be lower because of decreased sun exposure. In accordance with previous studies, we found that African Americans were significantly more likely to be vitamin D deficient.

We have shown previously that patients with HIV have a lower seroconversion rate than the general population after H1N1 vaccine administration [15]. Our study suggests that vitamin D levels do not explain the low frequency of vaccine responses in this population. The reasons that HIV infected individuals with well-controlled HIV infection respond poorly to influenza (and other) vaccinations are unknown. Different strategies have been used to address this problem including using higher doses and the use of adjuvant vaccines like ASO3 and MF59 [22-28]. Currently, however, the use of these adjuvant vaccines is not approved in the many countries, including the United States. In our study, we observed an unexpected result: when stratifying HIV disease by vitamin D levels, we found that patients with severe vitamin D deficiency were significantly more likely to be HIV viremic compared to patients with mild or no vitamin D deficiency (p=0.01). Obviously in this cross-sectional aspect of the study we cannot assess the causality of this association. It is possible that patients with uncontrolled HIV have a chronic state of inflammation that leads to lower vitamin D levels, or that low vitamin D levels are associated with worse virological control of HIV. Although many observational studies have shown an association between lower vitamin D levels and clinical progression of HIV disease, the design does not allow them to address the directionality of that association [29-31].

In summary, in this single-arm H1N1 vaccine study, we found no association between mean vitamin D levels and prior exposure to H1N1 and vaccine response.

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