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Effect of 25-Hydroxyvitamin D Status on Serological Response to Influenza Vaccine in Prostate Cancer Patients

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Abstract

BACKGROUND—Epidemiologic data suggest that there is an association between vitamin D deficiency and influenza infection. We conducted a prospective influenza vaccination study to determine the influence of vitamin D status on serological response to influenza vaccine in prostate cancer (CaP) patients.

METHODS—During the 2006–2007 influenza season, CaP patients treated at Roswell Park Cancer Institute were offered vaccination with the trivalent influenza vaccine (Fluzone[®], 2006–2007) and sera collected for hemagglutination inhibition (HI) assay titers before and 3 months after vaccination. Response to vaccination was defined as 1:40 titer ratio or a fourfold increase in titer at 3 months, against any of the three strains. Serum 25-hydroxyvitamin D (25-D₃) levels were measured using DiaSorin ¹²⁵I radioimmunoassay kits.

RESULTS—Thirty-five patients with CaP participated in the study. Median baseline 25-D₃ level was 44.88 ng/ml (range: 9.16–71.98 ng/ml). Serological response against any of the three strains was noted in 80%. There was a significant effect of baseline 25-D₃ level when tested as a continuous variable in relation to serological response ($P = 0.0446$). All patients in the upper quartile of 25-D₃ level responded by mounting a serological response ($P = 0.0344$). None of the other baseline variables (age, race, chemotherapy status, or white cell count) had an effect on serological response.

CONCLUSIONS—In this study in CaP patients, a replete vitamin D status was associated with more frequent serological response to influenza vaccine.

Keywords

influenza; vitamin D; titer; vaccine

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INTRODUCTION

Epidemiologic data suggest an association between vitamin D deficiency and influenza epidemics [1]. Hope-Simpson proposed that an unidentified “seasonal stimulus,” related to solar radiation, controlled the seasonality of influenza [2]. Influenza in North America and Europe, reaches epidemic peaks during December to March. These are the months during which the exposure to UVB sunlight rays and serum levels of 25-hydroxyvitamin D3 are lowest in the population [1–3].

Some authors suggest that vitamin D supplementation may protect against cold and influenza-like illnesses. In a post hoc analysis, Aloia and Li-Ng reported decreased incidence rates of influenza and common colds in patients on supplemental vitamin D [4].

Several studies have evaluated the serological response to influenza vaccine in cancer patients [5–12]. These studies indicate a diminished response to influenza vaccination. We have reported a high frequency of vitamin D deficiency among patients with prostate cancer [13] and colorectal cancer patients [14] and in a less formal analysis among individuals with lung, breast, head and neck cancers, lymphoma and leukemia. We postulated, based on clinical and epidemiologic data linking vitamin D deficiency and influenza, that vitamin D deficiency contributes to decreased immune response to influenza vaccine. We assessed the impact of vitamin D status on the serological response to influenza vaccine in prostate cancer patients.

METHODS

Patient Selection

During the 2006–2007 influenza season, CaP patients treated at Roswell Park Cancer Institute were offered vaccination with the trivalent flu vaccine (Fluzone®, 2006–2007). Fluzone vaccine was administered intramuscularly to all study patients. The vaccine is prepared as a sterile suspension of killed influenza viruses propagated in embryonated chicken eggs. Fluzone® contained 45 µg hemagglutinin per 0.5 ml dose, with 15 µg hemagglutinin each of the following three strains: A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004.¹

Institutional review board (IRB) approval was obtained and patients gave written informed consent for blood collection for hemagglutination inhibition (HI) assays before vaccination and 3 months post-vaccination to assess immune response to influenza vaccination. Baseline patient demographics including age, sex, race, cancer type, stage, chemotherapy use, vitamin D supplement use, and dose were collected.

Sample Collection and HI Assay

De-identified serum samples were collected. Three hundred-microliter of serum sample aliquot was shipped on dry ice to the Glennan Center for Geriatrics and Gerontology (Norfolk, VA) where HI assay was performed as previously described [5]. Samples were

¹<http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm112854.htm>, accessed on 9/22/2009.

assayed at baseline and 3 months post-vaccination. Response to vaccination was defined as 1:40 titer ratio or a fourfold increase in HI titer at 3 months post-vaccination, against any of the three strains [6,7]. Serum 25-D3 levels were measured using 25-hydroxyvitamin D125I RIA kits from DiaSorin Co. (Stillwater, MN) as previously described [8].

Statistical Analyses

Chi-square tests were performed to compare serological response between the groups with lowest and highest quartiles of baseline 25-D₃ level. Logistic regression models were used with covariates such as age, gender, cancer type, and chemotherapy as variables.

RESULTS

Table I summarizes baseline patient demographics. All patients had normal white blood cell count and no intercurrent illness or infection except CaP. Median baseline 25-D₃ level was 44.88 ng/ml (range: 9.16– 71.98 ng/ml). Serological response (1:40 titer ratio or a fourfold increase in HI titer at 3 months post-vaccination, against any of the three strains) was noted in 80% of the prostate cancer patients. Response to all three strains of vaccination (1:40 titer ratio or a fourfold increase in HI titer at 3 months post-vaccination, against ALL of the three strains) was rare with only six (17%) being responders.

Few patients (n = 9; 26%) were 25-D3 deficient (<20 ng/ml). Nineteen patients (54.3%) were receiving vitamin D supplement (D). Among the 14 patients in whom the supplement dose was known, the median daily D dose was 2,000 IU (800–9,000 IU). Among the 19 patients taking vitamin D supplement, 17/19 (89.5%) had response. For 16 patients not on D supplements, 11/16 (68.8%) had a serologic response. There was no significant difference in the response rates ($P = 0.21$).

There was a significant effect of baseline 25-D3 level when tested as a continuous variable in relation to serological response ($P = 0.0446$). There was a significant increasing trend in the probability of response to either of the three strains with increasing quartiles of baseline 25-D3 levels as tested by exact Cochran– Armitage trend test ($P = 0.0344$). All patients in the upper quartile of 25-D3 level responded by mounting a serological response. None of the other baseline variables (age, race, chemotherapy status, or white cell count) had an effect on serological response. Figure 1 shows response to different strains of influenza vaccine comparing lowest quartile to highest quartile of baseline 25-D3 levels.

DISCUSSION

Vitamin D may have profound effects on immunity [15]. It may act as an immune modulator and stimulate the expression of protective anti-microbial peptides (AMP) like cathelicidin and defensins in neutrophils and respiratory epithelium upon exposure to microbes [9,12]. Cathelicidin (LL-37) is an inducible AMP, which stimulates local angiogenesis and synergizes with the epidermal growth factor receptor to promote epithelial growth. These inducible AMPs also act as chemoattractants for macrophages and neutrophils through FMLP receptors [10,11]. The beta-defensins are small cationic AMPs that are widely expressed at mucosal surfaces, including airway and submucosal gland epithelia and are

products of individual genes that exhibit broad-spectrum activity against bacteria, fungi, and some enveloped viruses. Active vitamin D (25-D3) has been implicated as a major regulator of AMPs expression [16]. Vitamin D also suppresses production of pro-inflammatory cytokines, interferon γ , TNF α , and IL12 by macrophages, thereby reducing signs and symptoms of acute inflammation that may be associated with the cytokine storm seen with influenza infection [9–12,16].

Influenza epidemics are known to have a seasonal variation; peak incidence is in winter months when the exposure to UVB sunlight and hence, serum levels of 25-D3 are lowest. Limited clinical data indicate a link between vitamin D and respiratory illness. In a post hoc analysis, Aloia and Li-Ng [4] reported decreased incidence rates of influenza and common colds in patients on supplemental vitamin D. Two hundred eight African–American post-menopausal women aged 60 ± 6 (mean $\pm$ standard deviation) years participated in this double blind placebo controlled randomized study of vitamin D supplementation and correlation to bone loss. Half were given 800 IU per day of vitamin D3 in the first 2 years, and 2,000 IU per day in the third year, and half received a placebo. Every 3 months, the women were interviewed as to whether they had experienced a cold or influenza in the previous 3 months. Twenty-six women taking the placebo reported at least one of these illnesses, compared to seven women taking 800 IU/day, and only one taking 2,000 IU/day [4].

A randomized trial of co-administration of 1 μ g of 1,25-dihydroxyvitamin D3 (1,25-D3) intramuscularly with influenza vaccine was not found to enhance the humoral immune response as measured by change in HI titers in healthy young volunteers. Vitamin D status of the patients (replete or deficient) as measured by serum 25-D3 levels was not described in this study [17].

Based on the epidemiologic and preclinical evidence we hypothesized that baseline 25-D3 level may influence the serological response to influenza vaccine in patients with cancer. Results of our small patient study in CaP suggests that there is a trend towards higher response rates among patients with higher 25-D3 levels. There was a high rate of vitamin D supplementation among study participants. In the present study, a large number of patients were taking high dose of vitamin D with a median dose of 2,000 IU/day. Our patients had median 25-D3 level of 44.88 ng/ml. These levels are considerably higher than the non-supplemented general population or colorectal and prostate population [12,13]. In a prior study of prostate cancer patients, we have noted that median 25-D3 level was 22.5 ng/ml in a similar group of patients, 27.1 ng/ml in clinically localized prostate cancer, and 23.7 ng/ml in healthy controls [12]. Similarly low median 25-D3 of 21.3 ng/ml was noted in colorectal patients at our institution [13].

A beneficial effect of high baseline 25-D3 level was noted particularly for influenza A/H3N2 strain. These results correlate with findings of Urashima et al. [18]. In a randomized trial of vitamin D 3 supplementation (1,200 IU/day) versus placebo in school children, incidence of physician diagnosed influenza A infection was lower in vitamin D supplemented group (10.8% vs. 18.6%; relative risk (RR), 0.58; 95% CI: 0.34, 0.99; $P = 0.04$).

Prior studies have used different time endpoints to assess response to influenza vaccination—comparison of (1) pre- and post-immunization serum antibody concentrations, or (2) geometric mean titers, or (3) fourfold increase in antibody titers as an indicator of vaccine efficacy [19–22]. Furthermore, some consider a response in any single strain while others require a response in all three strains [23]. Whether these surrogate laboratory endpoints translate into the clinically relevant end point of prevention of influenza is unclear. Incidence of influenza like illnesses was not evaluated in our study population, but would be desirable clinical end-point in future studies.

There are limitations to the present study: the sample size was small and vitamin D levels were higher than most patient populations because of a high prevalence of supplementation. The results of the present study are intriguing and suggest an interaction between vitamin D signaling and the humoral immune response. Continued evaluation of the clinical role of low vitamin D level appears warranted. Delineation of the role of vitamin D in humoral immune response will be important. Future studies should address the question of whether vitamin D supplementation enhances immune response to influenza vaccination in nonsupplemented population with low baseline serum 25-D3 levels and prevents clinical influenza like illnesses. Prospective study in a larger cancer patient cohort is warranted.

Abbreviations

D	vitamin D supplement (cholecalciferol)
25-D₃	25-hydroxyvitamin D
HI	hemagglutination inhibition

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Fig. 1. Response to individual strains of influenza vaccine among prostate cancer patients with lowest and highest quartile of 25-D3 level.

TABLE I

Baseline Patient Demographics of Prostate Cancer Patients Who Received Influenza Vaccine

Characteristic	Number of patients (%)
Number	35 (100%)
Baseline 25-hydroxyvitamin D level (ng/ml)	
Median	44.88
Range	9.16–71.98
Vitamin D daily supplementation dose (n = 14)	
Median	2,000 IU
Range	800–9,000 IU
Age (years)	
Median	68
Range	53–79
White blood cell count (n = 26) ($\times 1,000$ cells/mm ³)	
Median	7.58
Range	4.30–13.08
Absolute lymphocyte count (n = 26) ($\times 1,000$ cells/mm ³)	
Median	1.77
Range	0.65–4.08
Chemotherapy	
Yes	3 (9%)
No	30 (86%)
Unknown	2 (6%)
Race	
African American	2 (6%)
White	33 (94%)