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Plasma levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and the risk of prostate cancer☆

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Abstract

In the US, prostate cancer (PCa) has the highest incidence rate of all cancers in males, with few known modifiable risk factors. Some studies support an association between the Vitamin D metabolites, 1,25-dihydroxyvitamin D ($1\alpha,25(OH)_2D_3$) and/or 25-hydroxyvitamin D ($25(OH)D_3$), and prostate cancer, while others have yielded conflicting results. $1\alpha,25(OH)_2D_3$ has anti-proliferative and pro-differentiating effects in prostate cancer cell lines, and levels of circulating $25(OH)D_3$ may be important as PCa cells possess 1-α-hydroxylase activity. Using a nested case–control design, we evaluated whether plasma levels of $25(OH)D_3$ and $1\alpha,25(OH)_2D_3$ were associated with prostate cancer risk in participants from the Nutritional Prevention of Cancer (NPC) trial. With 83 cases and 166 matched controls, we calculated the adjusted odds ratios for increasing plasma levels of $25(OH)D_3$ and $1\alpha,25(OH)_2D_3$. Compared to the lowest tertile of plasma $25(OH)D_3$ levels, the adjusted odds ratios were 1.71 (0.68–4.34) and 0.75 (0.29–1.91); the corresponding odds ratios for $1\alpha,25(OH)_2D_3$ were 1.44 (0.59–3.52) and 1.06 (0.42–2.66). Given the pivotal effects of the Vitamin D receptor on gene transcription, it is likely that the anti-carcinogenic effects of Vitamin D that have previously been described are related to the activity and expression of the Vitamin D receptor and should be investigated further.

Keywords: 1,25-Dihydroxyvitamin D; 25-Hydroxyvitamin D; Prostate cancer

1. Introduction

In the US, prostate cancer (PCa) has the highest incidence rate of all cancers, and the second highest mortality rate in men [1], with an estimated 220,900 cases and 28,900 deaths from this disease in 2003 [1]. While age and race are known risk factors for prostate cancer [2], few modifiable risk factors have been identified. Evidence indicates that PCa may have an environmental component [3], which has led to the extensive study of dietary risk factors, including calcium [4,5], lycopene [6], selenium [7], and animal fat [8], and their association with this disease. Schwartz and Hulka [9] observed that, in the US, mortality rates from PCa are relatively high in areas with low ultraviolet radiation, and hypothesized that Vitamin D deficiency might be associated with an increased risk of PCa. This hypothesis stimulated a great deal of interest in the potential association between Vitamin D and PCa. Vitamin D is a steroid hormone that can be obtained from dietary sources such as fatty fish and fortified dairy products, or synthesized endogenously from 7-dehydrocholesterol in the skin after exposure to UV irradiation from the sun [10]. The most abundant circulating Vitamin D metabolite is 25-hydroxycholecalciferol ($25(OH)D_3$), which is hydroxylated at the 1-carbon position by the enzyme 1-α-hydroxylase to form 1,25-dihydroxycholecalciferol ($1\alpha,25(OH)_2D_3$) [11]. This potent metabolite of Vitamin D can exert transcriptional effects on target genes after binding with the nuclear Vitamin D receptor (VDR), a member of the steroid nuclear receptor superfamily [12]. Both normal prostate and prostate carcinoma tissues express the VDR [13], and $1\alpha,25(OH)_2D_3$ has been shown to have anti-proliferative and pro-differentiating effects in normal human prostate and in PCa cell lines [14]. This hormone also inhibits tumor cell invasion, cell adhesion,

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and migration in prostate carcinoma cell lines [15]. In vivo, $1\alpha,25(OH)_2D_3$ and its analogs have been shown to inhibit tumor volume and metastases [16]. The presence of 1-α-hydroxylase activity was recently demonstrated in prostate cancer cell lines and in primary cultures of prostate cells [17]. If prostate cells can produce their own $1\alpha,25(OH)_2D_3$ from $25(OH)D_3$, it is biologically plausible that circulating levels of $25(OH)D_3$ may be of importance to prostate carcinogenesis. Low plasma levels of $1\alpha,25(OH)_2D_3$ [18] and $25(OH)D_3$ [19] have been associated with an increased risk of PCa in some epidemiological studies, while others have shown no relationship [20–22]. Therefore, the objective of this study was to further investigate whether an association exists between circulating levels of $25(OH)D_3$ and $1\alpha,25(OH)_2D_3$ and the risk of prostate cancer.

2. Materials and methods

2.1. Study design

This nested case–control study was conducted with participants from the Nutritional Prevention of Cancer (NPC) trial, the design of which has been described in detail [23]. Briefly, the NPC trial was a randomized, double-blind, placebo-controlled trial conducted among 1312 participants to examine the effects of 200 μg per day of selenium on the recurrence of non-melanoma skin cancer (NMSC). Beginning in 1983, Caucasian participants from seven clinic sites in the eastern US were randomized to receive either 200 μg of selenium in a 0.5 g high-selenium bakers' yeast tablet supplied by Nutrition 21 (La Jolla, CA) and Cypress Systems (Fresno, CA), or a yeast placebo tablet [23]. Participants completed questionnaires at baseline regarding health history, employment, and education [23]. Blood samples were collected at baseline and every 6 months thereafter, separated into plasma aliquots, and stored at −80 °C [23]. While no effect of selenium was observed for skin cancer [23], the results showed a significant reduction in PCa risk of 52% after an average of 7 years of follow-up [7]. In 1989, another treatment group of 424 participants was added to the NPC trial to test the effect of 400 μg of selenium on NMSC. The data collection methods were identical to those for the 200 μg group. In 1996, these trials were unblinded and participants from both the 200 and 400 μg studies were followed for disease events until 2002. For the current study, 96 prostate cancer cases were identified through March 2002. The first blood sample after randomization for each case was identified, then matched to the blood samples of two controls by age (within 5 years), treatment group (selenium or placebo), and clinic site. Of the 96 cases identified, 83 had samples available that were drawn prior to the diagnosis of prostate cancer. Therefore, plasma from a total of 83 cases and 166 controls was analyzed for levels of Vitamin D metabolites.

2.2. Analysis of Vitamin D metabolites

Plasma samples were assessed for levels of $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$ by established methods [24–26]. For isolation and quantification of $1\alpha,25(OH)_2D_3$, the samples were extracted with acetonitrile, vortex-mixed, and centrifuged [25]. Supernatant was added to borosilicate glass tubes, incubated with 12 mmol/l of sodium metaperiodate, applied to a C_{18}-OH cartridge, and washed for purification of $1\alpha,25(OH)_2D_3$ [25]. Fifty microliters of ^{125}I-labeled $1\alpha,25(OH)_2D_3$ derivative were added to 20 μl of calibrator or sample solution, along with buffer and primary antibody, mixed, and incubated [25]. After incubation, 0.5 ml of second-antibody precipitating complex was added, followed by vortex-mixing, incubation, and centrifugation. Radioactivity was counted with a gamma well-counting system [25]. For analysis of $25(OH)D_3$, acetonitrile extraction

Table 1
Baseline characteristics of prostate cancer cases ($n = 83$) and selected controls ($n = 166$) from the NPC trial

Variable	Cases	Controls	*P*-value
Age at randomization (years)	67.2 ± 5.9	66.7 ± 7.4	0.64[a]
Age at blood draw (years)	68.0 ± 6.1	67.6 ± 7.4	0.72
Body mass index (kg/m^2)	25.9 ± 3.7	26.1 ± 3.9	0.71
Baseline plasma selenium (ng/ml)	113.7 ± 19.5	116.0 ± 23.3	0.45
Cigarette smoking (pack-years)	59.2 ± 50.1	60.6 ± 36.9	0.86
Alcohol use (drinks per day)	1.8 ± 2.7	1.5 ± 2.4	0.41
Original treatment group			
Placebo	45 (33.3)	90 (66.7)	1.00[b]
200 μg Selenium	27 (33.3)	54 (66.7)	
400 μg Selenium	11 (33.3)	22 (66.7)	
Clinic site			
Augusta	14 (33.3)	28 (66.7)	1.00
Columbia	15 (33.3)	30 (66.7)	
Georgia	16 (33.3)	32 (66.7)	
Macon	23 (33.3)	46 (66.7)	
Miami	14 (33.3)	28 (66.7)	
Newington	1 (33.3)	2 (66.7)	
Wilson	0 (0.0)	0 (0.0)	
Education			
Completed high school or below	26 (28.0)	67 (72.0)	0.24
At least 1 year college	57 (36.5)	99 (63.5)	
Years on farm			
≤5 Years	43 (35.3)	79 (64.8)	0.59
>5 Years	40 (31.5)	87 (68.5)	
Sunscreen use			
Never	16 (32.0)	34 (68.0)	0.59
Sometimes	30 (30.6)	68 (69.4)	
Always	27 (36.0)	48 (64.0)	

Data is presented as mean ± S.D. or number (%).

[a] For continuous variables, a Student's *t*-test was used for statistical testing.

[b] The Kruskal–Wallis test was used for comparison of categorical variables.

Table 2
Crude and adjusted odds ratios for prostate cancer by tertile of plasma Vitamin D metabolites

	Cases, *n* (%)	Controls, *n* (%)	Odds ratio (95% CI)	Adjusted odds ratio[a] (95% CI)	*P*-for-trend value
$25(OH)D_3$ (ng/ml)					
8.1–25.3	26 (31.0)	58 (69.0)	1.00	1.00	0.51
25.4–32.7	33 (40.2)	49 (59.8)	1.50 (0.79–2.84)	1.71 (0.68–4.34)	
32.8–59.7	24 (28.9)	59 (71.1)	0.91 (0.47–1.76)	0.75 (0.29–1.91)	
$1\alpha,25(OH)_2D_3$ (pg/ml)					
13.7–27.5	27 (32.5)	56 (67.5)	1.00	1.00	0.90
27.6–32.9	29 (34.9)	54 (65.1)	1.11 (0.59–2.12)	1.44 (0.59–3.52)	
33.0–64.4	27 (32.5)	56 (67.5)	1.00 (0.52–1.91)	1.06 (0.42–2.66)	

[a] Models are adjusted for age at blood draw, clinic site, body mass index and cigarette smoking (pack-years).

was performed, and ^{125}I-labeled $1\alpha,25(OH)_2D_3$ derivative was added to the assay tubes [26]. After incubation with primary antibodies, 0.5 ml of the second-antibody complex was added, incubated, and counted with a gamma well-counting system [26]. The coefficients of variation for $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$ were 11.5 and 12.9%, respectively.

2.3. Statistical analyses

Characteristics of the cases and controls were compared by employing the Student's *t*-test for continuous variables and the Kruskal–Wallis test for significance for categorical variables. Two-sided *P*-values were considered significant at an $\alpha < 0.05$. Tertiles of plasma levels of Vitamin D were created based on the distribution for the entire sample, with the lowest tertile as the reference category for regression models. Stepwise logistic regression was used to determine the baseline variables that should be included in the multivariate logistic regression analyses for the association between plasma Vitamin D levels and prostate cancer risk. The variables that were significantly associated with prostate cancer using a likelihood-ratio test were included in the final model. The *P*-for-trend values of the logistic regression models were calculated using a categorical variable for each plasma Vitamin D metabolite.

3. Results

In the cases, the mean number of years (±S.D.) between the blood draw used for Vitamin D assessment and a diagnosis of prostate cancer was 5.1 ± 3.4. Table 1 presents a comparison of baseline characteristics for the cases and controls. With regard to the variables used for matching men with prostate cancer to control participants, the mean age at randomization for the cases and controls was 67.2 and 66.7, respectively ($P < 0.64$). The proportion of cases and controls was identical for each treatment group ($P = 1.00$) and across the seven clinic sites from which participants were recruited ($P = 1.00$), as would be expected for matched variables. The similar age at blood draw for the cases and controls is also relevant as it indicates that samples for both groups were assessed for Vitamin D levels after equivalent time on study. The mean number of months that had passed between randomization and the blood draw for analysis of Vitamin D metabolite levels for cases and controls was 9.8 and 11.0, respectively ($P < 0.46$; data not shown). There were no significant differences between the cases and controls for the remaining baseline characteristics, including body mass index, plasma selenium levels, pack-years of cigarette smoking, alcohol use, education, residence on a farm, or sunscreen use.

Table 2 shows the crude and adjusted odds ratios for prostate cancer by tertile of each plasma Vitamin D metabolite. Compared to the lowest tertile of plasma $25(OH)D_3$, the adjusted odds ratios (95% confidence intervals) for prostate cancer were 1.71 (0.68–4.34) and 0.75 (0.29–1.91) for the second and third tertiles, respectively (*P*-for-trend = 0.51). For plasma $1\alpha,25(OH)_2D_3$, the adjusted odds ratios (95% confidence intervals) for the second and third tertiles were 1.44 (0.59–3.52), and 1.06 (0.42–2.66), respectively (*P*-for-trend = 0.90).

4. Discussion

Currently, the risk factors for prostate cancer that have been identified [2], such as age and race, cannot be modified. However, there are some data that support an environmental component to the etiology of this disease [3], and it was hypothesized that Vitamin D might confer protection from prostate cancer [9]. Therefore, we sought to investigate whether plasma levels of $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$ were associated with the risk of prostate cancer. The results of this study do not support a relationship between circulating levels of these Vitamin D metabolites and prostate cancer risk.

Several investigations have been conducted to address the potential influence of $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$ on prostate cancer. Our results are in agreement with some reports [20–22], although not that of Corder et al. [18]. In the latter study, a significant reduction in prostate cancer risk was observed with higher serum levels of $1\alpha,25(OH)_2D_3$ among participants in northern California [18], while no

differences were observed for serum 25(OH)D_3. The reasons for the conflicting results between the current analysis and the northern California investigation are unclear. The mean plasma levels of 1α,25(OH)$_2$$D_3$ for the participants from the present study and for the Caucasian men in the northern California study were similar (31.4 and 31.3 pg/ml, respectively), as was the range of values observed. Because the primary protection observed with higher levels of 1α,25(OH)$_2$$D_3$ in the northern California study was found in men older than 57 years of age at baseline, we analyzed plasma levels of Vitamin D in that age group separately and observed no suggestive trends. The current investigation was limited by the small sample size of 83 cases and 166 controls, and this may be a factor in the disparate results observed in this study compared to the northern California sample.

With regard to 25(OH)D_3, our results were in agreement with some reports [20–22], but not with a Finnish investigation in which cases were observed to have significantly lower levels of 25(OH)D_3 compared to controls, especially among men younger than 52 years of age [19]. Lower serum levels were also associated with more aggressive cancer in the Finnish study [19]. The lack of agreement between the current results and those of the Finnish report may be related to the smaller sample size used for our study, or to differences in plasma Vitamin D levels due to geographical variation. The men in our investigation were primarily recruited from the southeastern US, where sun exposure is relatively frequent. Therefore, Vitamin D levels would be expected to be higher than those of men in Finland. It has been suggested that the lower limit of the normal range for circulating 25(OH)D_3 be set at 20 ng/ml [27]. By this definition, more than 85% of our sample had sufficient plasma 25(OH)D_3 levels; while for the Finnish study less than 50% of the participants reached adequacy [19]. It is possible that the risk of prostate cancer is increased only in Vitamin D-deficient populations.

As mentioned above, a major limitation of this study was its small sample size. Data from 249 participants were analyzed, which may have been insufficient to show a significant association between Vitamin D and prostate cancer. However, in this investigation there were no trends observed for plasma Vitamin D levels that suggested a link to protection from PCa. Another concern is the use of a nested case–control design within a nutritional intervention trial in which the intervention, in this case selenium, had a significant effect on the risk of prostate cancer. In the NPC trial, a 52% reduction of risk was observed in the group that received 200 μg of selenium per day [7]. To control for the potential effects of selenium on this analysis, we matched the cases and controls by treatment group, and the proportion of cases and controls from each group was identical. The use of data from participants in the NPC trial was a strength of this study. The NPC trial was a carefully conducted intervention trial, and endpoint ascertainment was confirmed by review of medical records after self-reports of illnesses by study participants.

In conclusion, the results of this study do not support a protective relationship for the Vitamin D metabolites, 1α,25(OH)$_2$$D_3$ and 25(OH)D_3, and prostate cancer in participants from the NPC trial. However, recent reports have indicated that genetic polymorphisms in the Vitamin D receptor may be related to the risk of prostate cancer [28,29]. Because the transcriptional effects of Vitamin D are mediated through the activity of the receptor, future studies should consider the effect of polymorphic variants on risk of this disease.

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