Vitamin D and Prostate Cancer: A Prediagnostic Study with Stored Sera[1]

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Abstract

This study evaluates the risk of prostate cancer in relation to serum levels of the major vitamin D metabolites, 25-hydroxyvitamin D (25-D[3]) and 1,25-dihydroxyvitamin D (1,25-D). Between 1964 and 1971, more than 250,000 serum samples were collected from members of the Kaiser Permanente Medical Care Plan in Oakland and San Francisco and stored for future use. Levels of 25-D and 1,25-D were measured in samples from 90 black and 91 white men diagnosed with prostate cancer before December 31, 1987 and controls individually matched on age, race, and day of serum storage. Mean serum 1,25-D was 1.81 pg/ml lower in cases than in matched controls ($P = 0.002$). Risk of prostate cancer decreased with higher levels of 1,25-D especially in men with low levels of 25-D. However, mean 25-D was not significantly different in cases and controls. The association of lower 1,25-D with prostate cancer was found in men above the median age of 57 years at serum storage but not younger men and was similar in black and white men. In men ≥ 57 years of age, 1,25-D was an important predictor of risk for palpable and anaplastic tumors but not for tumors incidentally discovered during surgery to treat the symptoms of benign prostatic hyperplasia or well differentiated tumors.

Introduction

Prostate cancer is the most frequently diagnosed cancer and the second leading cause of cancer death in American men (1, 2). Epidemiological and laboratory studies suggest that vitamin D metabolites and their analogues influence the growth of a number of forms of cancer (3–6). Higher serum levels of 25-D[3] and 1,25-D might be expected to reduce the risk of prostate cancer for several reasons; (*a*) prostate cancer death rates are lower in regions that receive more UV light needed for synthesis of previtamin D in the skin (7); (*b*) functional vitamin D receptors are present in normal prostate cells and in established prostate cancer cell lines (8); (*c*) 1,25-D inhibits the proliferation of cells in established prostate cancer cell lines and cultures of human prostatic epithelial cells established from normal, hyperplastic, and malignant tissues.[4,5]

Vitamin D_3 is synthesized in the skin from 7-dehydrocholesterol when exposed to sunlight (8, 9). Vitamin D_3 is not active until 25-hydroxylated to 25-D in the liver and then 1-hydroxylated to 1,25-D in the kidney. The liver has excess capacity to quickly 25-hydroxylate vitamin D_3 and the biological half-life of 25-D is several weeks. Thus, serum levels of 25-D reflect vitamin D status and are generally higher in the summer than in the winter especially in northern regions where little skin is exposed to sunlight during cold weather. Normal levels of 25-D are typically from 15 to 80 ng/ml and are not closely regulated.

Only a small proportion of 25-D is hydroxylated to the active hormone 1,25-D. Serum 1,25-D has a biological half-life of 6 to 8 h and levels are closely regulated by a number of factors, primarily serum calcium and phosphate, and is limited by poor renal function. Normal levels of 1,25-D are typically 15–60 pg/ml, roughly 1000 times less then 25-D levels. 1,25-D partitions into cells by virtue of its lipophilicity, binds to intracellular receptors, and translocates to the nucleus where the complex controls the transcription of a host of genes. Many of these genes are related to calcium metabolism: 1,25-D increases the absorption of dietary calcium; increases the mobilization of calcium from the bones; and reduces the urinary excretion of calicum. Other genes the transcription of which are regulated by 1,25-D appear to be important in cell differentiation.

Vitamin D_2 supplements such as ergocalciferol, which is used to fortify milk in the United States, are derived from plant sources. They are absorbed with dietary fats in chylomicrons. While the chemical structure of dermal and plant

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[3] The abbreviations used are: 25-D, 25-hydroxyvitamin D_2 or D_3; 1,25-D, 1,25-dihydroxyvitamin D_2 or D_3; ICDA, international classification of disease; CI, confidence interval.

[4] D. M. Peehl, R. Skowronski, G. K. Leung, S. T. Wong, T. A. Stamey, and D. Feldman. Antiproliferative effects of 1,25-dihydroxyvitamin D_3 on primary cultures of human prostatic cells, submitted for publication, 1992.

[5] R. J. Skowronski, D. M. Peehl, and D. Feldman. Vitamin D and prostate cancer: 1,25 dihydroxyvitamin D_3 receptors and actions in prostate cancer cell lines, submitted for publication, 1992.

vitamins differ slightly, their metabolism and activity closely parallel each other.

The availability of stored serum samples collected from 1964 to 1971 from members of the Kaiser Permanente Medical Care Plan of Northern California made an essentially prospective evaluation of the vitamin D hypothesis possible. Levels of 25-D and 1,25-D could be compared in men who were diagnosed with prostate cancer from 1 to 23 years later and controls not diagnosed with prostate cancer to determine whether the metabolites reduce the risk of disease. Levels were compared in stored sera from 90 black and 91 white men diagnosed with prostate cancer before December 31, 1987, and from pair-matched controls based on age, race, and day of serum storage.

Materials and Methods

Source Population. Starting in 1964, the Kaiser Permanente Medical Care Program of Northern California operated multiphasic screening centers in Oakland and San Francisco as part of its preventive health services program (10, 11). At the time of screening, members were ambulatory and, generally, healthy. From 1964 to 1971, more than 250,000 serum samples were collected at the screening centers and stored at −23°C or colder until 1980 when they were shipped to a frozen storage facility (−40°C) of the Orentreich Foundation for the Advancement of Science, Inc. (12, 13).

Case Selection. Cases were selected from men: (*a*) who had been screened; (*b*) whose sera had been stored; and (*c*) who were diagnosed with prostate cancer (ICDA-8 or ICDA-9 code 185) at a Kaiser facility before December 31, 1987. Thus, cases among men who were no longer Kaiser members at the time of diagnosis are not included in the study. Potential cases were identified by computerized searches of discharge records of northern California Kaiser hospitals and records of the Bay Area Resource for Cancer Control covering the five San Francisco Bay area counties (14).

Resources were not available to measure vitamin D metabolite levels in all 623 white, 121 black, and 13 oriental cases that were identified. Power calculations suggested that a relative protective effect of 0.5 could be detected with high power (from 80 to 95% depending on the presumed proportion exposed) using 200 case-control pairs (15). The desire to compare risk in black and white men dictated that blacks should be oversampled relative to whites. Hence, 100 white cases diagnosed at Oakland or San Francisco were randomly selected, and the 94 black cases diagnosed at Oakland or San Francisco plus 6 cases diagnosed at San Rafael were selected.

Control Selection. Controls were selected from men: (*a*) who had been screened; (*b*) whose sera had been stored; and (*c*) who were alive, Kaiser health maintenance organization members, and without a diagnosis of prostate cancer at the time of case diagnosis. Each selected case was matched to one control of the same race and age within 3 years when serum was stored. To account for the possible seasonal variation in 25-D, cases and matched controls were screened on or near the same day of the year. Most pair members were seen at the same screening center within 3 days of each other.

Exclusions. Nine cases were excluded because the complete medical record did not indicate prostate cancer. Four cases were excluded during histopathological review because invasive adenocarcinoma was not seen. Six cases and six controls were excluded because of missing sera. Thus, 19 cases and 6 controls were excluded. To avoid exclusion of all 19 controls and 6 cases paired to excluded cases and controls, 6 case-control pairs were salvaged from their respective pair members by forming the best age and race matches. Hence, the study includes 90 black and 91 white case-control pairs.

Histopathological Review. During histopathological review, primary and secondary Gleason scores ranging from 2 (well differentiated) to 5 (anaplastic) were assigned based on the predominant pattern in the cancerous tissue and a second pattern that is often more malignant (16). The total Gleason score (from 4 to 10) reported below is the sum of these scores. The study pathologist was blinded to stage and grade assigned at clinical diagnosis.

Seven cases that could not be reviewed by the study pathologist had been histologically confirmed at clinical diagnosis and are included in the study. Diagnosis was based solely on digital rectal examination and elevated serum acid phosphatase for two cases. Bone scans were not done on either of these patients. However, one of these patients was treated with diethylstilbesterol and referred for palliative radiation therapy suggesting the clear clinical impression of prostate cancer. The other patient died of lung cancer shortly after prostate cancer diagnosis.

Stage at diagnosis was obtained from cancer registry records based on evidence obtained within 4 months of clinical diagnosis. Date and cause of death were retrieved using the California Automated Mortality Linkage System (17). Symptoms at baseline and use of medical services between baseline and case diagnosis were retrieved from computerized screening and hospitalization records.

Laboratory Determinations. Serum calcium, phosphate, albumin, and creatinine had previously been determined using standard methods at the time of serum storage and were retrieved from computerized records. Adjusted serum calcium was calculated as calcium − albumin + 4.0 (18). Serum sodium concentration was within normal limits (135–148 mmol/liter) in each retrieved specimen (18) confirming that the samples had not been appreciably desiccated during storage. Competitive protein binding (19) and calf thymus receptor assays (20, 21) were used to measure the levels of 25-D (ng/ml) and 1,25-D (pg/ml) in the retrieved sera, respectively. These assays do not distinquish between metabolites of vitamin D_2 and D_3. Laboratory personnel were blinded to case and control status.

Statistical Analysis. Wilcoxon's signed rank test for paired samples was used to compare serum levels in cases and matched controls. Tests are two sided. Actual *P* values are reported with significance declared at the 0.05 level.

A conditional logistic model for paired data was used to evaluate whether vitamin D metabolite levels are related to the risk of prostate cancer, allowing for their possible interaction (22, 23). To illustrate the odds of prostate cancer across the physiological range, quartiles of 25-D and 1,25-D were identified in the 362 subjects and a second model was constructed in which the measured values were replaced by quartiles.

To identify possible major departures from a continuous trend in risk which would suggest the need for a revised model, the crude unmatched odds in each of the three higher quartiles of 1,25-D were compared to the odds in the lowest quartile.

To detect variation in risk based on the matching variables of age and race, separate models were constructed for

Table 1 Comparison of Case Characteristics According to Race

	Black	White
	mean (range)	mean (range)
Age at Baseline	53 (38–71)	61 (38–81)
Age at Diagnosis	66 (51–78)	72 (50–89)
Years Until Diagnosis	12 (1–23)	11 (2–22)
	n (%)	*n* (%)
Method of Diagnosis		
Needle Biopsy	60 (66.7%)	62 (68.1%)
TURP	26 (28.9%)	21 (23.1%)
Other	4 (4.4%)	8 (8.8%)
Total Gleason Grade		
4 or 5	10 (11.1%)	7 (7.7%)
6	33 (36.7%)	33 (36.3%)
7	21 (23.3%)	17 (18.7%)
8, 9, or 10	22 (24.4%)	26 (28.6%)
Unknown	4 (4.4%)	8 (8.8%)
Stage		
Localized	63 (70.0%)	54 (59.3%)
Regional	6 (6.6%)	15 (16.5%)
Remote	17 (18.9%)	12 (13.2%)
Unknown	4 (4.4%)	10 (11.0%)

black and white men, for men younger than and older than the median age of 57 years at serum storage, and for men younger than and older than the median age of 69 years at case diagnosis. The likelihoods for each pair of models were subtracted from the likelihood for the overall model. The resulting statistics are each asymptotically distributed as a χ^2 with 3 degrees of freedom (24). Analogously, variation in risk with method of diagnosis, grade at diagnosis, and stage at diagnosis (each dichotomized) were explored in men younger than 57 years of age at serum storage.

Linear and proportional hazard models were constructed to determine whether vitamin D metabolites predict total Gleason grade at diagnosis (from 4 to 10) and the risk of prostate cancer death (25), respectively, in cases.

Results

Description of Cases. Almost 90% of cases were diagnosed at least 5 years after serum was obtained; only 3 cases were diagnosed within 2 years of baseline. Although black cases were on average younger than white cases at the time of serum storage and at diagnosis, the proportion of cases identified by needle biopsy and transurethral resection of the prostate, the spectrum of grade and stage at diagnosis, and the interval from baseline to diagnosis were similar for black and white cases (Table 1). Four controls were known to have been diagnosed with prostate cancer after the matched case diagnosis.

Vitamin D Metabolite Levels. Despite prolonged storage which might degrade the samples, measured values of 25-D and 1,25-D were generally within or above normative limits for the laboratory used (Duke University Bone and Mineral Laboratory), 15–80 ng/ml for 25-D and 19–50 pg/ml for 1,25-D (Table 2).

In this ambulatory population, levels of 25-D and 1,25-D were similar in younger and older men. Mean 25-D was 5.52 ng/ml lower and mean 1,25-D was 5.03 pg/ml higher in black than in white controls. There was strong seasonal variation in 25-D but not 1,25-D. Mean January levels of 25-D were lower than July levels for both cases (18.5 *versus* 28.9 ng/ml) and controls (18.3 *versus* 27.8 ng/ml).

Table 2 Vitamin D metabolite levels in controls according to race and age at baseline[a]

	n	25-D, ng/ml			1,25-D, pg/ml		
		Mean	median	(range)	Mean	median	(range)
Black controls							
<57 Years	60	21.1	20.5	(8–44)	35.5	35.0	(17–81)
≥57 Years	30	18.6	19.5	(5–27)	38.1	37.0	(20–61)
White controls							
<57 Years	30	23.3	21.0	(12–44)	31.3	31.5	(17–45)
≥57 Years	61	27.0	25.0	(12–49)	31.3	30.0	(12–50)

[a] Black and white controls were dichotomized at the median age of 57 years at serum storage.

Case-Control Comparisons. Cases and matched controls had similar levels of 25-D ($P = 0.24$). Table 3 shows that cases and matched controls had similar levels of 25-D in each of the subgroups defined by race and age at serum storage. Cases and controls were equally likely to have had vitamin D deficiency at diagnosis: 27 cases (14.9%) and 24 controls (13.3%) had 25-D <15 ng/ml (18).

In contrast to 25-D, the active hormone 1,25-D was on average 1.81 pg/ml lower in cases than in matched controls ($P = 0.002$). Significantly lower levels of 1,25-D were found in both black and white cases over the median age of 57 years at serum storage compared to matched controls (Table 3).

In a conditional logistic model, the measured values of 25-D, 1,25-D and their interaction were jointly predictive of the risk of prostate cancer ($P = 0.02$). An analogous model was constructed using quartiles of 25-D and 1,25-D. The odds of prostate cancer in each quartile of 25-D and 1,25-D compared to their respective lowest quartiles are given in Table 4. The risk for prostate cancer tended to decrease with higher levels of 1,25-D especially in men with low levels of 25-D. Significantly reduced risk was found for each of the higher quartiles of 1,25-D in the lowest quartile of 25-D. The smallest risk was found for the highest quartile of 1,25-D and the lowest quartile of 25-D: OR, 0.15 (95% CI, 0.03–0.85).

In tests of heterogeneity, risk in relation to vitamin D metabolite levels depended on age at serum storage ($P = 0.002$) and age at case diagnosis ($P = 0.050$). However, the pattern of risk was similar in black and white men ($P = 0.51$). Looking at the separate strata, vitamin D metabolite levels were important predictors of risk in men older than the median age of 57 years at baseline ($P = 0.0001$) but not in younger men ($P = 0.98$). Thus, the protective effect of 1,25-D seen in Table 4 is likely to be attributable to men at least 57 years of age at serum storage. Table 5 shows the odds of prostate cancer in men at least 57 years of age at the time of serum storage in relation to vitamin D metabolite levels. As in Table 4, the smallest risk was found in the highest quartile of 1,25-D and the lowest quartile of 25-D: OR, 0.01 (95% CI, 0.00–0.33).

In men over 57 years of age at serum storage, the crude unmatched odds decreased with each higher quartile of 1,25-D from 0.66 in the second to 0.53 in the third to 0.37 in the fourth, highest, quartile compared to the first, lowest, quartile. These odds ratios are biased toward the null (26) and ignore the important interaction of 1,25-D with 25-D.

Table 3 Mean differences between cases and matched controls in vitamin D metabolite levels according to race and age[a]

	n	25-D ng/ml P	1,25-D pg/ml P
Blacks			
<57 Years	57	−0.75 (0.39)	−1.54 (0.11)
≥57 Years	33	2.45 (0.18)	−3.88 (0.03)
Whites			
<57 Years	32	2.31 (0.24)	1.59 (0.82)
≥57 Years	59	1.12 (0.41)	−2.76 (0.004)

[a] Wilcoxon's signed rank test was used to compare cases with matched controls.

Table 4 The odds of prostate cancer for each quartile of 25-D and 1,25-D compared to their lowest quartiles[a]

Quartile of 25-D (ng/ml)	Quartile of 1,25-D (pg/ml) 1 (5–26)	2 (27–32)	3 (33–39)	4 (40–81)
1 (3–18)	1.00	0.53[b]	0.28[b]	0.15[b]
2 (19–23)	0.96	0.58	0.35	0.21
3 (24–28)	0.93	0.63	0.43	0.29
4 (29–52)	0.90	0.69	0.53	0.41

[a] The risk for prostate cancer decreases with increasing levels of 1,25-D for all levels of 25-D. However, this trend is statistically significant only for men whose 25-D is in the lowest quartile. A conditional logistic model was constructed in which quartiles of 25-D and 1,25-D were used to predict the odds of prostate cancer. A term for the interaction of 25-D with 1,25-D was included in the model. Black and white men were included in the model.
[b] The 95% CI for this quartile did not include 1, the reference value for the lowest quartiles of 25-D and 1,25-D.

However, they are consistent with decreases in risk throughout the physiological range of 1,25-D.

In men over 57 years of age at serum storage, vitamin D metabolite levels were related to the risk of palpable tumors ($P = 0.0001$; $n = 56$) but not for tumors incidentally discovered at transurethral resection of the prostate to relieve the symptoms of benign prostatic hyperplasia ($P = 0.173$; $n = 28$) (Table 6). Vitamin D metabolite levels were also predictive of the risk for more anaplastic tumors with total Gleason grade 7–10 at diagnosis ($P = 0.0001$; $n = 44$) but not for more well differentiated tumors with total Gleason grade 4–6 ($P = 0.08$; $n = 38$). The risk for both localized and metastatic tumors was related to prediagnostic levels of vitamin D metabolites. However, greater protection was found for metastatic than for localized tumors.

Grade at Diagnosis. Vitamin D metabolite levels were predictive of total Gleason grade at diagnosis in cases at least 57 years of age at the time of serum storage ($P = 0.006$) but not younger cases ($P = 0.16$) (Table 7). In cases at least 57 years of age at the time serum was obtained, 15% of the variation in grade was explained by earlier vitamin D metabolite levels. Predicted total Gleason grade (from 4 to 10) was lowest, indicating relatively well differentiated tumors, in cases with high 1,25-D and low 25-D. This finding is consistent with the low risk for prostate cancer reported above in men with high 1,25-D and low 25-D. Curiously, predicted grade at diagnosis was highest in cases with high 25-D and high 1,25-D. Similar patterns were found for white cases at least 57 years of age ($n = 61$) and cases with palpable tumors at least 57 years of age ($n = 56$).

Table 5 The odds of prostate cancer for each quartile of 25-D and 1,25-D compared to their lowest quartiles in men at least 57 years of age when serum was obtained

Quartile of 25-D (ng/ml)	Quartile of 1,25-D (pg/ml) 1 (5–26)	2 (27–32)	3 (33–39)	4 (40–81)
1 (3–18)	1.00	0.22[b]	0.05[b]	0.01[b]
2 (19–23)	0.79	0.23	0.07[b]	0.02[b]
3 (24–28)	0.62	0.24	0.09	0.03[b]
4 (29–52)	0.48	0.24	0.12	0.06

[a] The risk for prostate cancer decreases with increasing levels of 1,25-D for all levels of 25-D in men at least 57 years of age when serum was obtained. The protective trend is more pronounced then when men less than 57 years of age were included in the model (Table 4). A separate model for men less than 57 years of age was not predictive of risk.
[b] The 95% CI for this quartile did not include 1, the reference value for the lowest quartiles of 25-D and 1,25-D.

Table 6 The odds of palpable or poorly differentiated prostate cancer for each quartile of 25-D and 1,25-D compared to their lowest quartiles in men at least 57 years of age when serum was obtained[a]

Quartile of 25-D (ng/ml)	Quartile of 1,25-D (pg/ml) 1 (5–26)	2 (27–32)	3 (33–39)	4 (40–81)
Palpable tumor				
1 (3–18)	1.00	0.14[b]	0.02[b]	0.00[b]
2 (19–23)	0.80	0.15	0.03[b]	0.01[b]
3 (24–28)	0.63	0.18	0.05	0.01[b]
4 (29–52)	0.51	0.20	0.08	0.03
Gleason Grade 7–10				
1 (3–18)	1.00	0.11[b]	0.01[b]	0.00[b]
2 (19–23)	0.55	0.10	0.02[b]	0.00[b]
3 (24–28)	0.30	0.09	0.03	0.01
4 (29–52)	0.16	0.09	0.05	0.03

[a] Separate models were constructed for pairs whose case had a palpable tumor ($n = 56$) or whose tumor was poorly differentiated ($n = 44$). A protective trend was found for increasing 1,25-D for all levels of 25-D. Significant decreases in risk were found for men in all but the highest quartile of 25-D despite the small sample sizes. Separate models for pairs whose case had an incidentally found or well differentiated tumor were not predictive of risk.
[b] The 95% CI for this quartile did not include 1, the reference value for the lowest quartiles of 25-D and 1,25-D.

Death from Prostate Cancer. Prostate cancer was reported as the underlying cause of death for 51 cases. Baseline vitamin D metabolite levels were unrelated to the risk for death due to prostate cancer overall and in subgroups of men < or ≥ 57 years of age at the time of serum storage. However, there was a strong association between total Gleason grade and risk for death from prostate cancer ($P = 0.005$). The odds of prostate cancer death doubled for each higher total Gleason grade from 4 to 10.

Factors Related to Vitamin D Metabolism. Mean serum calcium was similar in cases (9.77 mg/dl) and controls (9.82 mg/dl) ($P = 0.61$). After adjustment for serum albumin, no significant difference was found in free serum calcium in cases and matched controls ($P = 0.49$). Mean serum creatinine was similar in cases, 1.09 mg/dl, and controls, 1.12 mg/dl ($P = 0.23$).

Mean serum phosphate was 3.31 mg/dl in the 61 available cases and 3.15 mg/dl in the available 63 controls. This difference is notable even though it did not reach statistical

Table 7 Predicted total gleason grade at diagnosis (95% CI) in men ≥57 years of age at serum storage for each quartile of 25-D and 1,25-D[a]

Quartile of 25-D (ng/ml)	Quartile of 1,25-D (pg/ml)			
	1 (5–26)	2 (27–32)	3 (33–39)	4 (40–81)
1 (3–18)	7.50 [6.68-8.30]	6.86 [6.37-7.36]	6.22 [7.73-6.73]	5.59 [4.77-6.42]
2 (19–23)	7.19 [6.68-7.71]	6.86 [6.55-7.17]	6.52 [6.21-6.83]	6.19 [5.68-6.70]
3 (24–28)	6.89 [6.54-7.24]	6.85 [6.63-7.08]	6.82 [6.57-7.07]	6.78 [6.39-7.17]
4 (29–52)	6.59 [6.09-7.09]	6.85 [6.52-7.18]	7.11 [6.73-7.49]	7.37 [6.77-7.97]

[a] Total Gleason grade ranges from 4, well differentiated, to 10, anaplastic. Quartiles of 25-D, 1,25-D and their product were used in a linear model to predict grade at diagnosis.

significance at this reduced sample size ($P = 0.099$). Interestingly, variability in case levels was greater than in controls ($P = 0.018$) and was attributable to the 24 cases and 27 controls less than 57 years of age at baseline ($P = 0.001$). In men less than 57 years of age at baseline, mean serum phosphate was 3.51 mg/dl in cases and 3.23 mg/dl in controls ($P = 0.11$).

Health and Use of Medical Services. At the multiphasic screening visit, at which time the sera were obtained, cases were no more likely than controls to have reported conditions likely to disclose prostate cancer. Fewer cases (35.8%) than controls (48.0%) reported waking from sleep to urinate in the last year ($P = 0.050$). Conditions queried included burning or pain with urination, trouble starting urination, loss of bladder control, urine blood/dark, treatment for genitals, venereal disease, wakening to urinate, serious illness, ruptured hernia, and colon/bowel disease.

Between baseline and case diagnosis, 4 cases and 3 controls had a vasectomy; 17 cases and 14 controls had prostate surgery ($P = 0.58$), 26 cases and 19 controls had a diagnosis of benign prostatic hypertrophy ($P = 0.26$); and 91 cases and 71 controls had been hospitalized ($P = 0.03$). Hospital discharge records indicated that 8 of 31 case and 0 of 26 control hospitalizations in the year prior to case diagnosis were coded ICDA 185 or prostate cancer or were ruled out prostate cancer. Thus, the excess risk of hospitalization in the year prior to case diagnosis was directly related to the process of case diagnosis.

Discussion

Prior epidemiological studies suggest that the risk for a number of forms of cancer may be related to low levels of 25-D or factors, such as geographic variation in annual sunlight exposure, related to serum 25-D level (27–30). In contrast to these studies, cases and controls in this study had similar levels of 25-D and were equally likely to have had vitamin D deficiency. Notably, prior studies were either ecological studies, concerned other forms of cancer, or have not been confirmed by other groups of researchers.

On the other hand, results of this epidemiological study are consistent with prior laboratory studies in prostate and other cancers which suggested that the active hormonal form of vitamin D and its analogues limit the proliferation of tumor cells (3–6, 8). [4,5] Cases had significantly lower levels of 1,25-D than did matched controls.

The overall case-control difference in 1,25-D was attributable to men 57 years of age and older at the time serum was obtained. This suggests that prostate cancer is a heterogeneous disorder whose etiological heterogeneity is related to age. The plausibility of a protective effect for 1,25-D in men 57 years of age and older is supported by: (*a*) steady decreases in risk throughout the physiological range; (*b*) larger reductions in risk for palpable, disseminated, and anaplastic tumors compared to incidental, localized, and well differentiated tumors (which might have been present even in the controls); and (*c*) fewer anaplastic tumors in cases with high 1,25-D and low 25-D. These results were not found for younger men. However, the study does not suggest that decreases in 1,25-D are responsible for the increased incidence of prostate cancer with age because levels were similar in younger and older men.

The odds of prostate cancer in relation to earlier levels of vitamin D metabolites were similar for black and white men while 1,25-D tended to be higher in black compared to white men. Thus, the higher national prostate cancer incidence rate in blacks cannot be attributed to lower levels of 1,25-D or to lesser degrees of protection due to 1,25-D. However, the study was not large enough to precisely define small differences in risk related to race given the variation in risk with age, the tendency for blacks to have lower 25-D and higher 1,25-D, and the seasonal variation in 25-D which may be wider in whites than in blacks.

The intention of the study was to relate levels of 25-D and 1,25-D to the risk of clinically important prostate cancer many years later. The long average interval between baseline and diagnosis suggests that the measured vitamin D metabolite levels reflect levels of 25-D and 1,25-D prior to extensive disease which might have altered metabolite levels. However, the study is likely to have included cases with small foci of localized disease which would not have progressed to clinically significant disease and controls with undiagnosed prostate cancer at the time of case diagnosis. Given these limitations, which might tend to diminish the differences in baseline levels between cases and controls, the protective effect of 1,25-D may be underestimated.

Despite the seasonal variation in 25-D which would tend to obscure the relationship of 25-D with the risk for prostate cancer, the protective effect of 1,25-D was stronger in men with low levels of 25-D. As precursor and active hormone with very similar chemical stuctures, some degree of coordinate regulation and overlapping or competing activity of the two metabolites seems biologically plausible. The mechanism of this interaction and its importance with respect to the risk for prostate cancer needs to be further evaluated.

The protective effect of 1,25-D is unlikely to be directly mediated by calcium as cases and controls had virtually identical mean serum calcium levels. This finding provides *in vivo* evidence that regulation of cell growth and differentiation by 1,25-D is distinct from calcium homeostasis.

The protective effect of 1,25-D did not simply reflect poorer kidney function in cases because case and control creatinine levels were the same. Phosphate levels were slightly, although insignificantly, higher in cases than in controls in the small number of men with available values. These findings suggest that factors which regulate the hydroxylation of 25-D to 1,25-D such as parathyroid hormone and dietary phosphate might be related to risk and need to be evaluated.

Cases and controls had equal access to medical services as members of the same prepaid health care plan. The similar use of medical services by cases and controls suggests that case-control differences in 1,25-D did not derive from more frequent chronic disease in cases.

The study does not suggest that vitamin D supplementation will prevent prostate cancer. Cases and controls had similar levels of precursor 25-D which is rapidly formed from ingested as well as dermally synthesized vitamin D. The vast excess of 25-D ensures that substrate deficiency is not the reason that cases had lower 1,25-D levels. The issue seems to be how much of the active hormone is formed from the vast excess of available precursor.

Prophylactic treatment with 1,25-D (calcitriol) is at present of unproven value and likely to result in hypercalcemia in many men even when dietary calcium is restricted. A preventive trial of analogues less likely to cause hypercalcimia might be warranted if higher prediagnostic levels of 1,25-D are found to be protective in another population.

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