Vitamin D status and breast cancer in Saudi Arabian women: case-control study[1–4]

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ABSTRACT

Background: The role of vitamin D in breast cancer prevention is equivocal. Saudi Arabian women may be at greater risk of vitamin D deficiency because of a darker skin type and a greater likelihood of reduced ultraviolet B radiation exposure. Data regarding the vitamin D status of Saudi Arabian women and its relation to breast cancer risk are lacking.

Objective: The purpose of this research was to evaluate the association between circulating concentrations of 25-hydroxyvitamin D [25(OH)D] and breast cancer risk in Saudi Arabian women.

Design: A case-control study was conducted among 120 breast cancer cases and 120 controls. The study population was drawn from patients admitted to King Fahd Hospital in Jeddah, Saudi Arabia, from June to August 2009. Participants completed questionnaires on diet and medical history, and serum samples were collected from all women to measure circulating 25(OH)D concentrations.

Results: The participants had a mean age of 47.8 y and a mean body mass index (BMI; in kg/m^2) of 30.0. Breast cancer cases had significantly lower (mean ± SD) serum concentrations of 25(OH)D (9.4 ± 6.4 ng/mL) than did controls (15.4 ± 12.3 ng/mL; P = 0.001). In comparison with those in the highest category of vitamin D status for this population (≥20 ng/mL), the adjusted ORs (95% CIs) for invasive breast cancer were 6.1 (2.4, 15.1) for women with a serum 25(OH)D concentration <10 ng/mL and 4.0 (1.6, 10.4) for women with a serum concentration of ≥10 to <20 ng/mL (P-trend = 0.0001).

Conclusion: An inverse association exists between serum 25(OH)D concentrations and breast cancer risk in Saudi Arabian women. This trial was registered at clinicaltrials.gov as NCT01817231. *Am J Clin Nutr* doi: 10.3945/ajcn.112.054445.

INTRODUCTION

According to the Saudi Arabian National Cancer Registry, breast cancer was the most common cancer among women in 2007, accounting for 26% of all newly diagnosed cancers in females (1, 2). In Saudi Arabia, breast cancer is more commonly diagnosed in women younger than 40 y than in the United States (3). Vitamin D has been found to modulate breast cancer cell growth, and epidemiologic studies have suggested an inverse association between vitamin D status and risk of breast cancer (4, 5), although the results have been equivocal. Although the International Agency for Research in Cancer has suggested that an inverse association exists between vitamin D status and breast cancer risk, insufficient evidence is available to conclude that a causal effect exists (6). Several mechanistic studies have identified a potential mechanism of action for vitamin D in cancer prevention, including antiproliferation (7), prodifferentiation (8), and cell cycle stabilization (9). Whether these mechanisms translate to breast cancer risk reduction remains unclear. In 2010 the Institute of Medicine defined vitamin D deficiency as a 25-hydroxyvitamin D [25(OH)D][5] concentrations <12 ng/mL and insufficiency as 12–19 ng/mL (10). A sufficient concentration of vitamin D was defined as a serum 25(OH)D concentration ≥20 ng/mL.

Because vitamin D is acquired predominantly through endogenous synthesis in the skin after UVB radiation exposure, deficiency is common when exposure to sunlight is limited (11)—a hypothesis put forth in the 1990s when observational data suggested that geographic location in the United States was associated with risk of breast cancer (12) and that UVB exposure can be limited by cultural beliefs, clothing, and public health recommendations (13). Furthermore, whereas vitamin D is found in foods such as fortified milk, fatty fish, and cod liver oil, other food sources of vitamin D are limited, and dairy products in many countries are not fortified with vitamin D (13). After vitamin D is synthesized or ingested, it is hydroxylated in the liver to form 25(OH)D—the primary biomarker for vitamin D status. A second hydroxylation in the kidney is necessary to

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[2] This analysis of the de-identified data for epidemiological study was determined to be exempt according to regulations by the Human Subjects Committee at the University of Arizona.

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[5] Abbreviations used: KFH, King Fahd Hospital; 1,25(OH)D, 1,25-dihydroxyvitamin D; 25(OH)D, 25 hydroxyvitamin D.

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convert 25(OH)D to the active form 1,25-dihydroxyvitamin D [1,25(OH)D]. 1,25(OH)D binds to the nuclear vitamin D receptor, which is found in many cells, including both normal and cancerous breast cells (13, 14). In vitro and animal studies have shown that 1,25(OH)D promotes cell differentiation and apoptosis and inhibits cell proliferation (15).

Saudi women are thought to be at greater risk of vitamin D deficiency because of their darker skin pigmentation and their reduced UV exposure (16, 17). However, data in the Saudi Arabian population are limited (16, 18, 19). Thus, the objective of this study was to evaluate the association between circulating concentrations of 25(OH)D and breast cancer risk by using data collected from a case-control study in Saudi Arabian women. Our hypothesis was that circulating 25(OH)D concentrations would be significantly lower in Saudi Arabian women with a diagnosis of breast cancer than in controls.

SUBJECTS AND METHODS

Study population

Participants were recruited from King Fahd Hospital (KFH) in Jeddah, Saudi Arabia, with the use of a case-control study design to evaluate the relation between breast cancer risk and 25(OH)D concentrations. Jeddah is a city located in the Western region of Saudi Arabia (latitude 21.45 degrees north and longitude 39.82 degrees east). The current study was performed during the summer months of 2009, during which time the city has an average of 9 h of sunlight daily and a mean maximal temperature of 36.7°C. A sample size of 120 cases and 120 controls was assessed to provide >80% power to detect a significant difference ($\alpha = 0.05$) across groups in circulating 25(OH)D concentrations by using NQuery software.

A de-identified data set was provided for this analysis and was approved for epidemiologic study by the Human Subjects Committee at the University of Arizona. Cases were 120 female patients at KFH with newly diagnosed stage I-IV breast cancer who were between the ages of 18 and 75 y. The control group included 120 women matched on age, with no history of breast cancer, who visited the women's clinic at KFH for a regular clinical visit during the same data collection period. A single medical doctor recruited all control women during preventive care visits at the clinic.

Inclusion and exclusion criteria

All women presented with invasive breast cancer at the clinic or were receiving standard medical check-ups at the same women's clinic and were shown on medical record review to be free of cancer. Other eligibility criteria, selected to reduce variance in vitamin D status associated with environment, cultural behaviors, and diet, included the following: *1*) women between the ages of 18 and 75 y, *2*) BMI (in kg/m^2) ≤40, and *3*) residence in Saudi Arabia for >5 y, and *4*) absence of chronic diseases that could affect vitamin D metabolism, including renal, hepatic, endocrine, or autoimmune disease. All women were initially identified for study participation by physicians at the hospital and were provided care at this facility during the summer of 2009. Participants then met with the study coordinator to review eligibility before consent. Participants were also required to be able and willing to complete all study-related activities, such as weight and height measurements and blood draws, and were required to provide written informed consent before study enrollment.

Data collection

A lifestyle and medical history questionnaire was administered to each participant during their clinic visit to determine participant characteristics, such as overall health status; tobacco use; a medical history including medication use, history of cancer or benign breast cancer, family history of breast cancer, and menstrual history (regular or irregular in terms of days between menstrual periods); breastfeeding history; parity; oral contraception; age at first birth; education; and socioeconomic status. Age was assessed from medical records. Height and weight were measured by using a calibrated beam scale with a height bar; BMI was calculated as weight in kilograms divided by height squared in meters.

A short food-frequency questionnaire was administered that consisted of 15 questions regarding amount and frequency of intake of milk, dairy products, eggs, organ meats such as liver, other meats (beef, chicken, salmon, tuna, fish, and seafood), fruit, and vegetables. These items were included in the questionnaire to have a crude estimate of dietary vitamin D exposure and fruit and vegetable intake, which has been associated with a reduced breast cancer risk in some epidemiologic studies (4). The questionnaire asked respondents to report the frequency of daily intake per food item, with responses ranging from ≥3times/d to rarely/never. Participants were asked about their diet for the 12-mo period before the study, including use of supplemental vitamin D and calcium.

Sun exposure (face, hands, face and hands, both arms, both legs, and completely covered), use of sun protection, and style of dress were also assessed. Women were also queried about frequency of physical activity at enrollment, including questions regarding sedentary (sitting, standing, casual walking), moderate (regular walking or swimming), and vigorous activity (brisk daily jogging) and time walking outdoors. All self-reported questionnaires were completed during a single visit to the health center and were reviewed for completeness by the study coordinator before visit end.

Blood sample collection and measurement of 25(OH)D by HPLC

From June through August 2009, a single blood sample was collected from all 240 women (120 cases and 120 controls) to assess total serum 25(OH)D. Approximately 2 mL blood was collected via venipuncture. All tubes were protected from light, and the specimens were centrifuged at 3000 rpm for 10 min. The serum was separated and stored at −80°C until analyzed by HPLC in King Fahd Center for Medical Research. The method used has been described in detail elsewhere (20). Briefly, the method uses a reversed-phase HPLC technique that shows a clear resolution of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3. The mobile phase is an acetonitrile extract of serum by solid-phase extraction that uses C_{18}/OH cartridges. HPLC was performed by using a Shimadzu LC-10 system with a Shimadzu LC-10AT pump).

Statistical analysis

The statistical analyses were performed by using STATA 11.0 statistical software. Demographic and clinical characteristics of the cases and controls were evaluated by using descriptive statistics: means, SDs, and frequencies. For comparisons of baseline characteristics, *P* values were calculated with chi-square analyses for categorical variables and Student's *t* test for continuous variables.

Logistic regression modeling was used for assessment of the association between 25(OH)D concentration and risk of breast cancer. Potentially confounding variables that were evaluated for inclusion in the final model were age, BMI, history of cancer, parity, family history of cancer, exercise, location of exercise (indoors or outdoors), multivitamin use, presence of breast cancer in daughters, benign breast disease, menopause, and breastfeeding. Variables that changed the point estimate by ≥10% were added to the final model and included age, BMI, physical activity (walking outdoors), parity, education, and history of breastfeeding. All women provided blood samples for measurement of circulating vitamin D and completed the questionnaires at baseline.

RESULTS

Data from 240 women, including 120 breast cancer cases and 120 controls, were included in this analysis. The characteristics of the study population are shown in **Table 1**. The mean age was 47.7 y for cases and 47.9 y for controls. Age at menarche, height, and body weight did not differ between cases and controls. The mean BMI in both groups was 30.0 ± 5.2, which indicated that the study sample met the criteria for obesity (19). Most of the cases (n = 69) had stage III disease and 43 stage IV disease; none had stage I disease, and 9 had stage II disease. Family history of breast cancer was more common in cases (31.7%) than in controls (8.3%) (P = 0.0001). Moreover, the cases were less educated, had fewer live births, were more likely to report breastfeeding for >6 mo, and reported a lower use of oral contraceptive agents than did the controls. Controls were more likely to report current smoking than were cases.

Serum 25(OH)D concentrations were significantly lower in cases than in controls. The mean serum concentrations of 25(OH)D were 9.4 ± 6.35 and 15.4 ± 12.31 ng/mL in cases and controls, respectively (P = 0.002; data not shown). Notably, 60.8% of cases and 38.3% of controls had circulating 25(OH)D concentrations <10 ng/mL; the current recommended concentration for adequacy is ≥20 ng/mL (21). Concentrations of 25(OH)D of ≥10 and <20 ng/mL were present in 32.5% and 34.2% of cases and controls, respectively. Only 6.7% of cases, but 27.5% of controls, had 25(OH)D concentrations >20 ng/mL (**Table 2**).

The ORs for breast cancer in the total population by category of vitamin D status are shown in Table 2.The unadjusted and adjusted ORs showed a statistically significantly increased risk of breast cancer across decreasing 25(OH)D concentrations (P-trend < 0.0001). After adjustment for age, BMI, physical activity, parity, education, and history of breastfeeding, and compared with those women with 25(OH)D concentrations >20 ng/mL, the ORs (95% CIs) for breast cancer risk were 4.0 (1.6, 10.4) and 6.1 (2.4, 15.1) for women with 25(OH)D concentrations ≥10 and <20 ng/mL and <10 ng/mL, respectively.

TABLE 1
Demographic, lifestyle, and health characteristics of Saudi Arabian women with or without breast cancer (n = 240)[1]

Characteristic	All (n = 240)	Cases (n = 120)	Controls (n = 120)	P value[2]
Age (y)	47.8 ± 12.4[3]	47.7 ± 11.0	47.9 ± 11.0	0.87
Age at menarche (y)	12.9 ± 1.7	12.9 ± 1.6	12.9 ± 1.8	0.84
Weight (kg)	75.48	75.82 ± 9.42	75 ± 14.81	0.60
Height (cm)	158.7 ± 4.4	158.1 ± 4.0	159.2 ± 4.8	0.07
Weight at age 18 y (kg)	57.5 ± 10.2	57.3 ± 9.0	57.8 ± 11.3	0.71
Current weight (kg)	75.4 ± 12.4	75.8 ± 9.4	75.0 ± 14.8	0.61
BMI (kg/m^2)	30.0 ± 5.2	30.4 ± 4.2	29.6 ± 6.1	0.20
Nulliparous (%)	7.9	1.7	14.2	<0.001
History of BC in mother (%)	7.5	10.0	5.0	0.41
History of BC in sister (%)	12.5	21.7	3.3	<0.001
Benign breast disease (%)	18.8	32.5	5.0	<0.001
Breastfed <6 mo (%)	37.9	46.7	29.2	<0.001
Education (%)[4]				<0.01
High school or less	36.2	43.3	29.2	
Post high school	20.4	19.2	21.7	
Some college	33.8	35.0	32.5	
College graduate	5.4	1.7	9.2	
Annual income (%)				
≥5000 SAR	50.8	50.8	50.8	1.00
Current smoking, yes (%)	44.2	36.7	51.7	<0.05
Current use of oral contraceptives, yes (%)	16.7	31.7	1.7	<0.001
Walk >30 min/d, yes (%)	67.9	32.5	31.7	0.89
Walk outdoors, yes (%)	16.2	5.0	27.5	<0.01

[1] BC, breast cancer; SAR, Saudi riyal.
[2] P values calculated by using Student's t test for continuous variables and chi-square analysis for categorical variables.
[3] Mean ± SD (all such values).
[4] Values do not add up to 100% because of missing data.

TABLE 2
Crude and adjusted ORs (95% CIs) for the association between circulating concentrations of 25(OH)D and breast cancer

	Deficient (<10 ng/mL)	Insufficient (≥10 and <20 ng/mL)	Sufficient (≥20 ng/mL)	*P*-trend[1]
All cases and controls				
Total (*n*)	119	80	41	
Crude OR[2] (95% CI)	6.5 (2.8, 15.4)	3.9 (1.6, 9.5)	1.00 (referent)	<0.0001
Adjusted OR[3] (95% CI)	6.1 (2.4, 15.1)	4.0 (1.6, 10.4)	1.00 (referent)	<0.0001

[1] Tests for trend were conducted by using the final adjusted regression model and a categorical variable for circulating 25(OH)D category. 25(OH)D, 25-hydroxyvitamin D.
[2] Conditional logistic regression models with no adjustments.
[3] Conditional logistic regression models adjusted for age, BMI, walking outdoors, parity, education, and history of breastfeeding.

DISCUSSION

The risk of breast cancer has been observed to be greater in geographic areas with lower amounts of sunlight during the year (22). In addition, a protective association between UV exposure earlier in life and breast cancer risk, mostly during breast development (23), has been shown, which has led to the hypothesis that vitamin D may be associated with a reduced risk of breast cancer (24). Some studies support this hypothesis, but not all risk estimates are statistically significant (22, 24–29), both in relation to UV exposure/time spent outdoors (30, 31) and vitamin D status (24, 32); epidemiologic results are inconsistent (5, 33). Moreover, vitamin D status has been inversely associated with breast cancer stage, recurrence, and mortality in some studies (34), but not others (35). Most studies of vitamin D and breast cancer to date have been conducted in predominantly non-Hispanic white women. The current study is among the first, to our knowledge, to evaluate the association between serum 25(OH)D and breast cancer in women residing in Saudi Arabia, an area of high UV sunlight exposure, but potentially low vitamin D status related to skin type and cultural practices of dress. A study in Mexican women showed a 47% reduction in breast cancer risk in women with circulating concentrations of 25(OH)D >30 mg/mL (36). A study of women in the military did not show a significant relation between vitamin D status and breast cancer; however, among women sampled within 90 d of diagnosis, women in the lowest quintile had an OR of 3.3 for incident breast cancer (37) similar to our findings. Also in support of our findings (24), Abbas et al (24) found a protective association: vitamin D concentrations <30 nmol/L (12 ng/mL) were associated with an increase in risk of breast cancer, and a possible threshold effect was shown at concentrations >50 nmol/L (20 ng/mL) in a sample of premenopausal German women. An exploratory analysis in premenopausal and separately in postmenopausal women in our sample showed an increased risk in both groups, but CIs were large, with ORs (95% CIs) of 3.6 (0.43, 30.1) and 18.1 (2.2, 149.8) for premenopausal and postmenopausal women, respectively. Abbas et al performed a separate analysis in postmenopausal women as well. In this analysis (n = 289), risk of breast cancer was lower in women within the highest category (≥24 ng/mL) of 25(OH)D than in those with the lowest category (<12 ng/mL) (P-trend = 0.0006) (28).This study varied from our study in that the participants in the Abbas et al study were all postmenopausal women; 2 controls were matched per case on years of birth and study region (28). Furthermore, the blood samples were collected before breast cancer diagnosis for the cases, whereas our sampling was conducted at the time of diagnosis. A study in 2009 by Rossi et al (38) found a strong association between vitamin D intake (>190 IU/d) and a 64% reduced risk of breast cancer among women living in Southern Italy, but the relation was attenuated and not significant among women who lived in the North of Italy.

The Institute of Medicine has recommended a dietary allowance of vitamin D intake of 600 IU/d (or 15μg/d) for women aged 1–70 y and 800 IU/d for those aged ≥71 y (10). In our study population, dietary vitamin D intake was low; <34% of cases and 39% of controls consumed no more than a single serving of vitamin D–rich foods, eg, fish and dairy products (data not shown). Of note, the food supply in Saudi Arabia is not fortified with vitamin D as it is in the United States.

Sun exposure may be a strategy for meeting vitamin D requirements, particularly in people with a modest dietary intake of vitamin D. However, this recommendation is conditional to skin type, latitude, and other factors that may alter status, such as age (39–41). In determining the role of UV exposure in vitamin D status, time of day of exposure is also an important factor because the angle of the sun changes throughout the day; it is more difficult to produce vitamin D in the early morning or late afternoon (39). Women in Saudi Arabia, as well as in other Arab populations, have a high prevalence of vitamin D deficiency (18, 42–44). Among healthy Saudi women, vitamin D deficiency has been described in both premenopausal and postmenopausal women (18). In our sample, despite the fact that women reside in an area where the UV light levels are high yearround, we found mean concentrations of serum 25(OH)D to be quite low. Even with these relatively low circulating concentrations, the mean vitamin D concentration in this study was higher in women without breast cancer than in women with diagnosed breast cancer. Furthermore, Jeddah is a region of intense UV exposure. Given the associated heat, residents practice sun avoidance and spend little time outdoors (45, 46). Moreover, sociocultural practices related to dress play an important role in ensuring that women are not overexposed to the sun. Housing design and lifestyle choices (ie, living in an air-conditioned apartment and avoiding sun exposure) also can contribute to the greater time spent indoors, thus leading to an increased risk of vitamin D deficiency.

This study was novel in its focus on Saudi Arabian women—a group at greater risk of vitamin D deficiency and who have low concentrations of 25(OH)D. The short window for biosampling that reduced seasonal effects was an additional strength, as was the availability of demographic and lifestyle data during a face-to-face visit with each woman. The opportunity to have measured

BMI as a covariate in the analysis was an additional strength of our methods. Limitations included the lack of biosamples before diagnosis and a single measure of 25(OH)D to define status. Multiple measures of 25(OH)D would be better to assess long-term, average 25(OH)D status. The collection of detailed dietary intakes would also be beneficial. However, there is sparse evidence that dietary vitamin D alone significantly modifies circulating concentrations in depleted individuals (47), and our emphasis on objective measures compared with self-reported dietary sources is a significant strength of the study. Our study did not afford an opportunity to evaluate the association between vitamin D status and specific breast cancer subtypes, as has been suggested by others (48). In addition, we were not able to complete a genotype analysis to determine the role of vitamin D polymorphisms and risk of breast cancer, as has been suggested by McCullough et al (49) in a nested case-control study.

In summary, the issue of vitamin D and breast cancer risk is a field of intense study, and many aspects of this association require further investigation. Here we provided evidence of a significant association between low concentrations of circulating 25(OH)D and a higher risk of breast cancer with the use of a case-control study design of women residing in Saudi Arabia—a high-risk population. Importantly, although mean serum 25(OH)D concentrations were very low in the study sample overall, significant differences between cases and controls were observed.

Conclusion and future directions

The results of this case-control study showed a high prevalence of vitamin D deficiency in women in Jeddah, Saudi Arabia. Low vitamin D status is associated with a greater risk of breast cancer. These findings provide important clinical information. Efforts to more routinely assess vitamin D status and possibly provide supplementation to correct depletion in this at-risk group should be evaluated. The association between vitamin D and breast cancer risk clearly requires further investigation.

The authors' responsibilities were as follows—FMY (Principal Investigator): wrote the manuscript; PTK: analyzed the data and wrote the manuscript; RMA: performed the statistical analysis; JMY: performed hands-on conduct of the experiments and collected the data; TAK: provided essential materials for the research and made clinical decisions; ETJ, SG, IAH, and CAT: contributed to the final content and provided significant advice or consultation; and CAT: provided consultation on the overall research plan and designed the experiment. None of the authors had a conflict of interest related to this research.

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