

RESEARCH ARTICLE

Levels of Serum 25-Hydroxy-Vitamin D in Benign and Malignant Breast Masses

Sadaf Alipour^{1*}, Maryam Hadji², Ladan Hosseini³, Ramesh Omranipour⁴, Azin Saberi¹, Akram Seifollahi⁵, Leila Bayani⁶, Nooshin Shirzad⁷

Abstract

Background: The true association between breast cancer and vitamin D is currently under investigation. We compared serum 25-hydroxy-vitamin D levels in women with benign and malignant breast masses and controls. **Materials and Methods:** Levels of vitamin D were measured by electrochemiluminescence. Serum levels >35 ng/ml, 25-35 ng/ml, 12.5-25 ng/ml and <12.5 ng/ml were considered as normal, mild, moderate and severe vitamin D deficiency, respectively. **Results:** Overall, 364 women were included in the control, 172 in the benign and 136 in the malignant groups. The median serum vitamin D level was significantly lower in breast cancers than controls. Levels were also lower in malignant than benign cases and in benign cases than controls although statistically non-significant. **Conclusions:** Multinomial logistic regression analysis showed that severe vitamin D deficiency causes a three-fold increase in the risk of breast cancer while this was not the case for moderate and mild deficiency.

Keywords: Breast neoplasm - breast mass - cancer - vitamin D - 25(OH)-vitamin D

Asian Pac J Cancer Prev, 15 (1), 129-132

Introduction

The role of vitamin D in calcium hemostasis and bone health is well-known, while its effects on other body functions and disease states are being investigated. One issue is its probable impact on carcinogenesis; recent literature has widely discussed the matter. Receptors of vitamin D exist in various organs, including the breast. Relation of breast cancer risk and vitamin D status has been defined in many studies, but the true association is still not understood and needs further research (Chlebowski, 2011; Gupta et al., 2011; Jacobs et al., 2011; Yao et al., 2011; Lopes et al., 2012; Shao et al., 2012).

Sources of vitamin D include sun exposure and dietary intake. Conversion to the main serum metabolite, 25-hydroxyvitamin D (25(OH)D), is completed mainly in the liver, which is then further hydroxylated in the kidney to produce 1,25dihydroxyvitamin D. The best measurement for assessing vitamin D status is plasma level of 25(OH)D (Eliassen et al., 2011; Gupta et al., 2011; Jacobs et al., 2011; Lopes et al., 2012; Shao et al., 2012).

We measured serum vitamin D levels in women without breast disease, cases of benign breast masses and breast cancers in order to evaluate the association of vitamin D and benign and malignant breast disease.

Materials and Methods

In women attending the breast clinic of Arash

Women's Hospital for breast cancer screening or any breast complaint, clinical breast examination was done for all women. Mammography was carried out for women above 40 years of age; ultrasonography was undertaken as indicated by the radiologist in this age group, and in all patients less than 40 years of age. The clinical exam was undertaken by one of two breast surgeons of the clinic. Mammography interpretation and ultrasonographic scans were performed by one expert radiologist. In patients with one or more breast masses in the examination or imaging studies, the surgeons decided whether or not the patient needed histologic assessment. If required, core needle biopsy for palpable masses was undertaken by the same surgeons; non-palpable masses were biopsied under ultrasonographic guidance or by stereotactic biopsy by the radiologist. All specimens were reviewed by one pathologist expert in gynecologic pathology. Patients with normal breasts in examination and imaging were entered in the study as the control group (group 1); those harboring breast masses which had undergone histologic assessment were entered in the study as the case group. The latter group was classified further into cases with benign mass (group 2) and those with breast cancer (group 3) according to the histologic report. Age, age at first birth and menarche, parity, menopausal status, history of breastfeeding and family history of breast cancer as well as previous consumption of vitamin-containing compounds were inquired from participants and recorded by a trained interviewer.

¹Department of Surgery, ³Research Development Center, ⁵Department of Pathology, ⁶Department of Radiology, Arash Women's Hospital, ²Cancer Research Center, ⁴Department of Surgery, Cancer Institute, ⁷Endocrinology and Metabolism Research Institute, Dr. Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran *For correspondence: sadafalipour@yahoo.com

Women who had used any form of Vitamin D in the last two years, those who were under treatment for osteopenia or osteoporosis, patients with the history of any cancer or renal failure and those with equivocal histologic results were excluded from the study.

Serum samples were taken by the laboratory technician from all the patients and refrigerated in -40 degrees centigrade before undertaking the test. No sample was kept for more than four weeks. Levels of vitamin D were measured by the electrochemiluminescence method in all samples in one laboratory.

In the statistical analysis, the mann-whitney test was used for comparison of deficiency levels among the three groups and T-test and χ^2 test were used for data with normal distribution. The association between risk of benign/malignant breast mass and plasma vitamin D levels was assessed by multinomial logistic regression; adjustments for age, age at first birth, menarche, parity, menopause status, history of breastfeeding and family history of breast cancer were done. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated using plasma vitamin D levels as a categorical variable divided into four categories; serum level >35 nannogram per milliliter (ng/ml) was regarded as normal and levels between 25-35 ng/ml, 12.5-25 ng/ml and <12.5 ng/ml were considered as mild, moderate and severe vitamin D deficiency, respectively. P value <0.05 was considered significant. All analyses were performed using SPSS version 16.

Results

Overall, 672 women were included in the study, 364 in the control and 308 in the case group. Of these, 172 had benign mass (47.25%) and 136 (44.2%) had malignant masses. The mean age in control and case groups was 44.2 and 43.2 years, respectively. Some demographic features of the case and control groups, their age at first birth and menarche, the parity and menopause status, their histories of breastfeeding as well as their family history of breast cancer and any form of multivitamin consumption are demonstrated in Table 1.

The histology of 4 of the 136 breast cancers (5.3 %) was in situ ductal carcinoma, the others were invasive ductal carcinomas. Histologies of benign cases consisted of fibroadenoma in 98 cases (57%), fibrocystic changes in 37 (21.5%), ductal hyperplasia without atypia in 12 (7%), intraductal papiloma in 9 (5.2%), sclerosing adenosis in 6 (3.5%), mastitis in 6 (3.5%), and ductal ectasia in 4 (2.3%).

The prevalence of vitamin D deficiency in the three groups is shown in Table 2. The median serum vitamin D level in the case group was lower than the control group (7.7 vs 8.7 ng/ml). Median serum levels of vitamin D were higher in benign (7.9 ng/ml) compared with malignant cases (7 ng/ml) (group 2 and 3). When comparing each of these two groups with controls, the median serum vitamin D level was higher in group 1, lower in group two and the lowest in group 3. Serum vitamin D levels in cases of fibroadenomas alone were compared with breast cancers. Results showed lower levels in fibroadenomas (7.4 ng/ml vs 7 ng/ml).

Table 1. Features of Women in Case and Control Groups

Characteristic	Yes/No	Case	Control	p value
BMI (Kg/m ²)		26.76±4.77	26.76±4.24	0.93*
Age at 1st birth (yr)		18.8±7.5	18.7±7.5	0.83*
Parity		2.7±2.2	2.5±1.7	0.46*
Age at Menarche (yr)		13.4±1.9	14.4±8.8	0.08*
Menopause	Y	213 (74.5)	262 (72.4)	0.54**
	N	73 (25.5)	100 (27.6)	
Vitamin consumption	Y	37 (12)	69 (19)	0.1**
	N	203 (65.9)	266 (37.1)	
	missing	68 (22.1)	29 (8)	
FH of breast cancer	Y	56 (18.2)	52 (14.3)	
	N	169 (63.6)	287 (78.8)	0.03**
	missing	56 (18.2)	25 (6.9)	
Breastfeeding	Y	189 (61.4)	267 (73.4)	0.79**
	N	64 (20.8)	86 (23.6)	
	missing	55 (17.9)	11 (3)	

*T-Test, ** χ^2 Test

Table 2. Prevalence of Vitamin D Deficiency in Groups 1, 2 and 3

Study group	sev def	mod def	mild def	normal	Total
Breast cancer	90 (66.2%)	28(20.6%)	9(6.6%)	9 (6.6%)	136
Benign mass	111 (64.5%)	32(18.6%)	13(7.6%)	16 (9.3%)	172
Control	222 (61.0%)	67(18.4%)	36(9.9%)	39(10.7%)	364

normal (VitD>35.1), mild def= mild deficiency (35<VitD<25), mod def moderate deficiency (25<VitD<12.5),sev def=severe deficiency (VitD<12.5), vitamin D levels expressed in ng/ml (* χ^2 Test)

Data were missing about the menopausal status of 22 of the breast cancers. Nevertheless, nearly half of the malignant cases were premenopausal in the known cases and values were compared. The median serum vitamin D was 5.6ng/ml in 59 premenopausal women and 11.4ng/ml in 55 menopause cases.

In comparison with subjects having sufficient vitamin D levels, the odds ratios for breast cancer were 3 (95%CI 1.11-8.1), 1.79 (95%CI 0.9-3.5), and 0.96 (95%CI 0.3-2.8) in subjects with severe, mild and moderate vitamin D deficiency respectively after adjustment for age, age at first birth, menarche, parity, menopausal status, history of breastfeeding and family history of breast cancer. The Odds ratio for benign breast mass in subjects with severe, mild and moderate vitamin D deficiency compared to those with sufficient vitamin D levels was 0.6 (95%CI 0.3-1.1), 0.6 (95%CI 0.3-1.1), and 1.7 (95%CI 0.8-3.5) respectively.

Discussion

Although many studies have addressed the issue of breast cancer and vitamin D, research regarding the condition in benign lesions is scarce. The comparison of dietary factors in patients 40-59 years of age affected by fibrocystic breast disorders with normal women in the National Breast Screening Study in Montreal, Canada had showed a higher intake of vitamin D in the case group (Vobecky et al., 1993). The contrary was revealed when an inverse association was detected between proliferative benign breast disorders and amounts of vitamin D consumption in the Nurses' Health Study II (Su et al., 2012). A randomized controlled trial comparing the effect of calcium and vitamin D prescription versus

placebo in normal women showed no difference between the two groups in development of proliferative benign breast disorders after seven years (Rohan et al., 2009). Nevertheless, some abnormalities were noticed by Lopes et al in the expression of vitamin D receptors in some benign breast diseases (Lopes et al., 2012). Low levels of serum 25-hydroxyvitamin D has been implicated as a risk factor for some infectious diseases, probably via its effects on the immune system. With the aim of evaluating this effect on breast inflammation, Lippolis et al have showed the benefits of local injections of vitamin D in mastitis in cows (Lippolis et al., 2011)

In our study, the differences between median vitamin D levels in benign cases and controls as well as benign cases and cancers were not statistically significant (p values=0.3 and 0.1, respectively). Because fibroadenoma is a distinct, well described entity among benign breast masses, they were analysed separately and the differences were not statistically significant ($p=0.43$).

Literature contains various types of study which have assessed the relation between vitamin D and breast cancer. Many studies regarding the association linking vitamin D synthesis secondary to sunlight exposure or serum levels of the vitamin to breast cancer have shown an inverse relationship, but others have not (Lopes et al., 2012; Chlebowski, 2013).

Grant et al. (2010) have undertaken a meta-analysis of seven prospective works about the incidence of breast cancer regarding vitamin D status. They demonstrated a 50% increase in incidence when levels fell from 31.2 ng/ml to 9.6 ng/ml. Engel et al. (2010) performed a nested study in the French E3N cohort and detected 636 cases of breast cancer whom they compared with 1272 controls in terms of serum vitamin D concentrations. They concluded that higher levels could reduce the risk of the disease (Engel et al., 2010). The review of related papers up to 2011 by Mohr et al. (2011) found out a decrease in breast cancer risk in higher ranges of serum vitamin D in 11 case-control trials. As well, a 50% reduction in risk was associated with serum levels above 47 ng/ml (Mohr et al., 2011). Pazdiora et al. (2011) investigated serum levels of vitamin D in 170 patients affected by different types of cancers-43 of whom were breast cancer-and 214 healthy people. Levels of vitamin D were lower in cancers (Pazdiora et al., 2011). Kermani et al. (2011) in Tabriz, Iran, assessed the relation between breast cancer prognostic factors and serum vitamin D levels in 119 cases and found a probable association with disease prognosis (Kermani et al., 2011). Chung et al. (2011) analyzed the results of 19 randomized controlled trials through July 2011 and deduced that the consumption of high dosages of vitamin D decreased the risk of cancer (Chung et al., 2011). From 2003-2008, Yao et al. (2011) measured circulating vitamin D levels in 579 patients affected by breast cancer and 574 controls in their institute. They showed an inverse relationship for breast cancer risk in regard to vitamin D levels (Yao et al., 2011). Peppone et al. (2012) measured serum vitamin D levels in 194 cases and the same number of controls, regarding their phenotypes and prognostic features. They demonstrated lower levels of the vitamin in more aggressive, less favorable tumors

(Peppone et al., 2012). Shao et al. (2012) reviewed the literature for *in vitro* or animal studies upon the relation of breast cancer and vitamin D up to February 2011. They concluded that although the association is still uncertain, the present evidence is in favor of the role of vitamin D as a risk factor for breast cancer (Shao et al., 2012). Shamsi et al. (2013) investigated the use of vitamin D supplements in 297 breast cancer patients and compared them with 586 controls in Karachi, Pakistan between January 2009 and December 2010; the vitamin showed to be protective against the disease (Shamsi et al., 2013). The study of Bilinsky et al. (2013) in Sydney, Australia in 214 cases of breast cancer and 852 controls from 2008 to 2010 showed a significantly higher risk of breast cancer with levels of serum vitamin D below 75 nmol/L.

On the other hand, in a cohort of 36282 women assigned to daily servings of calcium and vitamin D or placebo for seven years, 6731 got breast cancer and no association was demonstrated between the prescription or circulating vitamin D level and breast cancer risk (Chlebowski et al., 2008). Freedman et al. (2008) found no association between serum levels of vitamin D and breast cancer risk in their cohort of postmenopausal women followed for four to 12 years, with 1005 cases of incident breast cancer (Freedman et al., 2008). The overall meta-analysis showed no significant association between breast cancer and vitamin D levels (Chung et al., 2011). Eliassen et al. (2011) assessed levels of vitamin D in serum samples collected around 10 years sooner from 613 breast cancer cases and 1218 matched controls out of the Nurses' Health Study II. They demonstrated no significant association between the levels and risk of the disease (Eliassen et al., 2011). Chlebowski et al. (2011) showed no association between breast cancer risk and consumption of vitamin D in their meta-analyses of five case-control studies. However, their meta-analysis of six cohort studies out of ten showed contrary results, demonstrating an inverse relationship between these issues. Again, results of 2 more recent large cohorts were against any association (Chlebowski et al., 2011). Ordonez-Mena et al. (2013) followed 9949 people for eight years after the measurement of vitamin D levels in their sera. There were 873 cases of cancer, 137 of them in the breast. Vitamin levels were lower in all cancers but not in site-specific tumors (Ordonez-Mena et al., 2013).

In our study, serum vitamin D levels were significantly lower in the case group compared with the control group ($p=0.03$). Median serum vitamin D levels in cancer cases were significantly lower than controls ($p=0.01$) but the difference with benign cases was not statistically significant as mentioned before. Multinomial logistic regression analysis showed that severe vitamin D deficiency causes a three-fold increase in the risk of breast cancer while it is not the case for moderate and mild deficiency.

The association of vitamin D and breast diseases in regard to menopausal status has not been considered extensively, and the subject has seldom been investigated in developing countries (Fedirko et al., 2012). The reverse association Yao et al. (2011) found between breast cancer risk and serum vitamin D was stronger in premenopausal

women with poor prognostic indicators (Yao et al., 2011). Engel et al. (2011) demonstrated in 2871 breast cancer among women of the French E3N cohort during 10 years follow-up that breast cancer risk in postmenopausal patients was lower when there was a greater use of vitamin D intake (Engel et al., 2011). Lee et al. (2011) showed in 299 cases of breast cancer and in 200 controls that higher consumption of vitamin D could protect from breast cancer in premenopausal Taiwanese patients (Lee et al., 2011). A significant association was demonstrated only in premenopausal and perimenopausal cases (Chlebowski, 2011). Fedirko et al. (2012) compared serum levels of vitamin D in 1000 breast cancer cases with 1074 controls and detected an inverse association between the vitamin levels and risk of breast cancer in both premenopausal and postmenopausal women. Bener and El Ayoubi (2012) found a high frequency of vitamin D deficiency in 635 postmenopausal breast cancer patients.

Nearly half the breast cancers in our study were premenopausal. This is expectable in Iranian patients. Research regarding the epidemiology of the disease in Iran has shown a ten years lower median age distribution in comparison with developed countries (Mousavi et al., 2007; Harirchi et al., 2011). In our study, the median serum vitamin D was significantly lower in premenopausal women compared with menopause cases ($p < 0.05$).

In conclusion, our study demonstrates a reverse association between vitamin D levels and breast cancer risk especially in levels under 12.5 ng/ml.

References

Bener A, El Ayoubi HR (2012). The role of vitamin D deficiency and osteoporosis in breast cancer. *Int J Rheum Dis*, **15**, 554-61.

Bilinski K, Boyages J (2013). Association between 25-hydroxyvitamin D concentration and breast cancer risk in an Australian population: an observational case-control study. *Breast Cancer Res Treat*, **137**, 599-607.

Chlebowski RT, Johnson KC, Kooperberg C, et al (2008). Calcium plus vitamin D supplementation and the risk of breast cancer. *J Natl Cancer Inst*, **100**, 1581-91.

Chlebowski RT (2011). Vitamin D and breast cancer: interpreting current evidence. *Breast Cancer Res*, **13**, 217.

Chlebowski RT (2013). Vitamin D and breast cancer incidence and outcome. *Anticancer Agents Med Chem*, **13**, 98-106.

Chung M, Lee J, Terasawa T, Lau J, Trikalinos TA (2011). Vitamin D with or without calcium supplementation for prevention of cancer and fractures: an updated meta-analysis for the U.S. Preventive Services Task Force. *Ann Intern Med*, **155**, 827-38.

Eliassen AH, Spiegelman D, Hollis BW, et al (2011). Plasma 25-hydroxyvitamin D and risk of breast cancer in the Nurses' Health Study II. *Breast Cancer Res*, **13**, 50.

Engel P, Fagherazzi G, Boutten A, Dupre T, Mesrine S, Boutron-Ruault MC, Clavel-Chapelon F (2010). Serum 25(OH) vitamin D and risk of breast cancer: a nested case-control study from the French E3N cohort. *Cancer Epidemiol Biomarkers Prev*, **19**, 2341-50.

Engel P, Fagherazzi G, Mesrine S, Boutron-Ruault MC, Clavel-Chapelon F (2011). Joint effects of dietary vitamin D and sun exposure on breast cancer risk: results from the French E3N cohort. *Cancer Epidemiol Biomarkers Prev*, **20**, 187-98.

Fedirko V, Torres-Mejia G, Ortega-Olvera C, et al (2012). Serum 25-hydroxyvitamin D and risk of breast cancer: results of a large population-based case-control study in Mexican women.

Cancer Causes Control, **23**, 1149-62.

Freedman DM, Chang SC, Falk RT, et al (2008). Serum levels of vitamin D metabolites and breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Cancer Epidemiol Biomarkers Prev*, **17**, 889-94.

Grant WB (2010). Relation between prediagnostic serum 25-hydroxyvitamin D level and incidence of breast, colorectal, and other cancers. *J Photochem Photobiol B*, **101**, 130-6.

Gupta D, Vashi PG, Trukova K, Lis CG, Lammersfeld CA (2011). Prevalence of serum vitamin D deficiency and insufficiency in cancer: Review of the epidemiological literature. *Exp Ther Med*, **2**, 181-93.

Harirchi I, Kolahdoozan S, Karbakhsh M, et al (2011). Twenty years of breast cancer in Iran: downstaging without a formal screening program. *Ann Oncol*, **22**, 93-7.

Jacobs ET, Thomson CA, Flatt SW, et al (2011). Vitamin D and breast cancer recurrence in the Women's Healthy Eating and Living (WHEL) Study. *Am J Clin Nutr*, **93**, 108-17.

Kermani IA, Kojidi HT, Gharamaleki JV, et al (2011). Association of serum level of 25 hydroxy-vitamin D with prognostic factors for breast cancer. *Asian Pac J Cancer Prev*, **12**, 1381-4.

Lee MS, Huang YC, Wahlqvist ML, et al (2011). Vitamin D decreases risk of breast cancer in premenopausal women of normal weight in subtropical taiwan. *J Epidemiol*, **21**, 87-94.

Lippolis JD, Reinhardt TA, Sacco RA, Nonnecke BJ, Nelson CD (2011). Treatment of an intramammary bacterial infection with 25-hydroxyvitamin D(3). *PLoS One*, **6**, 25479.

Lopes N, Paredes J, Costa JL, Ylstra B, Schmitt F. (2012). Vitamin D and the mammary gland: a review on its role in normal development and breast cancer. *Breast Cancer Res*, **14**, 211.

Mohr SB, Gorham ED, Alcaraz JE, et al (2011). Serum 25-hydroxyvitamin D and prevention of breast cancer: pooled analysis. *Anticancer Res*, **31**, 2939-48.

Mousavi SM, Montazeri A, Mohagheghi MA, et al (2007). Breast cancer in Iran: an epidemiological review. *Breast J*, **13**, 383-91.

Ordonez-Mena JM, Schottker B, Haug U, et al (2013). Serum 25-Hydroxyvitamin D and Cancer Risk in Older Adults: Results from a Large German Prospective Cohort Study. *Cancer Epidemiol Biomarkers Prev*, **22**, 905-16

Pazdiora P, Svobodova S, Fuchsova R, et al (2011). Vitamin D in colorectal, breast, prostate and lung cancer: a pilot study. *Anticancer Res*, **31**, 3619-21.

Peppone LJ, Rickles AS, Janelsins MC, Insalaco MR, Skinner KA (2012). The association between breast cancer prognostic indicators and serum 25-OH vitamin D levels. *Ann Surg Oncol*, **19**, 2590-9.

Rohan TE, Negassa A, Chlebowski RT, et al (2009). A randomized controlled trial of calcium plus vitamin D supplementation and risk of benign proliferative breast disease. *Breast Cancer Res Treat*, **116**, 339-50.

Shamsi U, Khan S, Usman S, Soomro S, Azam I (2013). A multicenter matched case control study of breast cancer risk factors among women in Karachi, Pakistan. *Asian Pac J Cancer Prev*, **14**, 183-8.

Shao T, Klein P, Grossbard ML (2012). Vitamin D and breast cancer. *Oncologist*, **17**, 36-45.

Su X, Colditz GA, Collins LC, et al (2012). Adolescent intakes of vitamin D and calcium and incidence of proliferative benign breast disease. *Breast Cancer Res Treat*, **134**, 783-91.

Vobecky J, Simard A, Vobecky JS, et al (1993). Nutritional profile of women with fibrocystic breast disease. *Int J Epidemiol*, **22**, 989-99.

Yao S, Sucheston LE, Millen AE, Johnson CS, et al (2011). Pretreatment serum concentrations of 25-hydroxyvitamin D and breast cancer prognostic characteristics: a case-control and a case-series study. *PLoS One*, **6**, 17251.