

## RESEARCH ARTICLE

# Association of Vitamin D Level with Clinicopathological Features in Breast Cancer

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### Abstract

A population-based relationship between low vitamin D status and increased cancer risk is now generally accepted. However there were only few studies reported on prognostic impact. To determine the effect of low vitamin D on progression of breast cancer, we conducted a cross-sectional analysis of vitamin D levels and clinicopathological characteristics in 200 cases of breast cancer diagnosed during 2011-2012 at the National Cancer Institute of Thailand. Vitamin D levels were measured by high-performance liquid chromatography (HPLC). Clinical and pathological data were accessed to examine prognostic effects of vitamin D. We found that the mean vitamin D level was  $23.0 \pm 6.61$  ng/ml. High vitamin D levels ( $\geq 32$  ng/ml) were detected in 7% of patients, low levels ( $< 32$  ng/ml) in 93%. Mean vitamin D levels for stages 1-4 were  $26.1 \pm 6.35$ ,  $22.3 \pm 6.34$ ,  $22.2 \pm 6.46$  and  $21.3 \pm 5.42$  ng/ml respectively ( $P=0.016$ ) and 24.1 and 21.3 ng/ml for lymph node negative and positive cases ( $P=0.006$ ). Low vitamin D level ( $< 32$  ng/ml) was significantly found in majority of cases with advanced stage of the disease ( $P=0.036$ ), positive node involvement ( $P=0.030$ ) and large tumors ( $P=0.038$ ). Our findings suggest that low and decreased level of vitamin D might correlate with progression and metastasis of breast cancer.

**Keywords:** Vitamin D - prognosis - breast cancer

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### Introduction

Breast cancer confers significant morbidity and mortality among women worldwide. Due to the magnitude of this disease, considerable research effort has been directed toward identifying breast cancer risk factors to target for prevention. Vitamin D is a fat soluble vitamin and can be obtained from exposure to sunlight and through diet or supplements. Although vitamin D function is most closely associated with the control of calcium and bone metabolism, it is proposed to have a variety of other biological roles including cellular proliferation, apoptosis, immunity to fight against bacteria and probably anti-cancer effects (Garland et al., 2006; Holick et al., 2006). Epidemiologic studies and others implied their protective role against cancers as the low vitamin D level linked to several cancers risk, including breast cancer (Lowe et al., 2005; Gorham et al., 2007; Crew et al., 2009; Bao et al., 2010; Yin et al., 2010; Bolland et al., 2011; Yousef et al., 2013; Ananthakrishnan et al., 2014).

In addition, inverse correlation between circulating vitamin D levels measured at diagnosis and following breast cancer recurrence and mortality have also been demonstrated (Palmieri et al., 2006; Goodwin et al., 2009). Lower serum vitamin D levels have also been associated with more advanced stages of breast cancer. Serum levels of vitamin D were lower in patients with locally advanced

or metastatic breast cancer than in those with early-stage disease (Palmieri et al., 2006). Cancer cells undergo certain physiological changes, which decrease their susceptibility to vitamin D. Malignant cells have decreased intracellular levels of  $1\alpha$ -hydroxylase (the activating enzyme encoded by *CYP27B1*) compared to normal cells, which increases intracellular vitamin D production. Furthermore there is increased breakdown of vitamin D in tumor cells, causing resistance to the antitumor effects of vitamin D (Larriba et al., 2010).

In a multi-ethnic cohort of breast cancer survivors, women with localized or regional breast cancer had lower serum vitamin D levels than those with *in situ* disease (Neuhausser et al., 2008). Recently, a study revealed that low levels of serum vitamin D at diagnosis were significantly associated with larger tumors (Hatse et al., 2012). Therefore, we examined whether serum levels of vitamin D at the time of breast cancer diagnosis correlate with various clinical pathological characteristics.

### Materials and Methods

#### Patients

In the present study peripheral blood was collected from two hundred cases of newly diagnosed breast cancer were evaluated for age, menopausal status, tumor stage, number of lymph node involvement, tumor size and

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**Table 1. Vitamin D Levels and Clinicopathological Features**

Variables	Vitamin D (ng/ml)					Distribution of vitamin D (ng/ml)					
	No.	%	Mean		P	<32		≥ 32		P	
			Mean	SD		No.	%	No.	%		
All patients	200	100	20.66	6.61		186	93	14	7		
Age, years	≤ 50	105	52.5	22.06	5.91	0.029	102	97.14	3	2.86	0.016
	> 50	95	47.5	24.09	7.19		84	88.42	11	11.58	
Menopausal status	Pre	105	52.5	22.06	5.91	0.029	102	97.14	3	2.86	0.016
	Post	95	47.5	24.09	7.19		84	88.42	11	11.58	
Tumor stage	1	35	17.5	26.05	6.35	0.016	29	82.86	6	17.14	0.036
	2	95	47.5	22.25	6.34		89	93.68	6	6.32	
	3	56	28	22.17	6.46		55	98.21	1	1.79	
	4	5	2.5	21.3	5.42		5	100	0	0	
Lymph node	0	74	51.39	24.12	6	0.006	69	93.24	5	6.76	0.03
	≥1	70	48.61	21.33	6.06		69	98.57	1	1.43	
Tumor size (cm)	≤2	50	38.76	24.12	5.88	0.02	46	92	4	8	0.038
	>2-5	70	54.26	21.11	6.14		70	100	0	0	
	>5	9	6.98	20.88	3.12		9	100	0	0	

SD, standard deviation

vitamin D level at National Cancer Institute of Thailand. The study was approved by institutional review board and ethic committee.

#### *Serum collection and determination of vitamin D level*

Serum was isolated from peripheral blood after centrifugation and frozen at -80°C until measurement. Levels of vitamin D were determined by HPLC with UV detection (Neyestani et al., 2007).

#### *Statistical analyses*

Statistical analyses in this study were done using the SPSS 18.0 statistical software package (SPSS Inc., Chicago, IL, USA). The cut-off value of vitamin D level was 32 ng/L (Dawson-Hughes et al., 2005; Hart et al., 2006), used to groupings. The association between vitamin D levels and clinical pathological data of the patients was described with percentages and means, and the means were compared using t-test. Correlation between vitamin D levels and clinicopathological characteristics of the patients was evaluated using chi-square test. The results were considered statistically significant at  $P < 0.05$ .

## Results

In this study, mean vitamin D level was  $23.02 \pm 6.61$  ng/ml. High vitamin D levels ( $\geq 32$  ng/ml) were detected in 14 patients (7%), while low levels ( $< 32$  ng/ml) in 186 cases (93%). They were significantly higher in post-menopause patients than pre-menopause cases ( $P = 0.029$ ; Table 1). Vitamin D was significantly inversely correlated with patients' tumor stage ( $P = 0.016$ ), number of lymph node involvement ( $P = 0.041$ ) and tumor size ( $P = 0.02$ ; Table 1).

In addition, when compared vitamin D with clinicopathological parameters of the patients, we found that low vitamin D level ( $< 32$  ng/ml) was significantly mainly detected in cases with poor prognosis-high stage of the disease ( $P = 0.036$ ), positive-nodal involvement ( $P = 0.030$ ) and large tumor size ( $P = 0.038$ )-as shown in Table 1.

## Discussion

In this study, the majority of patients (93%) had low vitamin D levels ( $< 32$  ng/ml). These findings are similar to other reports that the majority of patients with breast cancer had low levels of vitamin D (Goodwin et al., 2009; Neuhouser et al., 2008). Our observation revealed that low levels of vitamin D were associated with advanced stage, positive-nodal involvement and large tumors, suggesting that the prognostic effect of vitamin D may be due to the aggressiveness of tumors in low vitamin D patients, consistent with a potential role of vitamin D in breast carcinogenesis. Low vitamin D levels in patients with breast cancer have been associated with increased risk of cancer and mortality (Freedman et al., 2007; Neuhouser et al., 2008; Goodwin et al., 2009; Mohr et al., 2014). Vitamin D levels have been reported to be significantly lower in women with locally advanced or metastatic breast cancer compared with women with early-stage disease (Palmieri et al., 2006). Metastasis is a complex, multistep process, during which circulating tumor cells (CTC) spread from the primary tumor mass, in the reversible epithelial-to mesenchymal transition (EMT) form, to the distant organs. Once distant organs are reached, these mesenchymal tumor cells reverse to an epithelial identity via mesenchymal-to-epithelial transition (MET) to regain the ability to proliferate (Yang et al., 2006). Vandewalle B found the association between intracellular calcium and breast cancer cell growth and proliferation of the metastatic breast cancer cells MCF-7. The study revealed that MCF-7 cells have retained some calcium dependency and that agents that can increase calcium concentration in breast tumor cells may limit their proliferation and trigger at least a partial differentiation (Vandewalle et al., 1993). The regulation of intracellular calcium in breast cancer may be important in modulating cell proliferation, differentiation, apoptosis and cytotoxicity, as well as contributing to mechanisms of action of anticancer agents. Vitamin D is intimately involved in maintaining cellular calcium homeostasis. The role of vitamin D in the regulation of intracellular calcium in the estrogen-receptor negative human breast

cancer cell line BT-20 was observed in a study which showed that voltage-insensitive Ca<sup>2+</sup> channels (VICC) and the thapsigargin-sensitive endoplasmic reticulum Ca<sup>2+</sup> stores are the principal pathways for Ca<sup>2+</sup> entry and Ca<sup>2+</sup> mobilization in the breast cancer cell line. Vitamin D rapidly increases Ca<sup>2+</sup> influx through VICC and after a chronic treatment, depletes endoplasmic reticulum Ca<sup>2+</sup> stores. Targeting of Ca<sup>2+</sup> signaling mediated by VICC and endoplasmic reticulum Ca<sup>2+</sup> stores may represent a novel approach to the treatment and chemoprevention of breast cancer (Sergeev et al., 1998).

Furthermore, vitamin D levels shortly after diagnosis were significantly lower in American women with local versus regional breast cancer. Recently, lower vitamin D concentration was found to be associated with poorer overall survival and distant disease-free survival in post menopausal breast cancer patients (Vrieling et al., 2011).

Our findings provide the evidence that vitamin D may be used as a prognostic factor in patients with breast cancer. Although women with breast cancer will probably benefit in conditions of overall health from having high vitamin D levels, further investigation in larger studies is needed in recommending that vitamin D intake in patients with breast cancer be increased to high levels in order to improve breast cancer outcomes.

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