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Plasma 25-Hydroxy Vitamin-D and Risk of Breast Cancer: A Case Control Study

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Case Study

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ABSTRACT

Objective: To evaluate the association between low serum levels of 25 $(OH)_2$ D and the risk of breast cancer among the female population.
Methods: A prospective case control study was done, which recruited female patients with newly diagnosed breast cancer, follow up cases of treated breast cancers, and recurrent or metastatic breast cancer (Group A). Another group of control volunteers, presenting with benign breast diseases, in the surgical clinics was incorporated (Group B). Serum vitamin D levels of both groups were compared.
Results: 50 patients were included in each group. Age ranged 21-76 years with a mean of 47.6 years. The levels of vitamin D in Group A were significantly lower than those in Group B (Odd ratio 55.5); two-tailed *p* value <0.05. This signified a substantial difference of vitamin D levels between the patients with established breast cancer and those not suffering from breast cancer.
Conclusion: There is significant relation between breast cancer disease and low levels of vitamin D. This finding testifies the hypothesis that low serum levels of vitamin D plays an important role in the pathogenesis of breast cancer.

Keywords: Vitamin D; breast cancer; benign breast disease;

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1. INTRODUCTION

The anti-cancer properties of the active form of vitamin D, 1,25 $(OH)_2$ D has generated tremendous interest in the recent past. The antiproliferative effects include the induction of differentiation and apoptosis in addition to inhibition of cancer cell growth. High residential and occupational sun exposure has been associated with lower breast and colon cancers. Exposure to ultraviolet radiation accounts for 90 percent of 25-hydroxyvitamin D (25 (OH)D, calcidol), the major circulating vitamin D metabolite, which is the substrate for 1,25 $(OH)_2$ D, the biologically most active form of vitamin D. To the researcher's knowledge, no study so far assessed these issues in our population. Most of the recent published literature identified ecological, case-control and prospective studies on the incidence and mortality of colorectal, breast, prostate and non-Hodgkin lymphoma reported a significantly inverse association with sun exposure (Rhee et al., 2009; McCullough et al., 2005; Christakos et al., 2003). The metabolism of vitamin D is well known. Vitamin D, from sunlight and dietary sources, is transported in the blood by the vitamin D binding protein, to the liver where it is hydroxylated to C-25 resulting in the formation of 25-hydroxyvitamin D_3 ($25(OH)D_3$). This $25(OH)D_3$ is transported by vitamin D binding proteins to the kidneys where $25(OH)D_3$ is hydroxylated to the active form of vitamin D (Christcakes et al).

Vitamin D has been hypothesized to reduce cancer mortality through its effects on incidence and/or survival (Govannucci, 2005). Support for this hypothesis comes from a diversity of scientific approaches. Animal and in vitro studies have shown that vitamin D plays a variety of biological roles that potentially reduce cancer incidence and promote survival including suppressing tumor progression by reducing cell proliferation and stimulating apoptosis and cell differentiation (Tramp et al., 2004; Freedman et al., 2007). Exposure to sunlight is one of the major sources of vitamin D and various ecological and observational studies have documented an inverse gradient for solar radiation surrogates and cancer-related mortality (Moan et al., 2005). Recent analytical epidemiological studies found improved survival for several cancers if diagnosis or treatment occurred during nonwinter seasons, compared with winter, leading to the speculation that therapy may positively interact with vitamin D (Liz et al., 2006). The observation that obese and dark-skinned people tend to have lower vitamin D levels and higher cancer mortality has led some investigators to suggest that these associations may be mediated by vitamin D levels (Robsahm et al., 2004). The present study verifies the inverse relation between the occurrence of low levels of vitamin D and breast cancer.

2. METHODOLOGY

A prospective case control study was conducted in Ohud Hospital, Taibah University Al Madina Al Munawara and King Khalid Hospital Najran, Saudi Arabia during the period 2006-2009. Female patients with newly diagnosed breast cancer, follow up cases of treated breast cancers, and recurrent or metastatic breast cancer were included in the study group (Group A). Those patients with compromised renal and hepatic function and post renal and hepatic transplant patients were excluded from this group. Another group of control volunteers, presenting with benign breast diseases, in the surgical clinics was incorporated (Group B). Patients with history of breast cancer were excluded from this group.

Aliquots of whole blood was taken from all participants of both groups for the determination of serum 1,25 $(OH)_2$ D levels. A written informed consent was taken from each participant of both groups. The instrument of information from all patients of Group A was based on a well-designed questionnaire (ANNEX 1). Family history of breast cancer will be determined by

using the formula; None (no family history), Weak (one second-degree relative, defined as aunt, cousin, or grandmother with breast cancer), and Strong (one or more first-degree relative, defined as mother or sister, or more than one second-degree relatives with breast cancer).

After the completion of questionnaire, 10-ml blood was extracted from participants of each group. Blood was centrifuged, separated into plasma, red cells and white cell components, and archived at -130° C for further analysis. The plasma was used to measure the circulating 25(OH)D concentration by enzyme immunoassay (Immuno Diagnostic Services UK). This is a competitive assay in which biotin-labeled 25(OH)D was added to standard samples which were then incubated in microtitre wells coated with a highly specific sheep 25(OH)D antibody. This antibody is 100% specific for 25-hydroxy vitamin D_3 and 75% specific for 25-hydroxy vitamin D_2. Each sample was measured in duplicate and 25(OH)D levels were determined with a standard curve using standards supplied with the kit. Patients and volunteer samples were run on the same plates. At the end of study, each breast cancer patient sample was matched to one control volunteer. The main criteria for matching the samples was the time of the year the blood sample was taken, since vitamin D is derived from cutaneous synthesis in response to sunlight exposure. Other soft criterion for sample matching was the age at the time of sampling. All the collected data was analyzed by the student *t*-test to determine the significance level of the difference and a two-tailed *p* value ≤ 0.05 was considered significant. Relationship between variables was examined by Pearson correlation and considered significant if a two-tailed significance level ≤ 0.05 was attained. Analysis was undertaken by SPSS version 13.0 software (Chicago, IL, USA).

3. RESULTS

A total of 100 subjects were included in the study; 50 in Group A and 50 in Group B. Age ranged 21-76 years with a mean of 47.6 years. The levels of vitamin D in Group A were significantly lower than those in Group B (Odd ratio 55.5); two-tailed *p* value < 0.05 as outlined in Table 1.

Table 1: Vitamin D levels in the case control and volunteer groups

Groups	Vitamin D ≤ 20 ng/ml	Vitamin D ≥ 20 ng/ml	Total
Group A	47	3	50
Group B	11	39	50
Total	58	42	100

Similarly, Table 2 shows the range of vitamin D levels in both groups depicting significant difference among two groups; *p* value < 0.05. The 95% Confidence Interval was found to be 16.06.

Table 2: Detailed analysis of vitamin D levels in two groups

Groups	No.	Minimum	Maximum	Mean	Std. Deviation
Vitamin D levels in Group A	50	0.43	23.20	8.4	6.008
Vitamin D levels in Group B	50	2.7	44.6	27.6	9.704

4. DISCUSSION

Vitamin D and its metabolites reduce the incidence of cancer by inhibiting tumor angiogenesis (Newmark, 1994), stimulating mutual adherence of cells (Palmar et al., 2001), and enhancing intercellular communication through gap junction, thereby strengthening the inhibition of proliferation that results from tight physical contact with adjacent cells within a tissue (Lipkin and Newmark, 1985). Vitamin D metabolites help maintain a normal calcium gradient in the colon epithelial crypts and inhibit mitosis of breast epithelial cells (Garland et al., 2006). Pulsatile release of ionized calcium from intracellular stores, including the endoplasmic reticulum, induces terminal differentiation and apoptosis, and 1-25$(OH)_2$D enhances this release.

Welsh et al proposed that the 1,25$(OH)D_3$ makes a complex with vitamin D receptor in the normal mammary tissue which induces a program of genes that suppresses proliferation and stimulates differentiation in the normal mammary gland. This hypothesis predicts that dysregulation of vitamin-D-receptor-mediated gene expression in the mammary gland will alter mammary gland development or function and possibly predispose cells to transformation. Breast cancer death rates tended to be higher in areas with low winter sunlight levels and lower in sunny areas (Gorham et al., 1989). Women regularly exposed to sunlight and consumers of above average amounts of vitamin D had significantly lower incidence of breast cancer (John et al., 1999). Women in the lower quartile of serum 1, 25$(OH)_2$D had a risk of breast cancer 5 times higher than those in the higher quartile (Janowsky et al., 1999). Lower 1,25$(OH)_2$D levels were also associated with faster progression of metastatic breast cancer (Benito et al., 1990). Mortality rates of perimenopausal ovarian cancer were also lower in sunny regions (Grant, 2003), although one study found no geographical association within a single country (Grant, 2002; Jacobson et al., 1989). Another study categorically confirmed that high intake of vitamin D and calcium markedly reduced incidence of mammary cancer in mice and rats (Robsahm et al., 2004).

In more recent studies (Anzano et al., 2000; Mehta et al., 2000), synthetic vitamin D analogs were shown to prevent N-methyl-Nnitrosourea-induced mammary carcinogenesis. It is increasingly becoming apparent that genetic variability can influence individual responsiveness to dietary or pharmacological interventions. There is considerable interest in the genetically determined differences in the mammary vitamin D receptors' signaling pathway in relation to disease susceptibility (Zmuda, 2000). A number of common allelic polymorphism in the human mammary vitamin D receptor-gene have been identified and extensively studied with respect to a variety of diseases including breast cancer (Ingles et al., 2000). Various investigators identified specific alleles of the mammary vitamin D receptor that correlated with breast cancer incidence and/or metastases (Hou et al., 2002).

There has been a randomized controlled 5-year trial of triannual high oral doses of vitamin D that evaluated cancer mortality. It found a somewhat lower cancer mortality cancer (RR= 0.86%, 95% CI = 0.61 to 1.20) in subjects who were given vitamin D, although the case numbers were small (n=135 cancer deaths in both arms combined). And the difference in risk was not statistically significant (Trivedi et al., 2003). At the same time, another study estimated a 29% reduction in total cancer mortality risk associated with an increment of 25 nmol/L in 25(OH)D based on a predictive model for vitamin D level (Giovannucci et al., 2006).

Despite the growing evidence of the inverse association of vitamin D levels and breast cancer, there are published data which suggest no statistically important inverse relationship

(Frazier et al., 2003; McCullough et al., 2009; McCullough et al., 2007). John et al. (1999) observed a non significant trend to an inverse association. Robien et al. (2007) found that the association of high vitamin D intake with breast cancer was strongest in the first 5 years after baseline dietary assessment (RR: 0.66; 95% CI: 0.46-0.94 compared with the lowest intake group). In another study (Lin et al., 2007), higher intake of vitamin D were moderately associated with a lower risk of premenopausal breast cancer; the hazard ratio in the group with the highest quartile of intake relative to the lowest quartile of intake was 0.65 (95% CI: 0.42-1.00; P_{trend}= 0.07). Meta-analysis of the intake of vitamin D and the risk of breast cancer found no association between the amount of vitamin D intake and the risk of breast cancer (Shin, M., et al., 2007). However, most studies reported on very low intake of vitamin D. Restricting the analyses to intakes ≥ 400IU/day yielded a trend towards less breast cancer (Gissel et al., 2008).

Concerning the serum vitamin D levels and the risk of breast cancer, two studies (Janowsky, et al., 1999; DeLyra et al., 2008) observed no relationship of statistical importance. Lowe et al reported that the risk was influenced by vitamin D receptors polymorphism. After adjustment for age and menopausal status, the breast cancer risk for women with insufficient level of 25-VD and the bb genotype was 6.82 (95% CI: 2.31-14.7), whereas women with insufficient 25-VD levels alone had a risk of 3.54 (95% CI; 1.89-6.61). In their prospective study, Bertone-Johnson et al., found that high levels of both 25-VD and 1,25-VD were associated with a non significant lower risk of breast cancer. Hiatt et al found no relationship between breast cancer and 1,25-VD serum levels at an average of 15 years before diagnosis. Certain interventional studies showed no significant difference in the incidence of breast cancer between women assigned to vitamin D supplementation and those assigned to placebo (Wactawski-Wende et al., 2008; Chlebowski et al., 2008). Lappe et al. (2007) established differences between these groups (four cases in the intervention vs. seven cases in the placebo group), but could not find the difference statistically significant.

Relatively few epidemiological studies have directly addressed the relationship between vitamin D and the incidence of breast cancer (Simand and Vobecky, 1991). Present study confirms the inverse association between the vitamin D levels in the serum and the risk of breast cancer. More case-control and evidence-based large clinical studies are needed to substantiate the findings of our study. Foregoing in view, the present study was designed to establish the inverse association between vitamin D and breast cancer. If such relationship is established, this can be used as a foundation for further interventional studies to monitor the role of vitamin D supplementation and exposure to sunlight in the prevention of cancer of the breast.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ANNEX 1

GROUP A; PROJECT RECORD NUMBER…….

DATED AND TIME OF COMPLETION OF QUESTIONAIRE……..

1. **Age at diagnosis/ recruitment (years)** ☐ 20-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ ≥ 60
2. **BMI (Kg/M^2)** ☐ ≤ 18.5 ☐ 18.5-29.9 ☐ ≥ 30
3. **Family History of Breast Cancer** ☐ Yes ☐ No
4. **History of Benign Breast Disease** ☐ Yes ☐ No
5. **Age at Menarche (years)** ☐ ≤ 12 ☐ 13-14 ☐ ≥ 15
6. **Number of children** ……
7. **History of Breast Feeding** ☐ Yes ☐ No
8. **Menopausal Status** ☐ Premenopausal ☐ Postmenopausal
9. **History of Hormone Therapy** ☐ Yes ☐ No
10. **Hormone Receptor Status of the Tumor**:
    - A. **Estrogen Receptor** ☐ Positive ☐ Negative
    - B. **Progesterone Receptor** ☐ Positive ☐ Negative