

Prognostic Evaluation of Vitamin D Deficiency in Breast Cancer Patients: A Pilot Study in India

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ABSTRACT

Background: Vitamin D deficiency has been associated with poor prognosis in breast cancer. A new molecular classification of breast cancer is being increasingly used that is based on expression of estrogen receptors, progesterone receptors and Her2/neu amplification and is found to correlate well with prognosis and outcome of disease. The present study was designed to determine serum vitamin D deficiency in these molecular subtypes of breast cancer.

Methods: Estimation of serum vitamin D levels by chemiluminiscence was done in a cohort of 99 breast cancer patients and 25 age matched healthy controls. Breast cancer patients were further grouped into luminal A, luminal B, Her2/neu enriched and triple negative subtypes based on immunohistochemistry results. Non-parametric tests were performed to compare vitamin D levels in different groups.

Results: 54.6%, 60%, 66.6% and 84.2% of patients with luminal A, luminal B, Her2/neu enriched and triple negative subtypes respectively were found to be vitamin D deficient (<20ng/mL) as compared to only 20% in healthy controls. More aggressive Her2/neu enriched and triple negative subtype patients had significantly lower serum vitamin D levels than the luminal subtypes of breast cancer.

Conclusion: Vitamin D deficiency is more prevalent and significantly lower levels are found in more aggressive subtypes of breast cancer. Vitamin D supplementation must be considered in these forms of breast cancer to improve outcome.

Keywords: Vitamin D Deficiency, Breast Cancer, Her2neu Enriched Breast Cancer, Triple Negative Breast Cancer

Introduction

In addition to maintaining calcium and phosphate homeostasis, vitamin D has been proposed to have anti-proliferative effects on various cancer subtypes. It is believed to retard tumor cell growth and proliferation, promote apoptosis, prevent tumor cell stimulation by growth factors and regulate inflammation, thus affecting tumor microenvironment^[1]. Low levels of vitamin D have been shown to be associated with increased mortality and poor prognosis in breast cancer in various studies in different parts of the world.^[2,3,4]

Breast cancer is the most common cancer among Indian urban females.^[5] It has classically been classified based on morphology or histological grade into various types taking into account tumor cell's mitotic index, degree of differentiation and nuclear pleomorphism.^[6] However, within morphological and histological subtypes, variation was observed in terms of prognosis and response to therapy. With the advent of microarray based gene expression profiling, various classification systems emerged. Recently, a microarray based molecular classification proposed by Perou and colleagues^[7] and validated by

others has become the most acceptable classification to determine prognosis. It classifies the breast cancer into luminal A, luminal B, Her2neu enriched and basal type/triple negative breast cancer (TNBC) based on the tumor cells expression of estrogen receptor(ER), progesterone receptor (PR) or Her2/neu amplification. The status of ER, PR and Her2neu expression is routinely determined by immunohistochemistry and fluorescence in situ hybridization(FISH).^[8]

Based on a case study in California, that has shown deficient levels of vitamin D in breast cancer patients subtypes according to the above mentioned classification,^[9] the present study was planned to determine vitamin D levels in the breast cancer patients as per the molecular classification, to compare the serum vitamin D levels among the various groups of breast cancer patients and also with age matched healthy controls in India.

Materials and Methods

A consecutive case series study was conducted at a tertiary care hospital in North India in the year 2012-2014. Blood samples of 99 newly diagnosed breast cancer patients

presenting to the hospital were collected in vacutainers. Blood was allowed to clot for 20-30 min and centrifuged at 3000Xg for 20 min. to separate the serum.

Serum total Vitamin D estimation was performed by fully automated chemiluminescence based autoanalyser, Advia centaur XP (Siemens, USA). The performance of the instrument and quality controls were satisfactory before analyzing the samples.

The cohort of breast cancer patients was divided into 4 groups based on the immunohistochemical analysis on breast cancer cells. Luminal A group showed positivity for Estrogen Receptors (ER) and/or Progesterone receptors (PR) and were negative for Her2/neu overexpression. Luminal B group showed positivity with ER, PR as well as Her2/neu overexpression. Her2/neu enriched group showed Her2/neu overexpression but negative for ER and PR. Triple negative breast cancer (TNBC) group showed negative status with ER,PR as well as Her2/neu.

Total 25 voluntary healthy controls were also enrolled in the study and their serum vitamin D levels were measured. Based on the serum vitamin D levels, these groups were subdivided into vitamin D deficient (vitamin D \leq 20ng/mL), vitamin D insufficient (vitamin D between 20-29 ng/mL), and vitamin D sufficient (vitamin D \geq 30ng/mL).^[10]

Statistical analysis was performed using SPSS v.16 (SPSS, USA). Non-parametric Mann-Whitney U test was carried out to compare the various groups of breast cancer patients with healthy controls. Unpaired student t- test was performed to match the age of breast cancer patients and healthy controls. Non-parametric kruskal- wallis test followed by Mann-Whitney U test was done to compare these groups with each other. P value $<$ 0.05 was considered statistically significant.

Results

The mean age of patients with breast cancer was found to be 55.8 with standard deviation of 10.09. Table 1 shows the sample size, mean, standard deviation and 95% confidence interval of serum vitamin D levels of all the breast cancer patients groups and healthy controls.

The mean serum vitamin D levels in all the groups of breast cancer patients was found to be significantly lower than the healthy controls with p value $<$ 0.05 as shown in table 2 and figure 1. Furthermore, serum vitamin D levels in Her2/neu enriched group and Triple negative breast cancer group were significantly lower (p $<$ 0.05) than vitamin D levels in the sera of Luminal A and B type of breast cancer patients.

On subdividing the groups based on the levels of vitamin D, it was observed that luminal A type breast cancer patients had 54.6% (n=35) patients with deficient vitamin D, 32.8% (n=21) had insufficient serum vitamin D levels and only 12.5% (n=8) were having sufficient vitamin D levels. Luminal B type breast cancer patients group had 60% (n=6), 30%(n=3) and 10%(n=1) patients showing deficient, insufficient and sufficient levels of serum vitamin D levels respectively. However, in the Her2/neu enriched and TNBC type breast cancer patients, none of the patients showed sufficient serum vitamin D levels. Rather 66.6% (n=4) of Her2/neu enriched and 84.2% (n=16) of TNBC patients were deficient in vitamin D levels. This is in contrast to the healthy controls group where only 20% (n=5) were deficient in vitamin D levels, whereas, 48%(n=12) and 32% (n=8) of the total subjects showed insufficient and sufficient levels of serum vitamin D levels as also depicted in figure 2. This clearly shows that vitamin D deficiency is more prevalent in breast cancer patients as compared to age matched healthy controls with Her2/neu enriched and TNBC patients showing even lower values of serum vitamin D than in luminal types of breast cancer.

Table1: Mean, SD, 95% Confidence interval of serum Vitamin D levels for Breast cancer patients groups and Healthy controls.

| Groups | Sample size | Mean \pm SD of Vitamin D (ng/mL) | 95% Confidence interval |
|------------------|-------------|------------------------------------|-------------------------|
| Luminal A | 64 | 19.2 \pm 8.57 | 17.05-21.34 |
| Luminal B | 10 | 18.9 \pm 6.52 | 14.26-23.59 |
| Her2neu enriched | 6 | 15.7 \pm 5.98 | 9.44-21.99 |
| Triple negative | 19 | 13.31 \pm 5.19 | 10.8-15.81 |
| Healthy Controls | 25 | 25.95 \pm 8.09 | 22.61-29.29 |

Table2: Mann whitney U test comparing Breast cancer patients groups with healthy controls.

| Groups | Mann-Whitney U | Wilcoxon W | Z value | P value |
|------------------|----------------|------------|---------|---------|
| Luminal A | 471.0 | 2.55 | -3.0 | 0.003* |
| Luminal B | 60.5 | 115.5 | -2.35 | 0.018* |
| Her2neu enhanced | 22.5 | 43.5 | -2.62 | 0.009* |
| Triple negative | 42.0 | 232.0 | -4.63 | 0.000* |

*indicates statistically significant at p $<$ 0.05

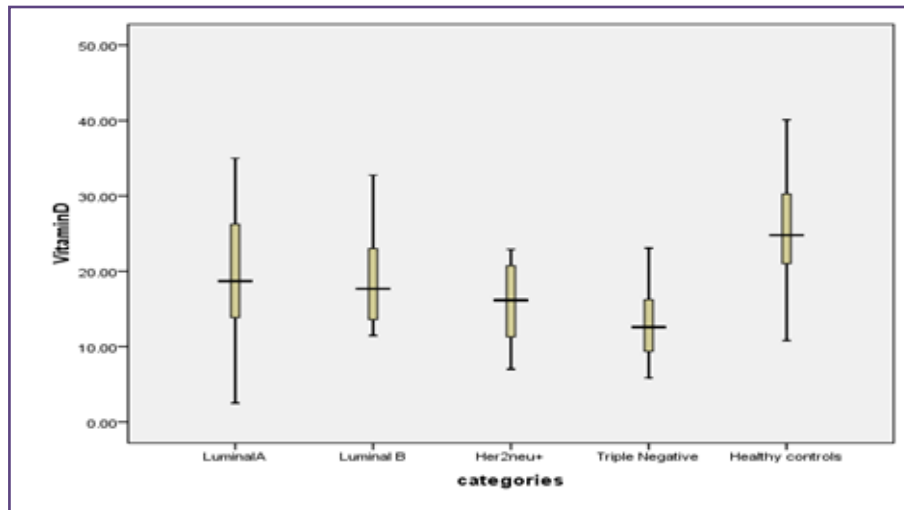


Fig. 1: Box plot showing Mean \pm SEM of Vitamin D levels (ng/ml) in Breast cancer patients groups and healthy controls. Solid bold line represents mean serum vitamin D in different groups.

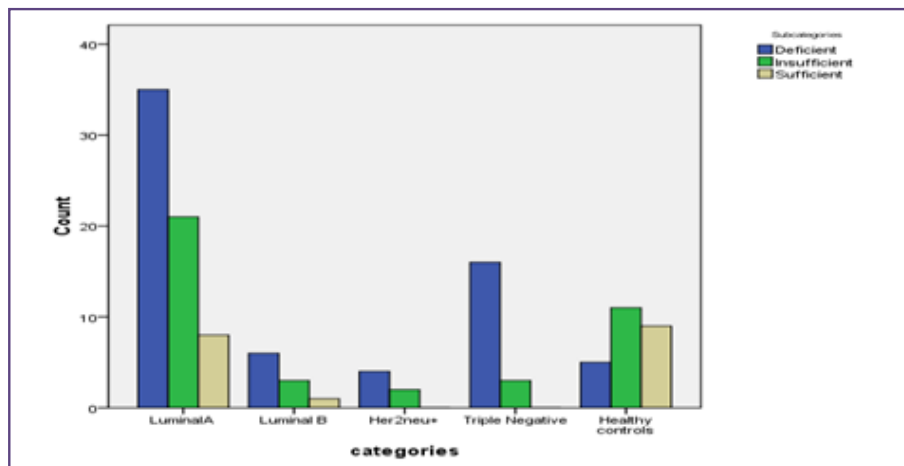


Fig. 2: Clustered Bar diagram showing number of subjects with Deficient Vitamin D levels (≤ 20 ng/mL), Insufficient Vitamin D levels (20-29ng/mL) and Sufficient Vitamin D levels (≥ 30 ng/mL) in Breast cancer patients groups and healthy controls.

Discussion

Despite the availability of a large number of studies on the role of vitamin D in breast cancer across the globe, the studies in India are limited. With the advent of new classification based on the receptor status in breast cancer patients, the current study was planned to look for the vitamin D deficiency in various subtypes of breast cancer.

In a country like India where skin pigmentation, milk intolerance along with lifestyle and avoidance of sun exposure already predispose to low vitamin D levels, the deficiency of vitamin D in breast cancer patients can result in poorer prognosis.

With a mean of 17.8 ng/mL of serum vitamin D in the breast cancer patients as compared to the mean of 25.9 ng/mL in healthy controls, the present study shows agreement

with previous studies in Saudi Arabia^[11] and in other parts of the world.^[2,3,4] The present study also shows that levels of vitamin D in triple negative breast cancer with mean of 13.3ng/mL is lowest among all the subtypes. This finding conforms to the earlier studies where vitamin D levels in TNBC have been found to be lowest.^[9] Furthermore, there are studies that show that TNBC has the worst prognosis than its other counterparts^[12]. Her2neu overexpression is long known to have contribution to aggressive behavior of breast cancer with poor outcomes^[13]. Her2neu enriched subtype of breast cancer has been also shown to have unfavourable prognosis as compared to the luminal types.^[14] Pertaining to the anti-proliferative and protective effect of vitamin D on cancer cells, its deficiency implies higher proliferation in cancer cells and this could explain lower

levels of vitamin D in the Her2/neu enriched and TNBC subtypes that are more aggressive and are associated with poor outcomes. Contrary to our study, a study by Kim HJ in Korea has shown relation of vitamin D deficiency with poor prognosis of luminal types but no relation was shown with her2/neu enriched or TNBC subtype. They have hypothesized it to be due to effect of vitamin D on suppressing estrogen mediated proliferation of breast cancers that are ER positive i.e. luminal type but they have not explained any mechanism for ER negative cancers.^[15] However, in a recent study, it was shown that by upregulating the vitamin D receptors in murine model of TNBC, antiproliferative effect is enhanced^[16] and thus explaining the effect of vitamin D in TNBC subtype breast cancer. Further studies explaining the exact molecular mechanism underlying such observations needs to be carried out. Moreover, studies with larger sample size involving larger geographical distribution have to be carried out to validate our results.

Studies are also available that show that vitamin D supplementation in breast cancer patients improved their prognosis.^[17] One such study showed that intake of high dose weekly vitamin D supplementation can be helpful not only in breast cancer but also in reducing treatment related arthralgias and fractures.^[18]

In the current study, a lower mean level of vitamin D was observed in both the breast cancer patients as well as healthy controls than the previous studies in western population. The plausible explanation for this could be socio-cultural taboos and vegetarian diet combined with lack of awareness regarding vitamin D supplements in Indian population as explained elsewhere.^[10] Their study also showed that mean levels of vitamin D in Punjab, Haryana are around 25.3ng/mL which is close to the mean vitamin D levels in healthy controls (25.95ng/mL) in our study.

The drawback of the current study includes the lower sample size, single geographical area and determination of a single parameter to evaluate prognosis in breast cancer patients. Therefore further studies considering these aspects must be carried out. However, to the best of our knowledge, this is the first study in India, evaluating role of vitamin D deficiency in breast cancer taking into account the molecular subtypes of breast cancer.

To conclude, Vitamin D deficiency is associated more with breast cancer as compared to age matched healthy controls. The Triple negative and her2/neu enriched subtypes that

carry poorer prognosis shows higher prevalence and significantly lower mean values of serum vitamin D than the luminal types.

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