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Determining Vitamin D Status: A Comparison Between Diabetic And Non-diabetic Women With Breast Cancer By RP-HPLC

Santosh Gunasekaran
JSS Academy Of Higher Education and Research, santosh.hastam@gmail.com

Suma M. Natraj
JSS Academy Of Higher Education and Research, mnsuma@jssuni.edu.in

Prashanth Kumar M V
JSS Academy of Higher Education and Research, pprash90@gmail.com

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Cover Page Footnote

Nil

Introduction:

Vitamin D refers to a group of fat-soluble secosteroids that are produced in 2 forms: D2 and D3. Both forms undergo hydroxylation in the liver by mitochondrial and microsomal 24hydroxylase (encoded by CYP24A1) to yield 25-hydroxyvitamin D (25(OH)D) or calcidiol. (1)

The 25(OH)D is then transported in the circulation by the vitamin D–binding protein and further metabolized in kidneys to produce 1,25 dihydroxyvitamin D (1,25(OH)2D) (by 1α-hydroxylase that is encoded by CYP27B1) or calcitriol. The half-life of 1,25(OH)2D is only 4 to 6 hours and 1000-fold less than the total 25(OH)D. (2) So, serum vitamin D is usually determined by measuring 25(OH)D biomarker that has a half-life of about 2 to 3 weeks. The Institute of Medicine guidelines suggest that individuals are at risk of vitamin D deficiency if 25(OH)D concentration is below 30 nmol/L, inadequacy at serum 25(OH)D concentration between 30 and 50 nmol/L, and individuals are considered sufficient at concentration 50 nmol/L or higher. In contrast, the Endocrine Society guidelines defined that 50 nmol/L is a cutoff value for vitamin D deficiency and the sufficient concentration exceeds 75 nmol/L. (3)

Suboptimal levels of Vitamin D, remains a common problem worldwide and its prevalence is high in India, ranging from 70-100%.(4) Apart from skeletal manifestations, low vitamin D level is associated with inflammatory conditions, diabetes, cardiovascular diseases and cancers. There is an inverse relationship between hypovitaminosis D and glycemic status, confirmed through several studies worldwide.(5) High incidence, aggressive histopathological variants, distant metastasis and poor prognosis of breast cancer is also linked with low Vitamin D levels in many observational studies.(6,7) Several molecular mechanisms have been found about the effects of Vitamin D in modulating glycemic levels and its protective nature in the development of breast cancer.(8,9) In addition, better exposure to sunlight is related to decreased incidence of several types of cancers.(10) Breast cancer in India is a common problem and studies regarding its association with Vitamin D levels among diabetics remain inconclusive. Early epidemiologic research showed that incidence and death rates for certain cancers were lower among individuals living in southern latitudes, where levels of sunlight exposure are relatively high, than among those living at northern latitudes. Because exposure to ultraviolet light from sunlight leads to the production of vitamin D, researchers hypothesized that variation in vitamin D levels might account for this association. (11) However, additional research based on stronger study designs is required to determine whether higher vitamin D levels are related to lower cancer incidence or death rates.

Insufficient Vitamin D levels is a silent epidemic even in tropical countries, with potential skeletal and extra-skeletal manifestations. Therefore, additional research on this topic assumes greater importance. This study aims to find a relationship of Vitamin D levels among diabetic and non-diabetic breast cancer patients.

Objectives:

1. To determine the levels of 25-hydroxy (OH) Vitamin D in women with & without diabetes and suffering from breast cancer.
2. To establish correlations between vitamin-D status, and diabetes and breast cancer.

Material and Methods:

The study has been performed after obtaining Institutional Ethical Clearance.

Sample size: 25 (20 Breast cancer patients+5 normal).

Inclusion criteria: Women above the age of 18 years diagnosed with breast cancer.

Exclusion criteria: Patients with renal failure, bone diseases, minor individuals and those taking Vitamin D supplements.

Reagents and Chemicals:

Vitamin D estimation Kit: MODULAR ANALYTICS E170, Roche cobas e 411

- working solutions

The pre-treatment reagents 1: Dithiothreitol 1 g/L, pH 5.5.

Pre-treatment reagent 2 : Sodium hydroxide 55 g/L.

M Streptavidin-coated microparticles, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

Vitamin D binding protein-BPRu, Ruthenium labeled vitamin D binding protein 150 µg/L; bistris propane buffer 200 mmol/L; albumin (human) 25 g/L; pH 7.5;

preservative. R2 2hydroxyvitamin D~biotin, 8.5 mL: Biotinylated vitamin D (2-OH) 14 μg/L; bis-tris propane buffer 200 mmol/L; pH 8.6; preservative.

Sample collection:

This study includes 20 female breast cancer patients (diabetic) and 5 normal samples (non-diabetic), Blood collected from patients before starting the chemotherapy, radiotherapy or any other treatment related to breast cancer. About 1 ml of blood samples will be drawn from the cubital vein, and then transported in an icebox. Serum separated by centrifugation at 10000 rpm for 10 min. Sterile disposable syringes used for the sample collection. All the serums transferred to 1.5mL micro-centrifuge tubes and stored at -80ºC before analysis.

Sample analysis:

The analyzer automatically calculates the analyte concentration of each sample (ng/mL or nmol/L).

Conversion factors: nmol/L x 0.40 = ng/mL

Assay

Resuspension of the microparticles took place automatically prior to use. Brought all the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer, To avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles. Calibration

Traceability: This method has been standardized against LC-MS/MS15 which in turn has been standardized to the NIST standard.16 Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Dilution:

Samples with vitamin D (25-OH) concentrations above the measuring range manually diluted with Diluent Universal or a suitable human serum with a low analyte concentration. The recommended dilution is 1:2. The concentration of the diluted sample must be > 30.0 ng/mL (>

75.0 nmol/L). After manual dilution, multiplied the results by the dilution factor 2. The endogenous analyte concentration of the human serum used for dilution has to be taken into account.

Health based reference values:

Currently there is no standard definition of the optimal vitamin D status. Many specialists consider the commonly used population based reference values too low. Health based reference values are recommended to replace population based reference values. Most experts agree that vitamin D deficiency should be defined as vitamin D (25-OH) of ≤ 20 ng/mL (≤ 50 nmol/L). Vitamin D insufficiency is recognized as 21-29 ng/mL. Similarly, the US National Kidney Foundation considers levels < 30 ng/mL to be insufficient or deficient.

The preferred level for vitamin D (25-OH) by many experts is now recommended to be ≥ 30 ng/mL (≥ 75 nmol/L).

Results :

1. Breast cancer patients had 50% low Vitamin D compared to non-diabetic controls.

Breast Cancer patients serum sample analysis report showed 50% low Vitamin D compared to normal individuals (P 0.018 by student t-test).

Fig 1: Vitamin D levels in the analysed samples.

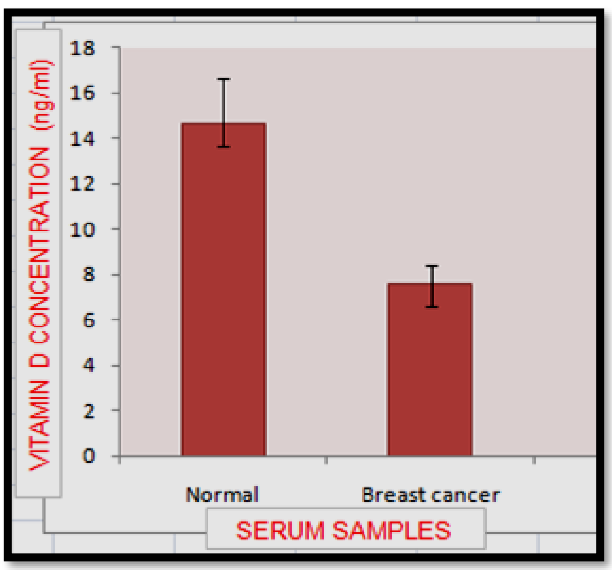

Table 1: Maximum number of Breast cancer patients are not suffering from diabetes and non-breast cancer patients (normal patients) are non-diabetic.

Comparison of Vitamin D levels between diabetic and non-diabetic patients with breast cancer is shown in Table 2

Diabetic (n=6)	4.34	(3-9.49)
Non diabetic (n=14)	9.92	(5.05-10.79)

a ~ interquartile range

Table 2: Comparison of Vitamin D levels between diabetic and non-diabetic individuals with breast cancer using Mann Whitney U test gives a p value of 0.062 which is not statistically significant.

Discussion:

Breast cancer has been considered as the most common type of cancer among the women within 161 countries, and the most common cause for cancer deaths, within 98 countries. Known and well-established risk factors for breast cancer include age, family history, the density of breast tissue, parity, overweight, alcohol intake, and genetic risk factors such as BRCA mutations.(14) Recently, vitamin D receptor (VDR) genes were reported to increase breast cancer risk. Several molecular breast cancer subtypes have been identified: luminal A and B (accounting for 50%-60% of breast cancer cases), basal-like or triple-negative (10%20% of breast cancer cases) and human epidermal growth factor receptor 2 (HER2)-enriched (10%- 15% of cases). Vitamin D receptor genes operated by vitamin D have important roles in the mammary gland through regulation of calcium transport during lactation, hormone differentiation, and milk production. Many efforts and enormous research have been directed toward identifying vitamin D as a breast cancer risk factor to be targeted for cancer prevention. (12).

This is because circulating vitamin D levels (levels ≥45 ng/mL) may protect against breast cancer and because breast cancer chemoprevention drugs that alternate the carcinogenesis process such as estrogen receptor modulators, tamoxifen, raloxifene, and aromatase inhibitor have high toxicities and not effective in the aggressive estrogen receptor–negative (ER−) breast cancers. (12) Although most case-controlled studies, meta-analysis, and pooled reviews found that 25(OH)D concentration was inversely related to breast cancer risk, only a few randomized controlled trials (RCTs) of vitamin D support this finding. Bolland et al, in their study of the Women's Health Initiative randomized trial showed that among 15646 women (43%) who were not taking personal calcium or vitamin D supplements at randomization, coadministered calcium and vitamin D significantly decreased the risk of total breast and invasive breast cancers by 14% to 20%. (13)

Many studies examined the association between vitamin D level and breast cancer risk, which generally show an inverse association. The meta-analysis conducted by Chen et al revealed that women with the highest quantile of circulating 25(OH)D was associated with a 45% (odds ratio [OR] = 0.55, 95% confidence interval [CI] = 0.38-0.80) decrease in breast cancer risk when compared with those women with the lowest quantile of blood 25(OH)D. Another metaanalysis serum 25(OH)D through the sun exposure and dietary intake more than 400 IU per day vitamin D supplementation decreased breast cancer risk and recurrence. (15)

Through this study, we see that lower levels of Vitamin D are found in breast cancer patients when compared to normal subjects, confirmed by the statistically significant results. This could contribute to furthering the research on understanding its mechanisms, benefits of Vitamin D supplementation on such patients, Vitamin D status in other cancers etc.

For this study, sample size could not be accurately determined beforehand as the actual prevalence of diabetes in breast cancer patients is not known. Although we could assume that prevalence of diabetes in the community would be the same for breast cancer patients too, we cannot safely conclude it, especially for diseases like cancer. Besides to select more number of fresh patients diagnosed with breast cancer, meeting all inclusion criteria is cumbersome. Therefore the patients who met the above inclusion criteria were enrolled during the study duration.

While this study strengthens the proposition that lower levels of Vitamin D is associated with breast cancer, more studies maybe needed to assess the effect of diabetic status on the levels of Vitamin D. Such studies need adequate number of breast cancer patients meeting the above inclusion criteria. Also, it could help us in knowing the subtype/s of breast cancer linked to low Vitamin D levels. Perhaps the limitation of this study is the inability to know if the statistically insignificant correlation between diabetic patients and low Vitamin D status, was due to fewer samples. Based on this study, further studies could also be done on the difference in variation of Vitamin D levels in diabetic vs non diabetic cancer patients and diabetic and non-diabetic normal patients.

Conclusion:

In Conclusion, low Vitamin D levels are very common among women suffering from breast cancer comparing to normal subjects. Although diabetic breast cancer women have lower Vitamin D levels when compared to non-diabetic women, the results are not statistically significant.

Acknowledgements: Nil

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