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Vitamin D Levels and Its Correlation with Severity of Breast Cancer

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Abstract

Introduction: Vitamin D has wide range of actions in the body which include immunogenic actions, differentiation of immune cells and keratinocytes, expression of growth factors etc. Genes encoding for proteins that regulate cellular differentiation, cell proliferation, apoptosis and angiogenesis contain vitamin D response element.

Materials and Methods: A total of 135 newly diagnosed cases of breast cancer and equal number of controls were recruited in the study. The histo-pathological grading of cancer, Hormone-receptor status (estrogen receptor-ER, progesterone receptor PR and HER-2 receptor) was done by immunohistochemistry, stage of tumor (based on TNM Classification), details of metastasis were noted. Vitamin D levels were done by HPLC method.

Results: The mean age, age at menarche, BMI, calcium intake, sun exposure, Ser PTH levels, Ser Calcium levels were insignificantly different in both the groups. Ser Vitamin D levels were 30.95±17.65 ng/Ml in cases and 27.21±7.97 ng/Ml in controls, with a p value of 0.198 which is not significant. The levels of Vitamin D in ng/Ml in Stage I, Stage II, Stage III and Stage IV were as follows: 26.06±11.45, 29.61±17.23, 47.33±21.13, 42.67±28.92 and a p value of 0.190(NS). Thus there was no significant difference in Vitamin D levels of cases and controls. The association between the biomarker status and stages showed significant results as indicated by p-value of 0.043.

Conclusion: This study fails to find any inverse association of vitamin D with severity of breast cancer and receptor status.

***Key Words**: Vitamin D, Breast Cancer, hypovitaminosis D, Receptor Status*

Introduction

Vitamin D receptors (VDRs) are found in many tissues other than kidneys, bones, and intestines like, skin, lymphocytes, cardiac muscle, breast tissue, and anterior pituitary gland.[1] In addition to its role in calcium metabolism, Vitamin D has wide range of actions in the body which include immunogenic actions, differentiation of immune cells and keratinocytes, expression of growth factors etc. Genes encoding for proteins that regulate cellular differentiation, cell proliferation, apoptosis and angiogenesis contain vitamin D response elements.[2,3] The factors associated with breast cancer are genetic mutation, reproductive factors, family history, breast

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density, increasing age and nutritional risk factors. Retrospective and prospective epidemiologic studies have revealed that vitamin D deficiency is associated with an increased risk of initiation, development, prognosis, progression and dying of breast cancer. Several recent reports have found vitamin D intake is beneficial not only for cancer prevention but also for women recently diagnosed with breast cancer. In contrast many studies including randomized controlled trials have failed to establish beneficial effects of Vitamin D in breast cancer.[4] Laboratory studies have demonstrated that vitamin D3 and its analogs inhibit cell proliferation and promote apoptosis in cancer cells in culture.[5,6] The major circulating form of vitamin D is 25-hydroxyvitamin D [25(OH) D] formed after the first hydroxylation of vitamin D3 or vitamin D2 in the liver. In the kidneys, 25(OH) D is converted to the active metabolite 1, 25(OH) D after second hydroxylation. 1, 25(OH) D has a short half life and tight homeostatic control. Once 1, 25(OH) 2 D completes the task of maintaining normal cellular proliferation, inhibition of angiogenesis, differentiation and apoptosis, it induces expression of the enzyme 24-hydroxylase (24-OHase) which enhances the catabolism of 1, 25(OH) 2D. This action of Vitamin D3 is also seen in breast tissue in vitro, where Vitamin D3 is shown to reduce cell proliferation, induce apoptosis and also promotes cell differentiation.[7-9]

Methods

This is a case-control study which was approved by the Institutional Ethics Committee. A total of 135 cases and an equal number of healthy age and sex matched controls were included in the study.

Statistical Analysis

All the analysis were performed using SPSS version 20.0 (IBM Corp.) and significance was evaluated at 5% level.

Results

Table 1 provides the descriptive statistics for various characteristics in cases and controls. The difference in mean age in two groups was statistically insignificant, as indicated by p-value of 0.15. Further, other characteristics like BMI were comparable between case and control groups as revealed by p-values > 0.05. As regards vitamin D, although the mean in cases (30.95 ± 17.65) was higher than that of control group (27.21 ± 7.97), the difference was statistically insignificant (p=0.198). The number of pre-menopausal cases is 90 and post-menopausal cases is 45. In control group premenopausal women are 99 and postmenopausal 36 in number. Similarly Ser PTH and Calcium levels were also comparable in both cases and controls.

Table 1: Descriptive statistics for cases and control group patients

Characteristics	Cases (N=135)	Controls (N=135)	P Value
Age in completed years	49.16±11.37	46.31±6.54	0.150(NS)
BMI in Kg/m2	21.79±3.56	28.21±30.08	0.162(NS)
Age at Menarche in years	12.8± 2.3	12.5±1.8	0.174(NS)
Calcium Intake in mg/day	760±265	780±236	0.231(NS)
Vitamin D Intake in mcg	2.3±1.1	2.2±1.3	0.182(NS)
H/O Smoking	Nil	Nil	-
Family h/o Breast Cancer	13%	0%	0.000
Use of Oral Contraceptives	21%	20%	0.44(NS)
Use of HRT post-menopausal state	0	0	-
Number of Children	2.1±0.9	2.0±1.3	0.53(NS)
Ser 25(OH)D3 ng/Ml	30.95±17.65	27.21±7.97	0.198(NS)
Ser Calcium mmol/L	2.24±0.58	2.36±0.76	0.137(NS)
Ser PTH pg/ml	28.5±11.6	27.96±12.54	0.179 (NS)

*Obtained using *independent samples t-test*; NS: Not significant

Table 2 shows the distribution of Vitamin D levels in both cases and controls.

Table 2: Distribution of Vitamin D levels across cases and Controls

	Vitamin D Levels, Deficiency, Insufficiency, Sufficiency and Toxicity			
	Deficiency Vitamin D3<10	**Insufficiency Vitamin D3 11-29**	**Sufficiency Vitamin D3 between 30-150**	**Toxicity Vitamin D3 > 150**
Cases	21(15.55%)	45(33.33%)	69(51.11%)	0
Controls	18(13.33%)	75(55.55%)	42(31.11%)	0

Table 3 shows comparison of biochemical parameters across patients with different stages of cancer. In the case group, the patients were sub-grouped based on the stages of cancer and the biochemical parameters were compared statistically across these stages. Vitamin D showed statistically insignificant difference across the stages as indicated by p-value > 0.05. The reason for higher vitamin D mean in case group was mainly due to stage 3 and 4 with levels higher than that of stage 1 and 2. However, the sample number of cases in 3 and 4 groups was much smaller, and hence insignificance was observed across comparison.

Table 3: Comparison of biochemical parameters across patients with different stages of cancer

Parameters	Stage I N=27	Stage II N=90	Stage III N=9	Stage IV N=9	P Value*
Vitamin D3 in ng/Ml	26.06±11.45	29.61±17.23	47.33±21.13	42.67±28.92	0.190(NS)

*Obtained using *ANOVA*; NS: Not significant

In conclusion, no relationship was observed between vitamin D levels and stage of cancer based on this sample study. The association of receptor status and stage of cancer was also observed on the studied sample. The results are shown in Table No 4. For HER2, out of 135 evaluations, 61.48% showed negative while 38.51% had positive result. The association between test result and stages of cancer was statistically insignificant with p-value of 0.585. For PR, in stage II, the proportion of positives was 85.5%,which mainly contributed to the significant association of PR outcome with stage of cancer(p=0.035). Further, for ER, 72.22%were positive. The association between the biomarker status and stages showed significant results as indicated by p-value of 0.043.

Table 4: Association of biomarker outcomes with stages of cancer

Biomarker	Stage1(n=27)	Stage2(n=90)	Stage 3 (n=9)	Stage4(n=9)	P-value*
HER2					
Negative (17)	4(14.81)	69 (76.66)	3 (33.33)	7(77.77)	0.585(NS)
Positive(8)	23(85.18)	21 (23.33)	6 (66.66)	2(22.22)	
PR Negative (5)	9(33.33)	13 (14.44)	8(88.88)	8(88.88)	0.035(S)
Positive (20)	18 (66.66)	77 (85.55)	1(11.11)	1(11.11)	
ER					
ER Negative (n=7)	0	25(27.77)	8 (88.88)	9(100)	0.043 (S)
Positive (n=18)	27 (100)	65 (72.22)	1(11.11)	0	

*Obtained using Chi-Square test; S: Significant; NS: Not Significant

*Figures in bracket indicate percentage

Discussion

The main aim of our study was to evaluate the co-relationship of breast cancer, its severity and biomarker status with the levels of vitamin D. The study fails to find any correlation between vitamin D deficiency, sufficiency and breast cancer severity and marker status. The difference in Vitamin D levels in cases and controls is insignificant. Also the levels were insignificantly different in stage I, stage II, stage III and stage IV. The main reason for this insignificance could be the small number of cases in Stage III and Stage IV. The receptor status of HER-2, Estrogen receptor ER and progesterone receptor PR were also assessed in this study and there was no difference in the levels of Vitamin D based on positive or negative status of either of the receptors. In this study we have taken into account the calcium intake, exposure to sun and PTH levels which are known confounders for the vitamin D levels and we have adjusted for these known confounders in our analysis. We have also excluded patients and controls who were or had been on calcium and Vitamin D supplements.

Two clinical trials of vitamin D and breast cancer incidence have been completed. In the Women's Health Initiative, a total of 36,282 women were randomly assigned to receive either 1000 mg calcium plus 400 IU vitamin D or placebo and were followed for an average of 7y. No effect of the intervention was observed for breast cancer incidence.[11] Similar inconsistent results were reported by Lowe et al.[3] Our study resembles these findings, though VDR and 1 alpha hydroxylase were not done in our study. Since the above mentioned follow up studies and interventional studies don't prove any significant difference in vitamin D levels and any beneficial effects of calcium and vitamin D supplementation, the role of vitamin D deficiency in cancer needs to be re-explored. Similar findings echoed by another study which aimed at studying the levels of vitamin D in breast cancer survivors post treatment and breast cancer specific mortality in multivariate analysis during a follow up period of 9 years.[12] This study design and end points were different from our study as we had enrolled newly diagnosed cases and ours was a cross-sectional study. Though the message conveyed by both studies is coherent. Many studies have reported Vitamin D deficiency as one of the risk factors for breast cancer and also inverse relationship of Vitamin D levels with the severity of breast cancer. These findings contradict our observations. A few of these studies have a very large sample size,5in which higher levels of Vitamin D3 were associated with lower risk of breast cancer specially in younger age group. In another trial by Lappe et al[13] women who were supplemented with1400–1500 mg calcium plus 1100 IU vitamin D had a significantly reduced risk of overall cancer incidence, with lower rates of breast cancer in the calcium- and vitamin D–supplemented group than in the placebo group. A few Asian studies from Pakistan and Korea have similar observations with low levels of Vitamin D in breast cancer patients, inverse relationship with severity of cancer and poor outcome with low vitamin D levels.[9,14,15] All these findings are contradictory to ours. It becomes all the more important to undertake multicentric project to define the role of Vitamin D in breast cancer specially when the trials from other Asian countries are reporting increased cancer risk with hypovitaminosis D. Some data available from India indicated that VDR poly-A polymorphism is significantly associated with BC risk in north Indians especially with early onset disease.[16] Analysis on VDR gene polymorphisms in breast cancer in Indian population has revealed that some VDR gene polymorphisms (Taq 1 genotypes) are associated with BC risk.[17] Asians have low exposure to sunlight, there is high prevalence of various malabsorption syndromes, hyper pigmentation and rare vitamin D supplementation. The different studies from India have reported a very high prevalence rate of Vitamin D deficiency and insufficiency ranging between 70-90%.[18]

Conclusion

This study has a main constraint of small sample size and thus fails to establish any cause effect relationship between Vitamin D levels and Breast Cancer though it may form a basis for designing a larger study to see the association of Vitamin D levels with severity of breast cancer and also with the marker status (ER, PR and HER-2) of breast cancer. Thus the role of vitamin D and its levels in severity, recurrence and prognosis is far from being definitive. In conclusion this study fails to find any inverse association of vitamin D with severity of breast cancer and receptor status.

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