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Serum Vitamin D Levels in Females with Breast Cancer and Normal Healthy Controls: A Case Control Study

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Abstract: ***Background:*** ***Vitamin D is not just a vitamin, it functions as a hormone in the body and it has a wide range of essential functions ranging from calcium homeostasis, immune regulations, cell differentiation to anticancer properties. Breast cancer is the most prevalent cancer in females throughout the world as well as in India. The present study was undertaken with an objective to estimate and compare serum vitamin D levels in females having breast cancer with normal healthy females.*** ***Method:*** ***The present hospital based, case control study evaluated a total of 100 females, forty years or above from the OPD and wards of the Department of Surgery, MM Institute of Medical Sciences and Research, Mullana, Ambala. Hundred (100) subjects included in the study were grouped as Group 1(cases) enrolling 50 breast cancer patients aged 40 years and above and Group 2 as controls with 50 age matched healthy females. Further, a detailed history was elicited from the patients and general physical examination along with local examination and systemic examination was performed for all. Breast cancer patients already on Vitamin D or Calcium supplements and those with renal disease were excluded from the study. Serum vitamin D [25(OH) D] was assayed by direct competitive chemiluminescence immunoassay (CLIA) for all subjects enrolled in the study.*** ***Results:*** ***Serum levels of Vitamin D in breast cancer patients ranged from 11.9±7.8 ng/ml while in healthy controls it ranged from 23.0±8.84 ng/ml and on comparison the levels in cases were found to be significantly lower with p<0.001.*** ***Conclusion:*** ***Considering the extra-osseous actions of vitamin D, it has a potential role in the regulation of immune functions and has anticancer properties. Significantly lower levels of serum Vitamin D levels in breast cancer patients as compared to age matched controls supports the fact that Vitamin D has beneficial role in the breast cancer patients so, vitamin D levels must be augmented in the patients of breast cancer.***

Keywords: Vitamin D, Breast Cancer, Calcium

1. Introduction

Vitamin D, a seco-steroid hormone is involved in the metabolism of bone and it also regulates calcium levels in the body. It regulates calcium homeostasis. It increases calcium absorption from the gastrointestinal tract and kidneys.[1]In recent years, there is growing interest in exploring extra-osseous actions of vitamin D because of its involvement in the regulation of immune functions and anticancer properties.[2]Vitamin D role has been proved to have role in protection against cancer as it is a potent inhibitor of proliferation (represses protooncogenes, stabilises p27) , inducer of apoptosis (induces BAX & BCL, releases cytochrome c). Further many studies have demonstrated the role of Vitamin D in promoting differentiation, decreasing inflammation (blocks proinflammatory cytokines, decreases levels of IL-1β, IL-6, IL-7, and NFκB). Further, several studies have shown that Vitamin D has a role as an inhibitor of invasion and metastasis (in breast cancer, it inhibits Wntsignalling through VDR or vitamin D receptors interaction with beta catenin) and angiogenesis (downregulates hypoxia-inducible factor 1 (HIF-1α) causing decreased secretion of VEGF).[3-14]

Considering the epidemiology of cancer worldwide it has been shown that Breast cancer is the most prevalent cancer in females all over the world with detection of 17 lakh new cases of breast cancer in the year 2018. Approximately 627000 women died because of breast cancer worldwide in 2018 and breast cancer accounted for 15% of all the cancer deaths in the females. Breast cancer is more prevalent in western countries but its prevalence is also increasing in the developing world also.[15]

Many studies undertaken in the recent past have demonstrated an inverse association between serum levels of Vitamin D and breast Cancer risk. Several epidemiologic studies have concluded the association of Vitamin D deficiency with increased risk for Breast Cancer. (A). Meta-analysis revealed protective role of high serum Vit D levels and development of Breast Cancer in premenopausal females.[16] The present study was undertaken to estimate and compare serum vitamin D levels in females having breast cancer with normal healthy females.

2. Material and Method

The present study was conducted in the Department of Biochemistry in collaboration with the Department of Surgery in the MM institute of Medical Sciences and research, Mullana, Ambala, Haryana. A matched case control study design was used by enrolling 50 females aged 40 years and above with pathologically and clinically diagnosed Breast Cancer from the Department of surgery as cases while 50 healthy age matched females were taken as control randomly. We excluded patients taking vitamin D treatment, Calcium supplements and those with renal disease. patients taking calcium supplement.

3. Ethical Consideration

Informed and written consent (in the language they best understand) was taken from each subject before collecting data and blood sample. Only those individuals, who volunteered to participate in the study, were included and the data was kept confidential. The study didn't impose any burden on the subjects and the Institute, therefore the study was ethically justified. The proposed study was undertaken after approval by Institutional Ethics Committee.

A detailed history regarding the present and past illness was elicitedfrom the cases and general physical examination, local examination and the systemic examination was done for all and the findings were recorded on a predesigned performa.

Collection of samples: 5 ml of venous blood sample was collected from the antecubital vein of the subjects in dry disposable syringe under aseptic conditions and transferred to a sterile, dry and acid washed vial for biochemical analysis. The blood was allowed to stand for half an hour. After the clot formation, the supernatant was centrifuged to perform the following biochemical investigations.

Serum vitamin D [25(OH) D]: It was assayed by direct competitive chemiluminescence immunoassay (CLIA). The investigation was carried out using Seimens ADVIA Centaur XP Immunoassay System fully-automated machine that is installed in the endocrinology laboratory of the biochemistry department in the hospital of MMIMSR of Mullana, Ambala, Haryana. Reference range for serum Vitamin D is 30 - 100ng/ml and it was interpreted as follows:[17]

Serum Vitamin D levels (ng/ml)	interpretation
<20	deficient
20 -30	insufficient
30-100	sufficient

Statistical Analysis
Data obtained was analysed statistically using SPSS version 20. The t-test and chi square test were applied to the relevant section. A p-value <0.05 was considered statistically significant

4. Results

Fifty female patients aged 40 years and abovewith Breast cancer (newly diagnosed and old cases) presenting to the Department of Surgery at MMIMSR, Mullana were enrolled in the study. Fifty healthy females were enrolled as controls for comparison. Patients group (newly diagnosed and old cases of braest cancer) and control group were similar in regard to ageas depicted in Table 1**.**

In the age group 40-49 years, there were 27 cases and 32 controls, while in the age group 50-59years, there were 13 cases and 8 controls and in the age group of ≥60, there were 10 of both cases and controls.

Table 1: Age wise distribution of cases and control

Age in years	Cases	Controls	total
40-49	27	32	59
50-59	13	8	21
≥60	10	10	20

Further on eliciting the occupational history of the cases and controls, it was seen that the maximum no of cases were among housewives with 8% of cases being in working ladies. The controls were also mainly housewives (82%) while 16% being working as illustrated in Table 2.

Table 2: Occupation of cases and control

Occupation	Cases Number (%)	Control Number (%)	P value
Housewife	46 (92)	42(84)	.218
Working women	4(8)	8(16)	
Total	50	50	

Detailed dietary history of both the cases and controls revealed that maximum number of subjects (75%) were vegetarians as depicted in Table 3.

Table 3: Food habits of cases and control

Food type	Cases Number (%)	Control Number(%)	Total Number(%)	P value
Vegetarian	36 (72)	38 (76)	74 (74)	.208
Non-vegetarian	14 (28)	12 (24)	26 (26)	
Total	50	50	100	

Detailed menstrual history of both the cases and controls was elicited and it revealed that 70% of the cases were menopausal and 52% cases were postmenopausal as illustrated in Table 4

Table 4: Menstrual status of cases and control

Menses	Cases Number(%)	Control Number (%)	Total Number (%)	P value
premenopausal	15 (30)	24 (48)	39 (39)	3.40
menopausal	35 (70)	26 (52)	61 (61)	
Total	50	50	100	

Table 5 represents the levels of Serum Vitamin D in both cases and controls. The maximum number of cases (96%) had their vitamin D levels less than 29.9 ng/ml with 46% having Vitamin D levels<10ng/ml in their serum. The majority of the controls (68%)were having insufficient Vitamin D levels in their serum. Only 16 subjects had normal levels of vitamin D indicating that majority of the enrolled subjects (84%) were deficient in serum vitamin D levels.

Table 5: Vitamin D levels of cases and controls

Vitamin D levels	Cases Number	Controls Number	Total
Severe Deficient(<10ng/ml)	23	2	25
Insufficient (10.1-29.9 ng/ml)	25	34	59
Normal (≥ 30ng/ml)	2	14	16
Total	50	50	100

Table 6 represents the Serum Vitamin D levels in both cases and controls. The serum levels of vitamin D in cases ranged from11.9±7.8 ng/ml while in controls it ranged from 23.0±8.84 ng/ml. The difference of serum vitamin D levels between cases and controls came out to be statistically

significant with p value < 0.001. this further indicates that levels of vitamin D is significantly lower in cases when compared to controls.

Table 6: Vitamin D levels of cases and control.

Vitamin D (ng/ml)	Cases (n=50)	Controls (n=50)	P value
	11.9±7.8	23.0±8.84	<0.001

5. Discussion

Many studies in literature have demonstrated an association between low serum levels of Vitamin D and increased risk for development of breast cancer, recurrence of breast cancer and increased mortality. Protective relationship of high levels of serum Vitamin D on development of breast Cancer in premenopausal women has been established in a meta-analysis by N. Estebanezet al.[16] Garland et al in their study on role of vitamin D for cancer prevention and concluded that maintaining a minimum year-around serum vitamin D level of 40 to 60 ng/mL would prevent approximately 58,000 new cases of breast cancer per year.[18]Further Knight et al in 2007 did a case-control study performed vitamin D measurements at three specified ages and concluded that vitamin D confers some protection from breast cancer.[19] Lopes et al, in 2012 did a review study on vitamin D and the mammary gland. They provided the evidence of an essential role of vitamin D in normal development of the mammary gland and breast cancer.[20] In the present study, we compared the levels of Serum Vitamin D in females with and without breast Cancer and observed that vitamin D levels of the cases ranged from 11.9±7.8 ng/ml while that in controls ranged from 23.05±8.8 ng/ml. The normal value of the serum vitamin D is taken as 30-100ng/ml. Based on the above findings, we observed that the levels of vitamin D levels were significantly lower in patients of breast cancer. Further we found that all the 50 patients with breast cancer enrolled as cases had low vitamin D levels except one. Though the levels of vitamin D in controls was also found to be low but it was substantially higher than the cases. Our study revealed that vitamin D levels is generally low in general population of North India despite having abundant sunlight throughout the year. This may be related to the dietary pattern of the people or due to clothing pattern. Our findings are in tune with the observations by Yousef et al wherein they performed a case-control study on 120 breast cancer cases and 120 controls and concluded that breast cancer cases had significantly lower serum concentrations of vitamin D than controls.[21]In a prospective study by Y Kimand Y Jee on the role of vitamin D in breast cancer patients; high vitamin D status was found to be weakly associated with low breast cancer risk but strongly associated with better breast cancer survival.[22]Similarly,Simon B. Zeichner et al in 2015 in a retrospective study on vitamin D supplementation during adjuvant chemotherapy in patients with HER2 non-metastatic breast cancerfound that vitamin D supplementation had a better outcome than the control group.[23]In a recent meta-analysis by Elizabeth T. Jacobs et al it was shown that vitamin D levels and breast cancer incidence had an inverse relationship.[24]

Similar findings were also demonstrated by Noureen Shaukat et al wherein the relationship between serum vitamin D levels and breast cancer risk was evaluated ina case control study enrolling 42 newly diagnosed breast cancer patients and 52 age matched female controls and it was concuded that though serum vitamin D levels were low both in cases and controls but the serum vitamin D levels were less than 20ng/ml in 86% of the cases and in 56% of the controls.[25]

Katie O'Briens et al published a cohort study in 2017 showing that females having vitamin D supplements had 11% lesser chances of having breast cancer and concluded that vitamin D supplementation can reduce the incidence of breast cancer in the general population.[26]Further Manar Atoum and Foad Alzoughool in their study done in 2017 found an inverse relationship between breast cancer and serum vitamin D levels.[27] Jemal Hussein Ahmed et al in their study done in 2018 in Ethiopia highlighted the importance of vitamin D in breast cancer patients undergoing chemotherapy.[28]

As reviewed by various authors, vitamin D is very important for maintaining the well-being of the human body. Healthy dietary pattern should be promoted to increase its availability from the food sources. The clothing pattern may need to be changed so as to permit some hours of sun exposure to the skin allowing endogenous vitamin D synthesis to occur.

6. Summary and Conclusion

In conclusion, our results confirmed that vitamin D deficiencies are common among North Indian females. Further, the serum Vitamin D levels in females with breast cancer was found to be significantly lower than the serum Vitamin D levels in age matched healthy females. It is now known that vitamin D has various roles in maintaining proper bodily and immune functions, so maintaining the proper vitamin D should be encouraged in the general population. Vitamin D also has beneficial role in the survival of breast cancer patients so vitamin D levels must be augmented in the patients of breast cancer.

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