Alteration of Vitamin D and Vitamin D Signaling Pathway in Breast Cancer: A Preliminary Study from Western India

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Summary

Recent evidences suggest protective mechanism of vitamin D against breast cancer by autocrine/paracrine manner and may modestly reduce risk of breast cancer. It also plays an important role in apoptosis, cell cycle regulation and metastasis. Therefore, the present study aimed to study vitamin D and its derivatives in breast cancer development.

Total 88 subjects including 51 breast cancer patients and 37 healthy individuals were enrolled for the study. Serum 25(OH) D levels were measured by HPLC. The transcript levels of CYP27B1, VDR and CYP24A1 were investigated in malignant tissues and adjacent normal tissues by RT-PCR. Statistical analysis was carried out using SPSS software. 'p' value <0.05 was consider as a statistical significant.

In the study, serum 25(OH) D was lower in breast cancer patients as compared to the controls. Based on serum 25 (OH) D levels, odds ratio analysis showed increased risk of breast cancer from mild to moderate to severe vitamin D deficiency. RT-PCR analysis showed that mRNA expression of CYP27B1 was lower whereas CYP24A1 and VDR were higher in malignant tissues as compared to adjacent normal tissues. ROC curve analysis for VDR suggested significant difference between malignant tissues and adjacent normal tissues. Multivariate analysis revealed that CYP24A1 was significantly associated with various clinicopathological parameters like menopausal status, stage, molecular subtypes, ER and HER2 receptors.

Best of our knowledge, this is the first Indian study in relation with vitamin D signaling pathway and breast cancer. It suggests that lower levels of 25(OH) D may be associated with breast cancer risk. Altered expression of VDR suggests its role in breast carcinogenesis. The data warrant in depth analysis with large number of sample to stamp influential role of vitamin D and signaling molecules in breast carcinogenesis.

Keywords: Breast cancer, CYP24A1, CYP27B1, 25 Hydroxyvitamin D [25(OH) D], VDR, Vitamin D signaling pathway

Introduction

Breast cancer ranks first among all other cancer with an incidence rate of 25.2% worldwide in case of female. Collectively, India accounts for almost one third of the global breast cancer burden which is 27.0%.[1] At The Gujarat Cancer and Research Institute (GCRI) which is the regional cancer center for western part of India, breast cancer emerged as major female health hazard. According to the population based registry of GCRI, out of 40% of female cancer cases registered, Various etiolological factors are associated with breast cancer including genetic factors, lifestyle, and diet.[2] Recently, it has been suggested that there is protective mechanism of vitamin D against breast cancer by autocrine/paracrine manner and various studies suggested that it may modestly reduced risk of breast cancer.[3,4] In autocrine/paracrine mechanism breast epithelium produces 1α 25(OH) 2D3 from the circulatory 25(OH) D with the help of anabolic enzyme 1α-OHase encoded by CYP27B1 gene. 1α 25(OH)2D3 is the biologically active metabolite and relatively small, lipophilic molecule that can easily penetrates by simple cell diffusion in the cell membrane and binds to the vitamin D receptor (VDR), thereby causing its dimerization with the retinoid X receptor (RXR) and its translocation to the nucleus. The ligand-bound 1α 25(OH)2D3-VDR–RXR complex binds to vitamin D response elements (VDREs) in multiple regulatory regions located in the promoters of target genes and this causes the recruitment of co-activators or co-repressors, which leads to positive or negative transcriptional regulation of gene expression. These target genes are involved in diverse molecular pathways, thereby resulting in a wide range of 1α 25(OH) 2D3 mediated anticancer actions in an autocrine/paracrine manner. Degradation of unneeded 1α 25(OH) 2D3 is accomplished by the catabolic enzyme 24 Hydroxylase (24-OHase) encoded by CYP24A1 gene for regulation of 1α 25(OH) 2D3 synthesis. In addition, 1α-OHase (CYP27B1) and 24-OHase (CYP24A1) also plays an important role in the vitamin D metabolic cascade.[5] Thus, alterations in vitamin D receptor and its associated anabolic enzyme CYP27B1 as well as catabolic enzyme CYP24A1 are important for maintenance of circulatory 25-hydroxyvitamin D levels, thus mRNA expression of CYP27B1, VDR and CYP24A1 as well as circulatory 25(OH) D plays crucial role in development of breast

cancer. However there is dearth of data from India, regarding circulating 25(OH) D levels and signaling molecules in breast cancer. Therefore, the aim of the present study was to evaluate role of circulating 25(OH) D levels and its associated genes involved in vitamin D signaling in breast cancer.

Materials and Methodology

Subjects

The study was approved by Institutional Review Board (IRB) and Institutional ethics committee of GCRI. Total 51 female breast cancer patients and 37 female controls were enrolled. Due consent was taken from all the subjects prior to enrollment in the study. Histopathologically confirmed breast cancer patients prior to any anti-cancer treatment were selected for present study. Moreover, any other illness as well as breast cancer patients supplemented with vitamin D or multivitamins were excluded from the present study. Premenopausal, postmenopausal and perimenopausal groups were divided according to subject questionnaires.

Blood and tissue collection and processing

Blood samples were collected into plain vials. Serum was separated and stored at -80^{0}C until analyzed. Malignant and adjacent normal tissues were collected after surgical resection. Adjacent normal tissues were selected from the remaining tumor free margins (at least 1-2 cm away) as defined by the pathologist and it was epithelial containing tissues (Fat tissues were avoided). The tissues were washed with ice-cold phosphate buffer saline (pH-7.4) and immediately stored at -80^{0}C in RNA stabilizing reagent. If sample contain small amount of fat tissues lipid was removed using lipid removal kit.

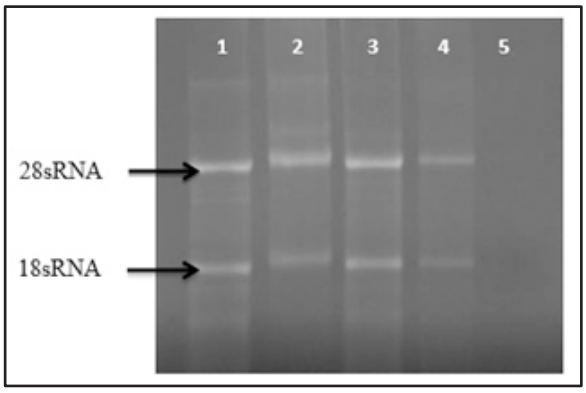

Figure 1: Separation of 18s RNA and 28s RNA on agarose gel electrophoresis. Lanes 1 and 3 shows intact bands of RNA from malignant tissues and lanes 2 and 4 shows intact bands of RNA from adjacent normal tissues. While lane 5 depicts negative control (NC)

Methodology

Circulatory 25(OH) D Levels by high performance liquid chromatography (HPLC)

Serum 25(OH) D levels were carried out by HPLC using recipe circulatory 25-hydroxy vitamin D2/D3 kit from Germany. Calibrator was used as a standard (component of kit), It is lyophilized pooled calf serum containing 25(OH) D3 and 25 (OH) D2 concentrations. Moreover, the mean values of calibrator are traceable to NIST-SRM972a (National Institute of Standard and Technology-Standard Reference Material 927a) (vitamin D metabolites in frozen human serum). According to manufacturer's instruction 25(OH) D levels were categorized into four type; severe deficiency (< 5µg/l), moderate deficiency (5-10µg/l), mild deficiency (10-20µg/l) and sufficiency (20-70µg/l).

Transcript Levels of CYP27B1, VDR and CYP24A1 by reverse transcriptase polymerase chain reaction (RT-PCR)

RNA isolation was done by RNAeasy mini kit from Qaigen, USA according to manufacturer's instructions. RNA integrity was carried out to check quality of RNA on 1% agarose gel electrophoresis. Figure 1 depicts 18s ribosomal RNA and 28s ribosomal RNA after separation of isolated RNA samples from malignant and adjacent normal tissues. RT-PCR was carried out using one-step RT-PCR kit (Qiagen, USA) for mRNA expression. 500ng of RNA was used for mRNA expression of CYP27B1, VDR and CYP24A1. PCR products were run on 1.5% agarose gel electrophoresis and bands were visualized under gel documentation system (Alpha Inotech Inc. USA) and quantified by integrated density values

Table 1: Primers used for mRNA expression of CYP27B1, VDR, CYP24A1 and 28sRNA

No	Parameter	Primer sequences	Reference
1.	CYP27B1	5'-GCTACACGAGCTGCAGGTGCAGGG -3'	Segersten et al 2005[6]
		5'-AGCGGGGCCAGGAGACTGCGGAGC -3'	
2.	VDR	5'-TGCCTGACCCTGGAGACTTTGACC -3'	
		5'-CATCATGCCGATGTCCACACAGCG -3'	
3.	CYP24A1	5'-GGCTTCTCCAGAAGAATGTAGGGGATGAAG -3'	
		5'-TGAGGCTCTTGTGCAGCTCGACTGGAG -3'	
4.	28 sRNA (HKG)	5'-GTTCACCCACTAATAGGGAACGTG-3'	
		5'-CATCATGCCGATGTCCACACAGCG -3'	

(IDV). 28s RNA was used as a house keeping gene (HKG). The primers were selected according to segersten et al.[6] The list of primers used for CYP27B1, VDR and CYP24A1 as well as 28 sRNA (HKG) for mRNA expressions is listed in Table 1, In which, gene bank number for CYP27B1 was AB006987, for CYP24A1 was NM_000782, for VDR was NM_000376.

Statistical analysis

Statistical analysis was carried out using SPSS statistical software (version 15.0; SPSS Inc., Chicago, IL, USA). Student's independent't' test was performed to assess the level of significance for circulatory 25(OH) D. Student's paired 't' test was used to compare the mRNA expression of CYP27B1, VDR and CYP24A1 between adjacent normal and malignant tissues of the breast cancer patients. Multivariate analysis was performed to correlate the markers like CYP27B1, VDR and CYP24A1 with various clinicopathological parameters. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for the risk estimation of breast cancer development in relation to 25(OH) D levels. Receiver's operating characteristic (ROC) curve was constructed to evaluate discriminatory efficacy of the circulatory 25(OH) D levels, CYP27B1, VDR and CYP24A1. The ideal cutoff was determined from multiple points on the ROC curve that resembled the mean value in the control group. Power analysis was performed for the study and it was greater than 80%. Moreover, effect size was also performed using z statistic test. The values were expressed as the mean ± Standard error of mean (SEM). 'P' value ≤ 0.05 was considered as statistically significant.

Results

Socio-demographical details of the subjects are depicted in Table 2. Age and menopause matched cases and controls were included in the present study. The age range of breast cancer patients was between 31-70 years, whereas the age range of controls was between 25-68 years. Most of the subjects were postmenopausal (49.1%) followed by premenopausal (43.1%) and perimenopausal (7.8%). Moreover most of the subjects were without family history of cancer.

Clinicopathological details of the breast cancer patients

As per Table 3, Pathological tumor, Node and Metastasis (pTNM) staging of breast cancer patients were determined as per American Joint Committee on Cancer (AJCC). Breast cancer patients also

Table 2: Details of breast cancer patients and controls

Characteristics of breast cancer patients		Characteristics of controls	
No. of breast cancer patients	**51 (100%)**	**No. of healthy individuals**	**37(100%)**
Age (Years)			
Mean	47	Mean	45
Median	46	Median	46
Range	31-70	Range	25-68
Menopausal status			
Premenopausal	22(43.1%)	Premenopausal	15(40.5%)
Perimenopausal	4 (7.8%)	Perimenopausal	6(16.2%)
Postmenopausal	25 (49.1%)	Postmenopausal	16(43.3%)
Familial history			
Yes	2 (3.9%)	Yes	1(2.7%)
No	49 (96.1%)	No	36(97.3%)

Table 3: Clinicopathological details of the breast cancer patients

Characteristics	N (%)
Diagnosis	
IDC	49(96.1%)
Others	2(3.9%)
Stage	
Early	23(45.1%)
Advance	20(39.2%)
Undefined	8(15.7%)
Lymph Node (LN) involvement	
LN positive	20(39.2%)
LN negative	12(23.5%)
Undefined	19(37.3%)
Estrogen receptor	
Positive	29(56.9%)
Negative	19(37.2%)
Undefined	3(5.9%)
Progesterone receptor	
Positive	25(49.0%)
Negative	23(45.1%)
Undefined	3(5.9%)
HER2	
Positive	26(50.9%)
Negative	16(31.4%)
Undefined	9(17.7%)
Molecular subtypes	
Luminal A	10(19.6%)
Luminal B	20(39.2%)
TNBC	9(17.6%)
HER2 enriched	3(6.0%)
Undefined	9(17.6%)

categorized according to hormone receptor status i.e. ER, PR and HER2 as well as molecular subtypes. Most of the breast cancer patients showed invasive ducal type of cancer (96.1%). 39.2% breast cancer patients had advanced breast cancer and 45.1% breast cancer patients had early stage of the disease. 39.2% cases were with lymph node (LN) involvement. Majority of patients were with luminal B subtype i.e. 39.2% followed by luminal A (19.6%), triple negative breast cancer (17.6%) and HER2 enriched (6.0%).

Comparison of circulatory 25(OH) D levels between breast cancer patients and controls

Figure 2 depicts mean levels of serum 25(OH) D in breast cancer patients and controls. It revealed that mean levels of 25(OH) D were 21.68µg/l or ng/ml and 19.86µg/l or ng/ml in controls and breast cancer patients respectively. Thus, serum 25(OH) D levels were lower in breast cancer patients as compared to the controls. However, the 25(OH) D levels were not statistically significant (p=0.57).

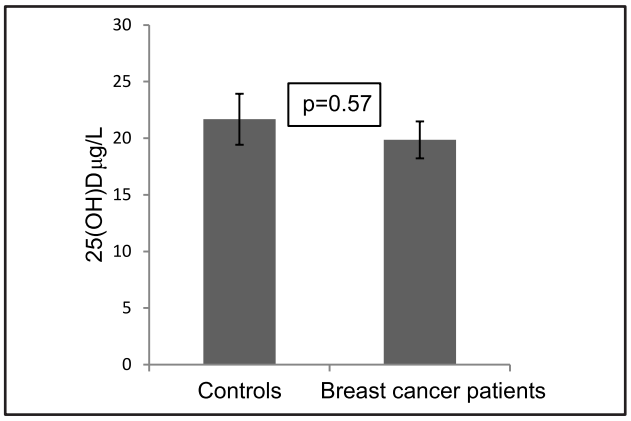

Figure 2: Mean levels of serum 25(OH) D in breast cancer patients and controls

Prevalence of 25(OH) D deficiency and sufficiency among subjects according to category

According to Figure 3, the prevalence of severe25 (OH)Ddeficiency was almost five fold higher in breast cancer patients as compared to controls (11.8% in breast cancer and 2.7 % among controls). Moreover, the prevalence of moderate 25(OH)D deficiency was also higher in breast cancer patients (17.6%) as compared to controls (13.6%).

Risk assessment of breast cancer by 25(OH) D levels

Table 4 shows association between 25(OH) D levels and breast cancer risk. Increased trend for odds ratio was observed from mild 25(OH) D deficiency (OR=0.67, 95% CI=0.25 to 1.79, p=0.43) to moderate 25(OH) D deficiency (OR= 1.30, 95% CI=0.36 to 4.65, p= 0.67) to severe 25(OH) D deficiency (OR=4.36, 95% CI= 0.47 to 39.89, p= 0.19). Further, odds ratio analysis for 25(OH)D levels in menopausal status showed that, the odds ratio was increased in post-menopausal breast cancer patients (OR=1.16, 95% CI=0.32 to 4.15, p=0.81) compared to pre (OR=0.96, 95% CI=0.25 to 3.66, p=0.95) and peri menopausal breast cancer patients (OR=0.33, 95% CI=0.02 to 5.32, p=0.43). However, there was no statistical significant difference was observed (Table 5). Sufficient Vitamin D levels were considered as a referent group for risk assessment.

Table 4: Odds ratio analysis for serum 25(OH) D levels and breast cancer

Circulatory 25(OH) D levels	Odds ratio	95% CI	p value
Total 25(OH) D deficiency	1.00	0.42 to 2.36	p= 0.99
Severe 25(OH) D deficiency	4.36	0.47 to 39.89	p= 0.19
Moderate 25(OH) D deficiency	1.30	0.36 to 4.65	p= 0.67
Mild 25(OH) D deficiency	0.67	0.25 to 1.79	p=0.43

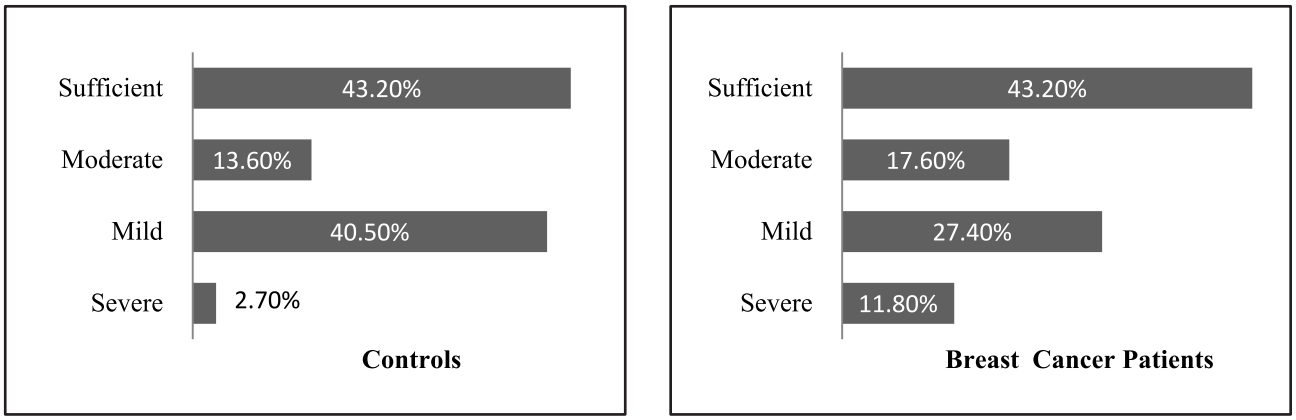

Figure 3: Prevalence of severe, moderate, mild 25(OH) D deficiency and 25(OH) D sufficiency among controls and breast cancer patients

Receiver operating characteristic (ROC) curve analysis for circulatory 25(OH) D levels

ROC curve plotted using SPSS version 15 and it is a meaningful statistical approach as it considers both sensitivity and specificity of the parameters. ROC curve was constructed for serum 25 (OH) D levels to evaluate their discriminatory efficiency between controls and breast cancer patients. Figure 4 shows ROC curve analysis for serum 25(OH)D levels, in which there was no significant difference have been found between breast cancer patients and controls (p=0.66).

Table 5: Odds ratio analysis for serum 25(OH) D levels and menopausal status of breast cancer patients

Menopausal status	Odds ratio	95% CI	p value
Premenopausal	0.96	0.25 to 3.66	p= 0.95
Perimenopausal	0.33	0.02 to 5.32	p= 0.43
Postmenopausal	1.16	0.32 to 4.15	p= 0.81

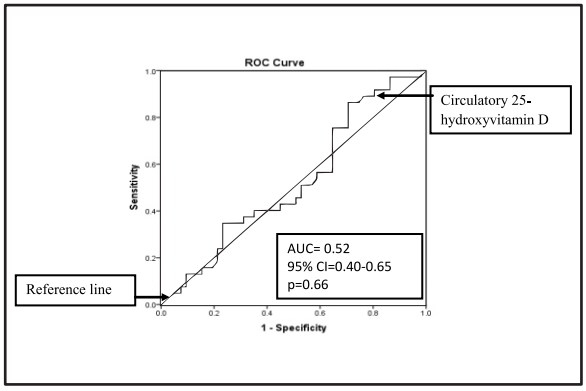

Figure 4: ROC curve analysis for 25(OH) D

Transcript levels of CYP27B1, VDR and CYP24A1 in malignant and adjacent normal tissues

Figures 5-7 show representative pattern of CYP27B1, VDR and CYP24A1, respectively. Mean integrated density value (IDV) of CYP27B1 (0.20 adjacent normal and 0.13 for malignant tissues), VDR (0.44 adjacent normal and 1.01 for malignant tissues) andCYP24A1 (0.44 adjacent normal and 0.64 for malignant tissues) in malignant tissues and adjacent normal tissues, in which the mean IDV values of CYP27B1 was lower in malignant tissues as compared to adjacent normal tissues (p=0.26). However, the difference was not statistical. Mean IDV of VDR and CYP24A1 was higher in malignant tissues as compared to adjacent normal tissues (p=0.04 and p=0.18).

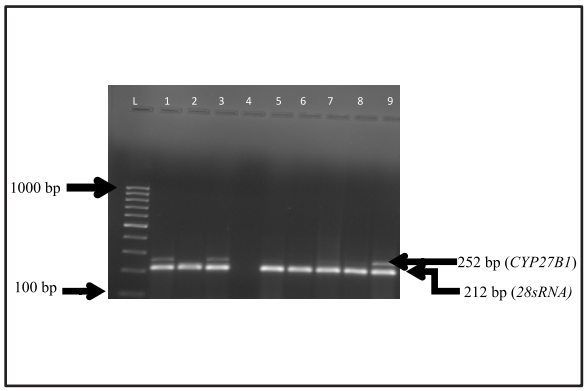

Figure 5: Representative pattern of CYP27B1 expression in malignant and adjacent normal tissues. Lanes 2, 6 and 8 represent the amplicon pairs of CYP27B1 and 28sRNA from malignant tissues, whereas lanes 1, 3, 5, 7 and 9 represents the amplicon pairs of CYP27B1 (252bp) and 28sRNA (212bp) from adjacent normal tissues. Lane L represents DNA Ladder (100-1000 bp) and Lane 4 represents negative control

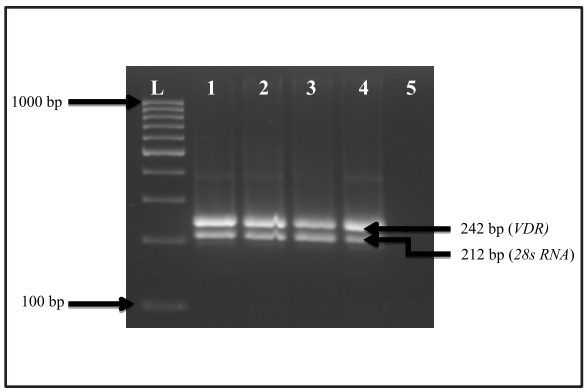

Figure 6: Representative pattern of VDR expression in malignant and adjacent normal tissues. Lane L shows 100bp ladder, lane 1 and lane 3 shows m RNA expression of VDR gene (242bp) as well as housekeeping gene 28s RNA (212bp) from malignant breast tissues, lane 2 and lane 4 shows m RNA expression of VDR gene as well as housekeeping gene 28s RNA from adjacent normal breast tissues, lane 5 shows negative control

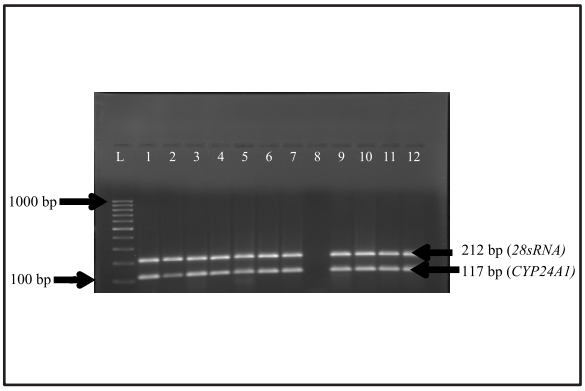

Figure 7: Representative pattern of CYP24A1 expression in malignant and adjacent normal tissues. Lanes 1, 3, 5, 7, 9 and 11 represents the amplicon pairs of CYP24A1 (117bp) and 28sRNA (212bp) from malignant tissues, whereas lanes 2, 4, 6, 10 and 12 represents the amplicon pairs of CYP24A1 and 28sRNA from adjacent normal tissues. Lane L represents DNA Ladder (100-1000 bp) and Lane 8 represents negative control

ROC curve analysis for CYP27B1, VDR and CYP24A1 in malignant and adjacent normal tissues

Table 6 depicts area under curve (AUC) and 95% Confidence interval (CI) for CYP27B1, VDR and CYP24A1. Among all transcript levels, VDR could significantly discriminate between malignant tissues and adjacent normal tissues (p=0.02) (Figure 8).

Table 6: Area under curve (AUC) for CYP27B1, VDR and CYP24A1

Parameter	Area	95% CI	p value
CYP27B1	0.43	0.11-0.75	p=0.67
VDR	0.82	0.62-1.00	p=0.02
CYP24A1	0.64	0.37-0.91	p=0.29

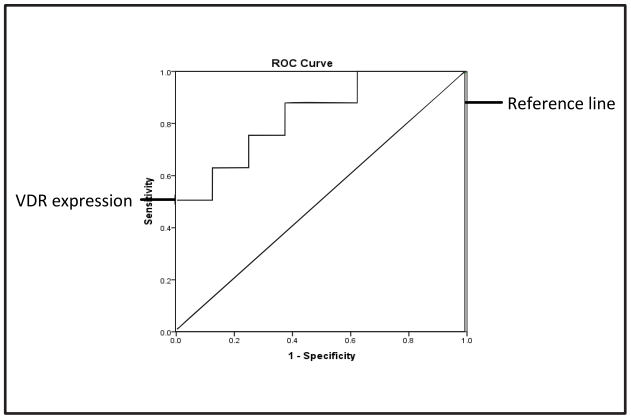

Figure 8: Reservoirs operating curve for VDRexpression between adjacent normal and malignant tissues

Multivariate analysis for CYP27B1, VDR and CYP24A1 transcripts levels with various clinicopathological parameters

The mRNA expression of CYP27B1, VDR and CYP24A1 were compared with menopausal status, age, molecular subtypes and various hormone receptor status (ER, PR, HER2). According to Table 7, F is factor and it is determined by degrees of freedom. Moreover, the test of main effect and the interaction effect are calculated by dividing the calculated variances by the variance (clinicopathological parameters) within the groups. However, for levels of significance we had considered p values. According to Table 7, CYP24A1 was associated with various clinicopathological parameter like menopausal status, stage, molecular subtype, ER and HER2 receptors (p=0.07, p=0.06, p=0.04, p=0.05 and p=0.03 respectively).

Table 7: Comparison of CYP27B1, VDR and CYP24A1with clinico-pathological parameters using multivariate analysis

Parameter	CYP27B1	VDR	CYP24A1
Menopausal status	F=0.43 p=0.62	F=0.74 p=0.54	F=66.76 p=0.07
Stage	F=1.82 p=0.40	F=2.02 p=0.39	F=99.21 p=0.06
Age	F=0.80 p=0.53	F=0.59 p=0.58	F=2.08 p=0.38
Molecular subtypes	F=0.03 p=0.88	F=1.19 p=0.47	F=193.32 p=0.04
Her2 Receptor	F=0.58 p=0.58	F=1.43 p=0.44	F=115.57 p=0.05
Progesterone Receptor	F=0.002 p=0.97	F=1.14 p=0.47	F=22.81 p=0.13
Estrogen Receptor	F=0.62 p=0.57	F=0.95 p=0.50	F=367.57 p=0.03

Discussion

Vitamin D, specifically in autocrine/ paracrine manner showed tremendous capacity to modulate important cancer features like regulation of proliferation and differentiation, angiogenesis, invasion, metastasis and enhance apoptosis suggesting its importance in cancer.[3,5,7, 21] In Indian population, there are very few studies which showed a relation between vitamin D levels and cancer.[8] Although there are no study documented regarding vitamin D and breast cancer especially molecular basis of vitamin D or genes in involved vitamin D signaling and breast cancer. Hence, the present study was concentrated to elucidate role of circulatory 25-hydroxyvitamin D, CYP27B1, VDR and CYP24A1 in breast cancer. To the best of our knowledge, this is first Indian study that showed vitamin D levels and its signaling molecules in breast cancer.

Various methods are available for estimation of 25-hydroxyvitamin D such as ELISA, RIA, LC-MS etc. However, determination of circulatory 25-hydroxyvitamin D by HPLC with UV detection can be considered the gold standard method.[9] In the present study, mean levels of circulatory 25-hydroxyvitamin D was lower in breast cancer patients as compared to the controls. Furthermore, odds ratio was increased in severe 25-hydroxyvitamin D deficiency followed by moderate and mild deficiency as compared to sufficient vitamin D levels, indicating that the risk of breast cancer increases when move from mild to

moderate to severe vitamin D deficiency. A study conducted by su x et al have shown that an inverse association was observed between proliferative benign breast disorders and amounts of vitamin D consumption.[10] In accordance with our results, two studies from India showed lower vitamin D levels in pediatric cancer and ovarian cancer patients compared controls.[8,11] Similarly, In Iran and other Middle East countries, the prevalence of vitamin D deficiency has been observed in approximately 30- 80% of breast cancer patients.[12] In another study by Rossi et al showed that circulatory 25-hydroxyvitamin D levels were significantly lower in patients than controls.[13] Shamsi et al and other studieshas also observed protective effect of vitamin D against breast cancer.[14-17] Moreover, in our study, we have also found that breast cancer risk was increased in post menopausal breast cancer patients as compared to pre and peri menopausal women. Contradictory, a significant association was demonstrated only in premenopausal and perimenopausal cases by Chlebowski.[18] Moreover, Bener and El Ayoubi found a high frequency of vitamin D deficiency in 635 postmenopausal breast cancer patients as compared to the pre and peri menopausal breast cancer patients.[19] Various studies have shown that responses to steroid hormones are modulated by crucial "pre-receptor" mechanisms involving tissue-specific activation or inactivation via locally expressed steroidogenic enzymes.[20] The enzymes 1α hydroxylase encoded by the gene CYP27B1 and 24-hydroxylase encoded by the gene CYP24A1 are important in vitamin D signaling pathway. CYP27B1 is responsible for the synthesis of the biologically active form of vitamin D (1, 25-dihydroxyvitamin D), whereas CYP24A1 mediates the catabolism of vitamin D.[21] In our study we found decreased mRNA expression of CYP27B1 in malignant breast tissues as compared to the adjacent normal tissues. Similarly various studies have demonstrated that CYP27B1 mRNA expression in breast tumors was decreased in comparison with normal mammary tissue.[22] It is speculated that tumors secrete endocrine/paracrine factors, which influence CYP27B1 expression, however other studies suggested that down regulation of CYP27B1 is caused by hypermethylation of its promoter.[23]

Present study demonstrated an increase CYP24A1 mRNA expression in breast cancer tissues as compared to adjacent normal tissues. Similarly, Segersten et al also showed that CYP24A1 mRNA expression was overall 2-fold higher in breast carcinoma as compared to normal tissues.[6] According to, Chen et al high CYP24A1 transcript levels seem to be a common feature of several solid tumors.[24] Thus, from the data we can presume that lower expression of CYP27B1 and higher expression of CYP24A1 resultant into alteration in optimal 25(OH) D levels.

In present study, mRNA expression of VDR was increased in malignant tissues as compared to the adjacent normal tissues. Moreover, ROC curve analysis revealed that VDR mRNA expression could significantly distinguish between malignant and adjacent normal tissues. VDR is expressed in the mammary gland and vitamin D has been shown to display anti-carcinogenic properties, this hormone has emerged as a promising targeted therapy. But in order to keep the homeostasis of the organism the amount of circulating vitamin D has to be tightly regulated. Some studies have demonstrated that the VDR protein is expressed in samples from normal breast tissues and also in breast cancer biopsy specimens.[6,25]

In our study, multivariate analysis results indicated that CYP24A1 expression was associated with menopausal status, stage, molecular subtype, ER and HER2 receptors. According to Albertson et al elevated tumor CYP24A1 expression is associated with a poorer prognosis of breast tumors and analysis of the data sets from the cancer genome atlas confirms that a subset of human breast cancers (10–13%) exhibit alterations in the CYP24 gene, with the most frequent changes being amplifications and up regulation at the mRNA level.[26] De Lyra et al has showed there were no differences in the expression of the CYP27B1, VDR and CYP24A1 mRNA in breast cancer and non-neoplastic mammary tissue.[27] Elevated as well as decreased CYP24A1 or CYP27B1 expressions are reported in different cancer cell lines.[28] Moreover, studies on human cancer biopsies agree with the hypothesis that the expression of VDR and CYP27B1 increases initially when a tumor develops, but while the tumor becomes more malignant and starts to dedifferentiate, the expression of VDR and CYP27B1 decreases while the expression of CYP24A1 strongly increases in human tissues of breast cancer and colorectal cancer.[29] This suggests that during early tumorigenesis the synthesis and signaling of 1,25(OH)2D3 are upregulated as a physiological defense system against epithelial tumor progression. When tumors dedifferentiate, VDR and

CYP27B1 levels drop while CYP24A1 expression increases, implicating that local 1,25(OH)2D3 concentrations decrease since less 1,25(OH)2D3 is synthesized while more is metabolized. The sequential acquisition of mutations that occur during tumor progression and metastasis could possibly negatively influence the expression of 1,25(OH)2D3-metabolizing enzymes.[30] Collectively these three genes i.e. CYP27B1, VDR and CYP24A1 are inter-correlated and play important role in vitamin D signaling pathway and ultimately play crucial role in development of breast cancer. The limitation of the study is small sample size though it is preliminary study. However, it is useful for public awareness and women health specially in Indian population. Moreover, the strength of the study is correlation of Vitamin D deficiency with various clinico pathological parameters such as correlation of vitamin deficiency with molecular subtypes of breast cancer.

Conclusion

Our findings suggest that low serum levels of 25(OH) D may be associated with an increased risk of breast cancer. Apart from that circulatory 25-hydroxy vitamin D deficiency is also associated with increased risk of breast cancer particularly, in postmenopausal women. Increased expression of VDR and CYP24A1 in malignant tissues suggests its role in breast cancer pathogenesis. The decreased expression of CYP27B1 in malignant tissues may be important in their predisposition to the development of breast cancer. Hence, vitamin D and its derivatives can evidently influence tumorigenesis and /or facilitate tumor progression.

Conflict of Interest: None

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