Vitamin D Receptor in Breast Cancer Tissues and Its Relation to Estrogen Receptor Alpha (ER-α) Gene Expression and Serum 25-hydroxyvitamin D Levels in Egyptian Breast Cancer Patients: A Case-control Study

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Abstract

This case-control study was performed on 40 patients with breast cancer and 40 controls to investigate the role of vitamin D receptor in breast cancer tissues and its relation to estrogen receptor-α gene expression in the same tissues and serum 25-hydroxyvitamin D. Females with decreased levels of serum 25-hydroxyvitamin D, increased tissues levels of vitamin D receptor and increased tissue expression of estrogen receptor-α gene expression had significantly increased risk for breast cancer incidence.

Introduction: This study aimed to explore the role of vitamin D receptor (VDR) in breast cancer tissues and its relation to serum 25-hydroxyvitamin D [25(OH)D] levels and estrogen receptor alpha (ER-α) gene expression in patients with breast cancer. **Patients and Methods:** Cancerous and normal breast tissues from 40 women with breast cancer were analyzed for quantification of VDR levels and ER-α gene expression. The serum levels of 25(OH)D were measured in patients with breast cancer and controls by radioimmunoassay. **Results:** Patients with breast cancer had serum levels of 25(OH)D significantly lower than normal control subjects. The levels of VDR and ER-α were significantly higher in breast cancer tissues than in normal breast tissues. The serum levels of 25(OH)D were indirectly and significantly correlated with the tissue levels of both VDR and ER-α gene expression. There was a significant direct correlation between the tissue levels of VDR and ER-α gene expression. The serum 25(OH) D levels, tissue VDR levels, and ER-α gene expression levels were inversely and significantly correlated with breast cancer histopathologic grade. Women with serum 25(OH)D levels $\leq$ 30 nmol/L, tissue levels of VDR $>$ 5 ng/mL, and tissue levels of ER-α gene expression $>$ 17.7 copies had significantly increased risk for breast cancer incidence. **Conclusion:** Women with low serum 25(OH)D levels, high tissue levels of VDR, and ER-α gene expression had increased risk for breast cancer. VDR are upregulated in breast cancer tissues thus it may be used for target therapy especially in hormone-negative breast cancer.

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Introduction

Breast and ovarian cancers, which largely, if not exclusively, affect women, caused significantly more deaths in 2015 than in 2005.[1] The rates of breast cancer around the world are greatly variable. In general, developed countries have higher rates than developing countries. Although all the reasons for these differences are not known yet, lifestyle and reproductive factors likely play a large role.[2]

In Egypt, breast cancer was estimated to be the most common cancer among women; it was also the leading cause of cancer-related

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mortality.[3] This was confirmed in many regional Egyptian cancer registries[4,5] and in hospital-based frequencies.[3]

Menopausal status and age as risk factors for breast cancer were thought to affect breast carcinogenesis by increasing the exposure of the breast to estrogens and other sex hormones. Because the effects of estrogens are mediated by the estrogen receptors (ERs), the magnitude of their effects may be determined by the level of ERs expressed in the breast, especially ER alpha (ER-α).[6]

Dandamudi et al[7] conducted a systematic review on the association between the dietary patterns and breast cancer risk. They concluded that diets containing vegetables, fruits, legumes, lean protein, and whole grains may lower breast cancer risk. However, those rich in saturated fats, red and processed meats, added sugars, fried foods, and refined grains are promoters for breast cancer. Narvaez et al[8] reported the mechanism by which dietary vitamin D might prevent the development and growth of breast cancer. In the past decade, vitamin D has been extensively studied as possible therapeutic alternative or adjuvant in cancer management. It was found that 25-hydroxyvitamin D [25(OH)D] is the major circulating and storage form of vitamin D that has a half-time of about 2 to 3 weeks.[9]

Epidemiologic studies of the association between 25(OH)D and breast cancer risk are limited and conflicting.[10] Among observational studies, case-control studies always find significant inverse correlations between 25(OH)D concentrations and breast cancer incidence. By contrast, prospective studies with long follow-up times generally fail to find significant inverse correlations between 25(OH)D concentrations and breast cancer incidence because breast cancer develops rapidly, so a single 25(OH)D concentration measurement rapidly loses predictive ability.

In 1969, the nuclear vitamin D receptor (VDR) was discovered. The role of VDR in the endocrine system and its presence and function in over 30 tissues and organs has been examined.[11] It has been found in nearly all tissues and organs in the human body.[12,13]

VDR, in addition to its pivotal role in regulation of calcium metabolism, bone mineralisation, calcium homeostasis and its related disorders, also regulates differentiation and division of various cell types. It has been suggested that vitamin D through VDR may play a role in many other physiological and pathophysiological processes including certain cancers.[14]

The aim of the current study was to explore the role of VDR in breast cancer tissues and its relation to ER-α gene expression and serum 25(OH)D levels in Egyptian patients with breast cancer.

Patients and Methods

Patients

Eighty females were included in this study and divided into 2 groups: Group I included 40 patients with recently diagnosed invasive ductal carcinoma of the breast of clinical stages II and III.[15] Patients were recruited from the Experimental and Clinical Surgery Department and Cancer Management and Research Department, Medical Research Institute, University of Alexandria, Egypt, in the period from May 2015 to October 2016. Group II included 40 normal healthy control females of matched age and menstrual and socioeconomic status with the group of patients with breast cancer. Controls were selected from female workers at the Medical Research Institute who underwent routine checkup including mammography; all controls were negative for breast cancer.

After having approval from the ethical committee, Medical Research Institute, Alexandria University, Egypt, signed informed consents were obtained from all subjects who agreed to participate in this study. A full history was recorded, and each patient underwent a thorough clinical examination, routine laboratory investigations, mammography of both breasts, and radiologic investigations including x-ray of chest, ultrasonography of abdomen, computed tomography of the chest and abdomen and bone scan were performed if indicated, and fine-needle aspiration cytology of breast mass was done to establish the pathologic diagnosis.

Patients with breast cancer were managed by either breast conservative surgery or modified radical mastectomy. The clinicopathologic data were obtained from patients' pathology reports. The collected data included tumor size, tumor pathological grade, axillary lymph node involvement, ER status, PR status, Her-2 expression and vascular invasion. The patient's clinical stage was determined by the oncologist according to the tumor-nodes-metastasis (TNM) classification system.[15]

Methods

Study Design. Case-control study

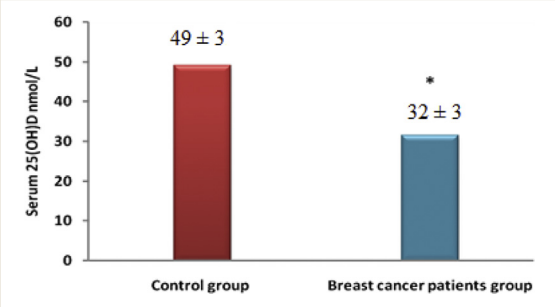
Samples Collection. *Blood Samples.* Blood samples were collected from the controls and breast cancer groups before surgery. The blood was centrifuged at 3500 rpm for 10 minutes at room temperature, and serum samples were separated and stored at −80°C until assayed for determination of 25(OH) D by a ready-for-use radioimmunoassay kit (DIAsource ImmunoAssays SA, Ottignies-Louvain-la-Neuve, Belgium).

Tissue Samples. Tissue samples were collected at the time of surgery from the patients with breast cancer. Two tissue samples were taken, one from the cancerous breast tissue and the other from the adjacent normal breast tissue. The specimens were stored at −80°C until analysis for quantification of ER-α mRNA gene expression by quantitative real time polymerase chain reaction (qRT-PCR) technique and determination of VDR levels by a ready-for-use enzyme-linked immunosorbent assay kits (USCNK Life Science, Inc, Wuhan, China).

Determination of Serum 25(OH)D Levels by Radioimmunoassay Kit

This is an in vitro radioimmunoassay for the quantitative measurement of serum 25(OH)D levels. At first, calibrators, controls, and sera were pretreated with acetonitrile to release 25(OH)D from vitamin D binding protein. A fixed amount of ^{125}I-labeled-25(OH) D competes with the 25(OH)D from treated samples, controls, or calibrators, for a fixed amount of specific monoclonal antibody sites immobilized to the lower and inner surface of plastic tubes. After 2 hours incubation at room temperature on a tube shaker, an aspiration step terminated the competition reaction. The tubes were then washed twice and aspirated again. The tubes were then washed with 2 mL washing solution and counted for 60 seconds in a

Figure 1 The Serum 25(OH)D Levels in the Control and Breast Cancer Patients Groups. *Significance Was Compared With the Control Group. Significance Was Considered at $P < .05$



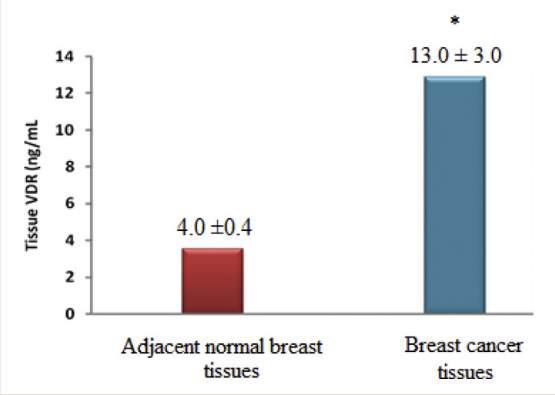
Abbreviation: 25(OH)D = 25-hydroxyvitamin D.

gamma counter (Perkin Elmer, Turku, Finland). A calibration curve was constructed, and the 25(OH)D concentrations of the samples were determined by interpretation from the calibration curve. Two internal quality controls included in the kit were assayed concurrently with the samples and their concentrations were within the range specified on the vial label. The sensitivity of the assay was 1.5 ng/mL.[16]

Determination of Tissue VDR Levels by Enzyme-linked Immunosorbent Assay Kit

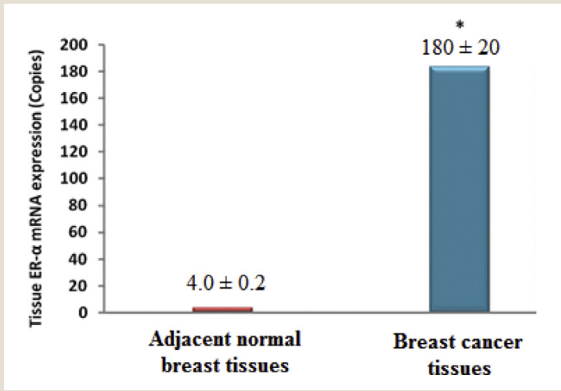
Sample Preparation. One hundred mg of each tissue sample was rinsed in ice-cold phosphate buffered saline (PBS) (0.02 mol/L; pH 7.2) to remove excess blood thoroughly then minced to small pieces and homogenized in 10 mL of PBS with a glass homogenizer on ice.

Figure 2 The Tissue VDR Levels in the Breast Cancer Tissues and Their Adjacent Normal Breast Tissues of the Same Patient. *Significance Was Compared With the Normal Breast Tissues. Significance Was Considered at $P < .05$



Abbreviation: VDR = vitamin D receptor.

Figure 3 The Tissue ER-α Gene Expression Levels in the Breast Cancer Tissues and Their Adjacent Normal Breast Tissues of the Same Patient. *Significance Was Compared With the Normal Breast Tissues. Significance Was Considered at $P < .05$



Abbreviation: ER-α = estrogen receptor alpha.

The resulting suspension was subjected to 3 freeze-thaw cycles to further break the cell membranes. After that, the homogenate was centrifuged for 5 minutes at 10000×g, and the supernatant was removed and store at −20°C until assayed.[17]

Principle. This technique is a sandwich enzyme linked immunosorbent assay for in vitro quantitative measurement of VDR in human tissue homogenates. The microtiter plate provided was precoated with an antibody specific to VDR. Standards or samples were added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific for VDR. Avidin conjugated to the horseradish peroxidase was added to each microplate well and incubated. 3,3',5,5'-Tetramethylbenzidine substrate solution was added. The enzyme-substrate reaction was terminated by addition of sulphuric acid, and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentrations of VDR in the samples were determined by comparing the optical density of the samples to the standard curve. The minimum detectable concentration of VDR was typically less than 0.113 ng/mL.

Quantification of Tissue ER-α Gene Expression by qRT-PCR

The RNA content of each tissue sample was extracted using spin/column rapid extraction kit according to the manufacturer's protocol (Qiagen). The primers for the ER-α target gene were synthesized with the assistance of the computer program Primer Express (Bioneer, Alameda, CA), which selected the theoretically optimized primer sequences for the target gene. The amplification primer pairs were:

Forward primer: 5′-ATCCTGATGATTGGTCTCGTCT-3′;

Reverse primer: 5′-TCTGGAAGAGAAGGAACCATATCC-3′.

qRT-PCR reactions were performed using Thermo Scientific Verso One-Step qRT-PCR Kit (Thermo Fisher Scientific,

Table 1 Comparing the Diagnostic Values of Serum 25(OH)D, Tissue VDR Levels, and ER-α Gene Expression Using the ROC Curve Analysis

Variables	AUC, %	*P* Value[a]	Cutoff	Sensitivity, %	Specificity, %
Tissue ER-α gene expression, copies	96.7	.000	17.7	75	85
Serum 25(OH)D, nmol/L	85.5	.000	30	55	90
Tissue VDR, ng/mL	78.5	.000	5	65	85

Abbreviations: AUC = area under the curve; ER-α = estrogen receptor alpha; 25(OH)D = 25-hydroxyvitamin D; ROC = receiver operating characteristic; VDR = vitamin D receptor.
[a]Significance was considered at $P < .05$.

Waltham, MA). The qRT-PCR reactions were carried out in a total volume of 25 µl containing 0.25 µl of Verso enzyme mix, 12.5 µl of one step PCR master mix, 1.75 µl of Forward primer, 1.75 µl of reverse primer, 1.25 µl of RT enhancer, 2.5 µl of water (PCR grade), and 5 µl of Template (RNA). The reaction mix was put in real time PCR machine (DTlite Real-Time System, Moscow, Russia). The PCR conditions for ER-α were established as follows: after incubation at 50°C for 2 minutes and predenaturing at 95°C for 10 minutes, 45 cycles were performed at 95°C for 30 seconds, at 60°C for 30 seconds, and at 67°C for 45 seconds.[18,19]

Statistical Analysis

The statistical analyses were carried out using the Predictive Analytics software (PASW statistics 18, Hong Kong). The non-parametric Mann-Whitney *U*-test was used for studying differences between the group of patients with breast cancer and the control group regarding serum levels of 25(OH)D and mean tissue expression of VDR and ER-α mRNA. The diagnostic values of assayed parameters were compared using the receiver operating characteristic (ROC) curve analysis. The cutoff point for each parameter was determined according to the best discrimination between patients and controls regarding optimal values of sensitivity and specificity using the ROC curve. Spearman correlation was carried to explore the possible correlation between different parameters. Relative risk with 95% confidence interval (95% CI) was calculated. *P*-values $< .05$ were accepted as significant.

Results

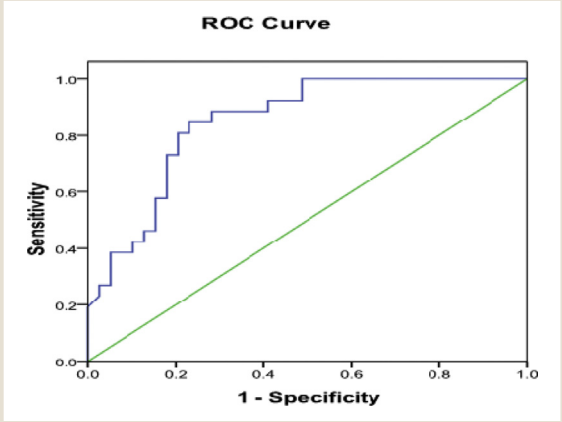
The Levels of Serum 25(OH)D, Tissue VDR, and Tissue ER-α Gene Expression

The mean levels of serum 25(OH)D in the patients with breast cancer group (32 ± 3 nmol/L) were significantly lower than that in the control group (49 ± 3 nmol/L; $P < .05$) (Figure 1). The mean VDR levels in breast cancer tissues (13 ± 3 ng/mL) were significantly higher than their levels in adjacent normal breast tissues (4.0 ± 0.4 ng/mL; $P < .05$) (Figure 2).In the breast cancer tissues, the mean ER-α gene expression levels (180 ± 20 copies) were significantly higher than that in adjacent normal breast tissues (4.0 ± 0.2 copies; $P < .05$) (Figure 3).

Comparison of the Diagnostic Values of 25(OH)D, VDR, and ER-α Gene Expression in the Group of Patients With Breast Cancer Using the ROC Curve Analysis

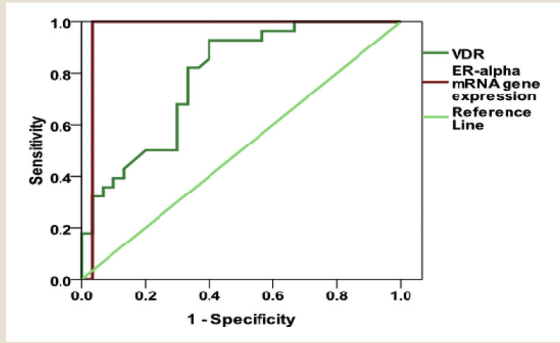
The ROC curve analysis was used to compare the diagnostic values of serum 25(OH)D, tissue VDR levels, and ER-α gene expression depending on the area under the ROC curve (AUC). The higher AUC corresponds to a better diagnostic marker. 25(OH)D showed significant AUC 85.5% ($P = .00$), with

Figure 4 Graphical Representation of the ROC Curve for Serum 25(OH)D in the Group of Patients With Breast Cancer



Abbreviations: 25(OH)D = 25-hydroxyvitamin D; ROC = receiver operating characteristic.

Figure 5 Graphical Representation of the ROC Curves for Tissue VDR and ER-α Gene Expression in Breast Cancer Tissues



Abbreviations: ER-α = estrogen receptor alpha; ROC = receiver operating characteristic; VDR = vitamin D receptor.

Table 2 Stratification of Serum 25(OH)D, Tissue VDR Levels and ER-α Gene Expression by Clinicopathologic Data of the Group of Patients With Breast Cancer

Clinicopathologic Data	25(OH)D, nmol/L	Tissue VDR, ng/mL	Tissue ERα Gene Expression, Copies
Histologic grade			
II	35.4 ± 4.5	15.8 ± 5.0	58.0 ± 5.7
III	20.9 ± 3.4[a]	8.3 ± 1.5[a]	33.5 ± 6.1[a]
PR status			
Negative	42.7 ± 3.8	5.1 ± 1.1	21.2 ± 4.5
Positive	35.4 ± 2.6	11 ± 2.9	63.0 ± 5.1[a]

Abbreviations: ER-α = estrogen receptor alpha; 25(OH)D = 25-hydroxyvitamin D; PR = progesterone receptor; VDR = vitamin D receptor.
[a]Significance was considered at P value < .05.

sensitivity 55% and specificity 90% at a cutoff of 30 nmol/L (Table 1, Figure 4). VDR levels showed significant AUC 78.5% ($P = .00$), with sensitivity 65% and specificity 85% at a cutoff of 5 ng/mL. ERα mRNA gene expression levels showed significant AUC 96.7% ($P = .00$), with sensitivity 75% and specificity 85% at a cutoff of 17.7 copies (Table 1, Figure 5).

Serum 25(OH)D, Tissue VDR and ER-α Gene Expression Levels Stratified by Clinicopathologic Data of the Group of Patients With Breast Cancer

In the current study, serum 25(OH) D levels, tissue VDR levels, and ERα-gene expression levels showed significant indirect correlations with breast cancer histopathologic grade (Table 2). On the other hand, ER-α gene expression levels showed significant direct correlation with progesterone receptor (PR) status. The correlations with other clinicopathologic characteristics like tumor size and axillary lymph nodes (LNs) were non-significant ($P > .05$).

Correlation Between the Assayed Biochemical Parameters

The results of the present study revealed significant indirect correlations between the serum 25(OH)D levels and both tissue levels of VDR ($r_s = -0.39$; $P = .007$) and ER-α gene expression ($r_s = -0.44$; $P = .004$). On the other hand, there was a significant direct correlation between the tissue levels of VDR and ER-α gene expression ($r_s = 0.40$; $P = .003$) (Table 3, Figure 6).

Table 3 Correlation Between the Assayed Biochemical Parameters

Biochemical Parameter	Biochemical Parameter	
	Tissue VDR	Tissue ER-α Gene Expression
Serum 25(OH)D		
r_s	−0.39[a]	−0.44[a]
P	.007	.004
Tissue VDR		
r_s		0.40[a]
P		.003

Abbreviations: ER-α = estrogen receptor alpha; 25(OH)D = 25-hydroxyvitamin D; PR = progesterone receptor; r_s = Spearman correlation; VDR = vitamin D receptor.
[a]Correlations were considered significant at $P < .05$.

Association of the Assayed Biochemical Parameters With Breast Cancer Risk

As shown in Table 4, women with serum 25(OH)D levels ≤ 30 nmol/L had significantly increased risk for breast cancer incidence. This risk was 11 (95% confidence interval [CI], 3-37) times more than women who had levels > 30 nmol/L. Also, women with tissue levels of VDR > 5 ng/mL had significantly increased risk for breast cancer incidence by a factor of approximately 11 (95% CI, 4-31) times more than women whose tissue levels of VDR ≤ 5 ng/mL. On the other hand, women with tissue levels of ER-α gene expression > 17.7 copies had significantly increased risk for breast cancer incidence by a factor of 17 (95% CI, 6-52) times more than women whose tissue levels of ERα gene expression was ≤ 17.7 copies.

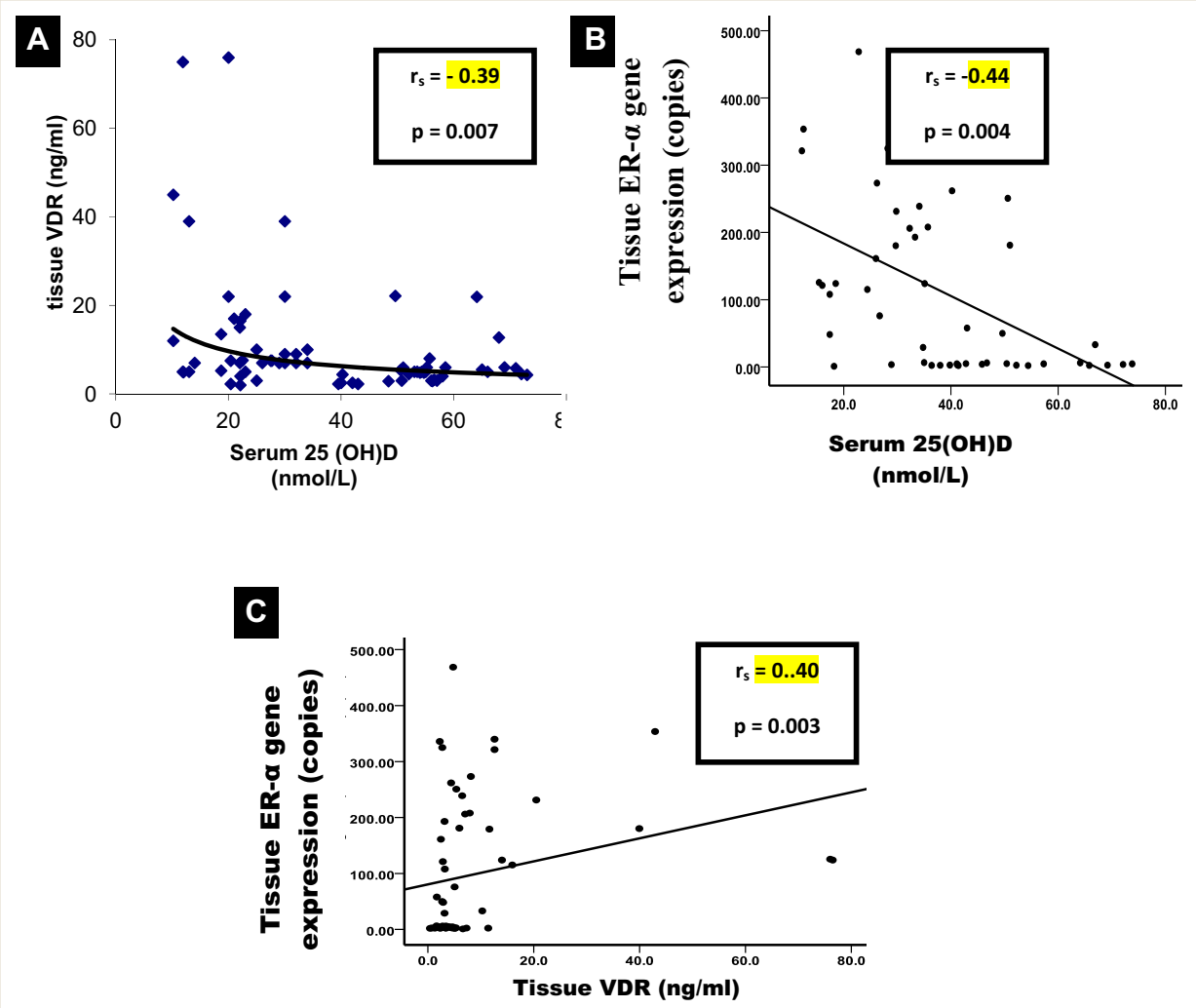
Discussion

The aim of this study was to explore the role of VDR in breast cancer tissues and its relation to serum 25(OH)D levels and ER-α gene expression, and their correlation with the breast cancer risk among women. In addition, their correlations with clinicopathologic data of patients with breast cancer were studied.

In the present work, the VDR levels were significantly higher in breast cancer tissues as compared with the corresponding levels in adjacent normal breast tissues. This result was in accordance with Santagata et al,[20] who confirmed that most human breast tumors are VDR-positive and documented that VDR is upregulated in breast carcinomas as compared with adjacent normal breast tissue. This may be explained by the fact that the breast cancerous tissues contain a high percentage of apoptotic cells,[21] and the increased expression of VDRs may be an involved mechanism in the induction of apoptosis in breast carcinomas.

Santos-Martínez et al[22] stated that patients with VDR-negative tumors relapsed significantly earlier than patients with VDR-positive tumors. Loss of VDR resulted in an increase in hypoxia-inducible factor-1, vascular endothelial growth factor, angiopoietin-1, and platelet derived growth factor levels. Moreover, in vivo, mice lacking VDR exhibited enlarged blood vessels to perfuse tumor lesions. This clearly implicates VDR in the control of

Figure 6 Correlation Between the Assayed Biochemical Parameters. A, Correlation Between Serum 25(OH)D Levels With Tissue VDR Levels. B, Correlation Between Serum 25(OH)D Levels With Tissue ER-α Gene Expression. C, Correlation Between Tissue VDR Levels With Tissue ER-α Gene Expression



Abbreviations: ER-α = estrogen receptor alpha; 25(OH)D = 25-hydroxyvitamin D; VDR = vitamin D receptor.

tumor-associated angiogenesis.[23] Weitsman et al[24] reported that 1,25(OH)D-VDR complex potentiates tumor necrosis factor-induced cytotoxicity in human cancer cells, and this is most likely the result of a VDR-mediated genomic effect and therefore may be potentiated in breast carcinomas by VDR upregulation.

The results of the current study revealed that serum levels of 25(OH)D in the group with breast cancer were significantly lower as compared with the control group (32 ± 3 nmol/L vs. 49 ± 3 nmol/L), which were in agreement with Kim et al,[25] who found that median serum levels of 25(OH)D in patients with breast cancer was 13 ng/mL (range, 4-46 ng/mL). At the same time, there was a significant indirect correlation between serum 25(OH)D levels and tissue levels of VDR ($r_s = -0.39$; $P = .007$); also, the analysis of the association between serum 25(OH)D levels and breast cancer risk revealed that women with serum 25(OH)D levels ≤ 30 nmol/L had significantly increased risk for breast cancer incidence. This risk was 11 (95% CI, 3-37) times more than women who had levels > 30 nmol/L. Our results supported the results reported by Kim et al,[26] who showed that serum 25(OH)D was inversely associated with breast cancer risk among women. Also McDonnell et al,[27] in their pooled analysis of 2 randomized studies, found that women with 25(OH)D concentrations ≥ 60 ng/mL had an 80% lower risk of breast cancer than women with concentrations < 20 ng/mL. Several studies supported that high 25(OH)D levels are associated with decreased risk of breast cancer.[28-30]

Regarding the association between tissue VDR levels and breast cancer risk, the results of the current study revealed that women with tissue levels of VDR > 5 ng/mL had significantly increased risk for breast cancer by approximately 11 (95% CI, 4-31) times than women whose tissue levels of VDR were ≤ 5 ng/mL. The results of

Table 4 Association Between Serum 25(OH)D Levels, Tissue VDR Levels, and ER-α Gene Expression With Breast Cancer Risk

The Assayed Parameters	N (%) Control Croup (n = 40)	N (%) Breast Cancer Group (n = 40)	Odds Ratio	95% CIs	*P* Value
Serum 25(OH)D, nmol/L					
> 30 (ref)	36 (90)	18 (45)	1 (Ref)	–	–
≤ 30	4 (10)	22 (55)	11	3-37	.0001[a]
	Adjacent Normal Breast Tissues (n = 40)	**Breast Cancer Tissues (n = 40)**			
Tissue VDR, ng/mL					
≤ 5 (ref)	34 (85)	14 (35)	1 (Ref)	–	–
> 5	6 (15)	26 (65)	11	4-31	< .0001[a]
Tissue ER-α gene expression, copies					
≤ 17.7 (ref)	34 (85)	10 (25)	1 (Ref)	–	–
> 17.7	6 (15)	30 (75)	17	6-52	< .0001[a]

Abbreviations: ER-α = estrogen receptor alpha; 25(OH)D = 25-hydroxyvitamin D; PR = progesterone receptor; ref = reference group; VDR = vitamin D receptor.
[a]Significance was considered at $P < .05$.

the current study supported those reported by Welsh et al,[9] who documented that there is a strong positive association between insulin growth factor-1 (IGF-1) levels and breast cancer. Brosseau et al[31] supported the finding of Welsh et al[9] and reported that IGF-1 has been found to upregulate VDR expression in breast cancer cells.

Also, the current study revealed a significant direct correlation between the tissue levels of VDR and ER-α gene expression ($r_s = 0.40$; $P = .003$), and the levels of ERα gene expression were significantly higher in breast cancer tissues as compared with its expression in normal breast tissues. Our results were consistent with the results of Williams and Lin,[32] who showed that ER-α is upregulated in most breast cancers, and its expression is a hallmark of hormone-dependent tumor growth.

It was reported that breast tumors with a high content of ER-α display features like tumor cellularity[33] and thymidine kinase activity,[34] which are indicative of an increased proliferative rate. The level of ER-α expression is an important factor in the natural history of breast cancer, and patients with high ER-α content who do not receive adjuvant therapy have lower recurrence-free survival than patients with undetectable ERα,[35] and the analysis of the association between ER-α gene expression and breast cancer risk in our study revealed that women with tissue levels of ER-α gene expression > 17.7 copies had significantly increased risk for breast cancer by 17 (95% CI, 6-52) times than women whose tissue levels of ER-α gene expression are ≤ 17.7 copies. Our results supported the results found by Hosseini et al,[36] who reported that overexpression of ER-α plays a major role in breast cancer pathogenesis via promoting cell growth and proliferation.

The ROC curve analysis was used to evaluate and compare the diagnostic accuracy of serum 25(OH)D, tissue VDR and ER-α gene expression in patients with breast cancer. The present study revealed that serum 25(OH)D, tissue VDR, and ER-α gene expression were good markers for predicting patients with breast cancer. Our results showed that tissue ER-α gene expression was the most superior diagnostic marker, followed by the serum level of 25(OH)D, followed by tissue VDR.

In the current study, serum 25(OH) D levels, tissue VDR levels, and ER-α gene expression levels showed significant indirect correlations with breast cancer histopathologic grade. The relatively small sample size of the study population limited our ability to detect significant trends of 25(OH)D deficiency, loss of VDR, and low ER-α gene expression with respect to lymph node involvement and tumor size of the breast cancer.

In conclusion, vitamin D status is a modifiable risk factor for breast cancer. Increasing 25(OH)D concentrations via supplementation is safe and affordable. It has a potential role in the prevention of breast cancer, and it may reduce its aggressiveness, and its deficiency is associated with increased risk of breast cancer. However, further larger studies are required to validate our findings. Also, the elevated levels of VDR in breast cancer tissues may open the door for further studies targeting these receptors for treatment or increasing the effectiveness of chemotherapeutic agents and thus reducing their doses especially in hormone-negative breast cancer.

Clinical Practice Points

- Before conducting this case-control study, we noted the indirect correlation between the serum levels of 25(OH)D and the risk of breast cancer incidence as it was reported in the literature.
- The new addition from the current study finds the correlation between serum levels of 25(OH)D and its breast tissue receptor VDR levels and the breast tissue gene expression of ER-α. We found that the levels of VDR and ER-α gene expression were significantly elevated in breast cancerous tissues compared with their corresponding normal breast tissues. The serum levels of 25(OH)D showed significant indirect correlations with each of

VDR levels and ER-α gene expression. Women with serum 25(OH)D levels ≤ 30 nmol/L, tissue levels of VDR > 5 ng/mL, and tissue levels of ER-α gene expression > 17.7 copies had significantly increased risk for breast cancer incidence.

- Thus, VDR and ER-α gene expression are upregulated in breast cancer tissues. This may open the door for further future studies that targeting these biochemical parameters for prevention of breast cancer. At the same time, the risk for breast cancer incidence can be modified by the supplementation of vitamin D, which is safe and has the ability to prevent breast cancer occurrence and aggressiveness.

Disclosure

The authors have stated that they have no conflicts of interest.

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