ORIGINAL ARTICLE

Vitamin D3 Receptor Interaction and Association with Androgen Receptor in Premenopausal Women Diagnosed with Localized Breast Cancer in Iraq

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ABSTRACT

Background : As the most frequent malignancy among women all over the world, breast cancer accounts for 25% of all cancers and the leading cause of female cancer-related mortality. It is the most commonly diagnosed malignancy among the Iraqi population, constituting about one-third of the registered female cancers and the leading cause of death from malignant neoplasm among women. Breast cancer women are also diagnosed with vitamin D3 deficiency, and low levels of serum vitamin D are reported to be associated with recurrence, invasiveness, and mortality. Moreover, women with higher levels of serum vitamin D3 are twice more likely to survive than those with low levels.
Aim: To evaluatie the association of vitamin D3 receptor with susceptibility to breast cancer in Iraqi women.
Method: This study was a retrospective-prospective study, carried out at Nanakali hematology/oncology hospital of Erbil on a sample of 74 cases who were randomly selected from among women who were diagnosed with breast cancer (stage II and stage III) from July to December 2016. The collected data were analyzed using Cho-square test and T-test through Graphpad Prism program version 6.
Results: Based on the results of the present study, 45% of patients were premenopausal, whose risk of ER+PR+ tumors was 20% lower. There were no significant differences between premenopausal, menopausal and postmenopausal breast cancer patients with different BMI groups regarding molecular subtypes. Moreover, there was a significant association between obesity and hormonal receptor subtype ER+/PR+.
Conclusion: Most of the Iraqi women with breast cancer are either obese or overweight at the time of diagnosis. There is a weak inverse association between vitamin D levels and breast cancer risk. Serum calcium and estrogen levels are positively associated with breast cancer risk.
Keywords: Perimenopause, breast cancer, androgen receptor, vitamin D receptor, diagnosis

INTRODUCTION

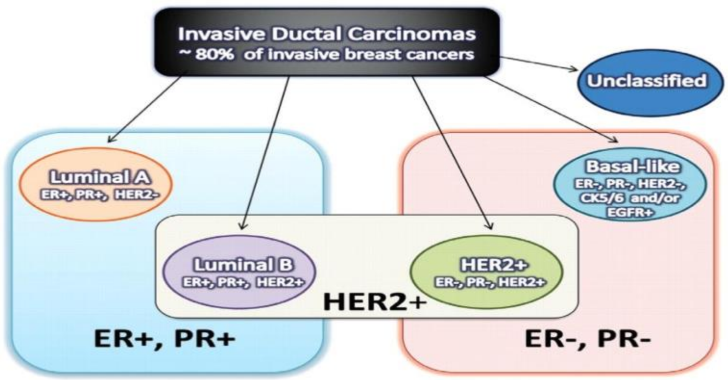
Breast cancer accounts for 25% of all cancers all over the world[1] and is the most frequently diagnosed type of cancer in women, about 80-90% of all breast cancer infiltrating ductal carcinoma[2]. In this cancer, the cells in breast tissues grow in an uncontrollably, resulting in formation of a lump in the breast. It is also the most commonly diagnosed malignancy among the Iraqi population, accounting for about one-third of the registered female cancers and the leading cause of death from malignant neoplasm among women[3]. According to the statistics by the latest Iraqi Cancer Registry, a total of 21,101 new cases of cancer were registered in 2012; 9,268 were in men and 11,833 were women[3]. Since 1986, breast cancer has been the most frequent and the leading cause of female death in Iraq; accounting for 34% of the registered cancers and 23% of cancer-related mortality among women. Middle-aged women have been reported with the highest level of prevalence particularly at relatively advanced stages[4].

Breast cancer women are frequently diagnosed with deficiency of vitamin D whse low levels are associated with recurrence, invasiveness, and mortality. In addition, higher levels of serum vitamin D cause breast cancer women to survive twice more than those with low levels [5]. Also, up to 78% of breast cancers cells contain vitamin D receptors which might prevent tumor growth and eradicate carcinogenic cells due to an increase in blood supply. Moreover, vitamin D receptors keep functioning until a tumor becomes very aggressive, which can be the reason for better survival of patients with higher blood levels of vitamin D[6].

There is no specific standard level of vitamin D; however, an optimal range is ≥32 ng/mL, insufficiency is at 20–31 ng/mL, and deficiency is at <20 ng/mL[7]. Even with a particular intake dose of vitamin D, different individuals have been reported to have different serum levels of 25 (OH) D. Levels of 25 (OH) D can be affected by a number of biological and demographic factors such as genetic variation, dietary fat amount and components, ethnicity, and some diseases[8].

Receptors are in charge of cell signaling. Receptors present on the surface of the cell are bound by hormones or other protein molecules which also cause the desired change within the cell. The presence of receptors are sometimes shown by breast cancer, helping with deciding the course of treatment. The presence of the receptors is always checked through biopsy samples. Three major receptors have been reported to be available in breast cancer cells[9,10]. As the first group, estrogen binds to estrogen receptor (ER). Breast cancer cells with estrogen receptor require estrogen for their growth and are denoted as ER+. Almost one-third of invasive breast cancer cases are ER+. Similarly, progesterone binds to progesterone receptors present in breast cancer cells, denoted by PR+. In case of HER2+, cancer cells overexpress HER2/neu protein which plays a significant role in growth of cancer cells[11] (Figure 1).

Figure 1. Breast cancer classification and its subtypes[11]



Vitamin D has two precursors: ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). While vitamin D2 is only obtained from the diet and its natural sources are rare[12], vitamin D3 can be retrieved from diet, mainly from oily fish or fish liver oil, and its major source exposure of the skin to sunlight[13]. Vitamin D classically plays a role in homeostasis of calcium and phosphate and mineralization of bones[12].

Accrding to preclinical reseach, vitamin D has beneficial effects on various diseases including cancer[14]. Since this discovery, vitamin D analogs, the active metabolite of vitamin D, and 1,25dihydroxyvitamin D3 (1,25(OH)2D3) have frequently been linked to the suppression of cancer cell metastasis, proliferation, and invasion[15-17]. In certain breast cancer cell lines, 1,25(OH)2D3 has been shown to increase the expression of E-cadherin resulting in the prevention of invasion and metastasis[18]. Moreover, vitamin D exhibits protective effects against breast cancer via the vitamin D receptor (VDR)[14]. There is growing evidence that breast cancer incidence is associated with various polymorphisms of the VDR gene[19]. The aims of the present study is to investigate the association of VDRwith vitamin D (25(OH)D3) serum levels of breast cancer in premenopausal women in Iraq.

MATERIALS AND METHODS

The present retrospective-prospective study was carried out at Nanakali hematology/oncology hospital of Erbil, the Kurdistan Region of Iraq. It consisted of a sample of 74 cases who were diagnosed with breast cancer (stage II and stage III). Their age range was 20-72 years. They were randomly selected from among the women diagnosed with breast cancer from July to December 2016 after informed consent was taken from each of them. A questionnaire was used to collected required demographic data including age, weight, height, and body mass index, and clinic-pathological assessment such as TNM staging, histology grade, and ER/PR and HER2 status.

For the purpose of comparison with the breast cancer patients, 30 females control subjects aged 20-70 years were also included in the study. The controls were selected from among subjects who were healthy, such that they did not have breast cancer, hypertension, endocrine disorders, metabolic kidney diseases, or acute illness or infection at the time of sampling.

After the sterilization procedure, 10 milliliters of venous blood were collected without tourniquet, of which 4 milliliters were preserved in tubes containing ethylene diamine tetraacetic acid disodium salt (EDTA Na2) at -20°C for DNA extraction purposes, and 6 milliliters were transferred into tubes containing ethylene diamine tetraacetic acid disodium salt (EDTA Na2). The blood samples were allowed for 10 minutes on the rack to clots, then centrifuged at 4000 rpm for 10 min. Then serum was separated from the other cells using a micro pipet, and divided into 5 Eppendorf, then frozen at -20 ºC until the use for assay.

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and rounded to the nearest tenth, according to the following equation:

$$\text{Body mass index (BMI)} = \frac{\text{Weight (kg)}}{(\text{Height (m)})^2}$$

Following current recommendations, normal weight was defined as BMI of 18.5-24.9, overweight as BMI of 25.0 to 29.9, and obesity as a BMI of 30.0 or highe[20].

The serum 25 (OH)D levels of the 74 patients with breast cancer and the 30 control subjects was measured through electrochemiluminescent immunoassay on an Elecsys automated analyzer (Roche Diagnostics, Mannheim, Germany) and using Elecsys Total Vitamin D(25-OH) kit (Roche Diagnostics)[19]. The same method was used to measured parathyroid hormone (PTH), calcium, and estrogen and progesterone hormones.

Data analysis was carried out using statistical software Graphpad Prism program version 6. The mean and standard deviation of each variable were estimated. The corrected Chi-square (x2) and T-test were used to compare the sensitivity and specificity among the various tests. VDR gene was classified as homozygous wild type G/C, heterozygous mutant G/C, and mutant C/C. P-values of ≤ 0.01 and ≤ 0.05 were considered statistically significant. Odds ratios for allele frequency between the patient and control groups were determined to clarify which acted as an etiological or a preventive factor.

RESULTS

The study consisted of a total of 74 breast cancer patients whose age range was 20–72 years. Most of the patients (78.4%) were over 40 years old (Table 1).

The participants were classified into three main age groups including premenopausal (women less than 45 years of age), menopausal (women between 45 - 50 years of age), and postmenopausal (women over 50 years of age) (Table 2).

Based on the data presented in Table 3, 81% of the breast cancer patients had a positive ER, and 82% had positive PR. Moreover, about one-third (38%) of the breast cancer patients had positive HER2, and 62% had negative HER2 (Table 3).

In addition, according to the data in Table 4, there is a highly significant association between obesity and positive ER among the breast cancer patients (p<0.001). Moreover, a significant association was observed between obesity and positive PR among breast cancer patients (p<0.05) (Table 4).

In accordance with the data in Table 5, it could be seen that there is a highly significant association between the women's BMI and ER/PR (P< 0.001) (Table 5).

Moreover, based on the data Table 6, it can be seen that there is a significant association between obese postmenopausal breast cancer patients and HR+/HER- subtype (p<0.001), and this association is more significant in postmenopausal obese women (p<0.001). On the other hand, there is not any considerable difference among premenopausal, menopausal, and postmenopausal breast cancer patients with different BMI groups regarding molecular subtypes (p>0.05) (Table 6).

Table 2. Age groups of breast cancer patients

Age groups	Frequency	%age
20-29	2	2.7
30-39	14	18.9
40-49	26	35.1
50-59	21	28.4
60 ≥	11	14.9
Total	74	100.0

Moreover, according to the data Table 7, estrogen and progesterone have a highly significant association with hormone receptor in breast cancer patients (p<0.001) (Table 7). Comparing the levels of vitamin D3 in the patients and controls indicated (Table 8)

ER/PR receptor distribution had a significant association with vitamin D and calcium level among the breast cancer patients (p<0.001) (Table 9).

Moreover, there was a significant difference between premenopausal patients and controls regarding their levels of calsium (Table 10).

Table 2. Menopausal distribution of breast cancer patients

Variable	n	%age
Premenopausal	33	45
Menopausal	21	28
Postmenopausal	20	27
Total	74	100

Table 3. Hormone receptors, HER2 receptors status of breast cancer patients

Variable	n	%age
ER		
Positive	60	81
Negative	14	19
Total	74	100
PR		
Positive	61	82
Negative	13	18
Total	74	100
HER2/neu		
Positive	28	38
Negative	46	62
Total	74	100

Table 4. Distribution of hormone receptor, and HER2 receptors according to BMI categories

Variables	Normal	Overweight	Obese	X²	P-value
ER					
Positive	3(43%)	24(89%)	33(82%)	60.08	<0.001
Negative	4(57%)	3(11%)	7(18%)		
PR					
Positive	5(71%)	23(85%)	33(82%)	6.628	0.030
Negative	2(29%)	4(15%)	7(18%)		
HER2/neu					
Positive	3(43%)	10(37%)	17(42%)	0.57	0.650
Negative	4(57%)	17(63%)	23(58%)		

Table 5. ER/PR receptor distribution according to BMI in breast cancer patients

BMI ER&PR	ER&PR +ve	ER+ve/PR-ve ER-ve/PR+v	ER & PR -ve
Normal	3(-.5%)	7(46.6%)	2(3%)
Overweight	23(42%)	3(20%)	27(39.1%)
Obese	29(57.5%)	5(33.4%)	40(57.9%)
Total	55(100%)	15(100%)	(69(100%)

X^2 96.1 P value <0.001

Table 6. Distribution of BMI according molecular subtypes for premenopausal breast cancer patients

Molecular subtypes	Premenopausal						Menopausal						Postmenopausal					
	BMI						BMI						BMI					
	Normal		Overweight		Obese		Normal		Overweight		Obese		Normal		Overweight		Obese	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
HR+/HER-	3	100	7	50	7	53.8	0	0	2	28.5	5	71.5	0	0	5	62.5	6	54.5
HR+/HER+	0	0	3	21.5	4	30.7	0	0	3	37.5	5	62.5	0	0	2	25	4	36.3
HR-/HER+	0	0	3	21.5	0	0	0	0	0	0	1	100	0	0	0	0	0	0
HR-/HER-	0	0	1	7	2	15.5	0	0	0	0	1	100	0	0	1	12.5	1	9.2
Statistical test	χ^2=6.123						χ^2=3.651						χ^2=3.715					
P-value	0.295						0.06						0.08					

Table 7: ER/PR receptor distribution according to estrogen and progesterone in breast cancer patients

ER&PR	ER & PR +ve		ER+ve/PR-ve ER-ve/PR+ve		ER & PR –ve		X^2	P value
	N.	%	N.	%	N.	%		
Estrogen								
Normal	11	18.6	1	14.2	3	23	22.67	<0.000
Abnormal	48	81.4	6	85.8	10	77		
Total	59	100	7	100	13	100		
Progesterone								
Normal	13	22	4	57.1	4	30.7	28.55	<0.000
Abnormal	46	78	3	42.9	9	69.3		
Total	59	100	7	100	23	100		

Table 8. Menopausal distribution according to vitamin D3 in patients and control

Period	**Sample**	**Mean**	**Std.Deviation**	**T test**	**P Value**
Premenopausal	Patients	42.57	28.87	2.69	0.0112
	Control	67.12	22.83		
Menopausal	Patients	8.48	6.716	10.37	< 0.0001
	Control	43.35	8.46		
Postmenopausal	Patients	6.540	4.052	19.18	< 0.0001
	Control	47.57	7.545		

Table 9. ER/PR receptor distribution according to vitamin D and calcium in breast cancer patients

ER&PR	ER & PR +ve		ER+ve/PR-ve ER-ve/PR+ve		ER & PR –ve			P value
	N	%	N	%	N	%		
Vitamin D								
Noramal	6	10.1	2	28.5	1	0.7	21.92	<0.000
Abnormal	53	89.9	5	71.5	12	93.3		
Total	59	100	7	100	13	100		
Calcium								
Normal	29	49.1	7	100	12	90.9	95.23	<0.000
Abnormal	30	50.9	0	0	1	9.1		
Total	59	100	7	100	13	100		

Table 10. Menopausal distribution according to ca in patients and control

Period	Sample	Mean	Std. deviation	T-test	P-value
Premenopausal	Patients	9.598	0.8676	3.42	0.0013
	Control	8.788	0.5280		
Menopausal	Patients	9.428	0.7610	0.43	0.6693
	Control	9.571	0.6987		
Postmenopausal	Patients	9.759	9.206	1.20	0.2383
	Control	1.114	0.8810		

DISCUSSION

The results of the present study in terms of association between obesity and hormonal receptor subtype ER+/PR+ are somewhat similar to the results of the study comparing the highest versus the reference categories of relative body weight, which showed that the risk for ER+PR+ tumors was 20% lower (95% CI = −30% to −8%) among premenopausal (2,643 cases) and 82% higher (95% CI = 55–114%) among postmenopausal (5,469 cases) women[21]. The results of the present study indicated a considerable association between obese postmenopausal breast cancer patients and HR+/Her- subtype; however, this result is less notable among premenopausal obese women. In this regard, the findings of the present study are in line with the results of another study carried out in Iraq[22].

Obesity has been recognized as a significant risk factor of breast cancer among postmenopausal women and is associated with poor prognosis. This result was in agreement with the results of other studies that show patients with high BMI are more frequently diagnosed with hormone receptor-positive tumors and suggest that triple-negative tumors may have distinct etiology[23,24]. As was seen, progesterone and estrogen have an essential role in the development and maintenance of female sexual characteristics and also play a crucial role in the pathogenesis and progression of breast cancer. However, the biological effects of estrogen such as growth stimulation and differentiation of normal mammary tissue are mediated primarily through high-affinity binding of estrogen receptors[25].

Several reproduction-related factors are reported to predispose women to breast cancer. For example, women with early onset of menarche (menstruation which begins before age of 12 years) or late menopause (menopause which occurs after 55 years) have an increased risk of developing breast cancer[26]. This indicates that breast cancer risk and elevated estrogen levels have a significant association. High estrogen exposure and high breast cancer risk during the years when women had functional ovaries have a clear relationship. Ghanim et al observed elevated levels of estrogen in 19 cases (63%) of the studied cases, which indicates a significant association between breast cancer risk and elevated estrogen levels[27]. The data of this study support the hypothesis that higher serum estrogen concentrations are associated with a higher risk of breast cancer in postmenopausal women[28]. Breast cancer was approximately five times more likely to develop in women with high estrogen levels than those with low values[29].

There are many studies that investigated the relationship between vitamin D levels and breast cancer, with slight variations from our study to another. In a study by Yao et al, it was found that increased serum levels were associated with decreased risk of breast cancer. Specifically, they found that those with sufficient levels had a 63% reduction in odds of breast cancer [30]. In our study, vitamin D levels were measured in stored blood. Clinical, pathologic, and dietary data were accessed to examine prognostic effects of vitamin D. On the other hand, it was concluded that those with decreased levels of vitamin D had an increased risk factors ER/PR.

Palmieri et al. conducted a study to clarify the role of vitamin D in breast cancer progression by comparing the levels of serum vitamin D in patients with early diagnosis and those with advanced breast cancer. They found a relationship between vitamin D levels and breast cancer. Though it was not causal, they simply concluded that women with early breast cancer had higher levels than those with advanced disease [31]. Other contributing factors to vitamin D deficiency include darker skin tone of the Middle Eastern population, use of sunblocks, avoiding performing activity in sunny areas, and the dietary regimen. Two pathways for vitamin D biosynthesis and action have been proposed in mammary carcinogenesis [32]. The first one involves 1,25(OH)2D3 and the second involves 25(OH)D3. In the first circulating pathway, 1,25(OH)2D3 reaches the breast tissue to exert its anti-carcinogenetic effect, while in the other pathway, circulating 25(OH)D3 reaches the breast tissue and is catalyzed to 1,25(OH)2D3 by the 1-α-hydroxylase in the breasts. All produced 1,25(OH)2D3 might bind to VDR and therefore regulate cell proliferation, differentiation, and apoptosis[33].

Regarding the association between breast cancer-specific mortality and lvels of calsium, it could be noted that there is an association between large tumors and high levels of calcium at diagnosis of breast cancer, and this may well be an effect of the tumor presence[34]. Previous findings suggest an increased incidence and more aggressive breast cancer tumor characteristics, associated with higher calcium levels[35,36.] In contrast, the explorative analysis in the present study found an association between high levels of calcium and a lower breast cancer-specific mortality. This finding needs further scientific attention, and the expression or activity of the calcium receptor may modulate the effect of calcium on breast tumors.

CONCLUSION

Most of the Iraqi women with breast cancer were either overweight or obese at the time of diagnosis. Women with high BMI presented with a more aggressive stage at the time of diagnosis. Their tumor usually show positive hormonal status (ER/PR), with HR+/HER2- being the most predominant molecular subtypes. Increased awareness of not only breast cancer patients but also their physician about the importance of weight control during the management of breast cancer patients and also breast cancer survivors. Hence most obese women have tumor hormonal status positive with favorable molecular subtype (ER+/HER2-), thus decreased weight will mainly contribute to decreasing tumor exposure to high endogenous estrogen especially in postmenopausal age, dramatically affect response to treatment in return. There was a weak inverse association between vitamin D levels and breast cancer risk. Serum calcium and estrogen levels were positively associated with breast cancer risk.

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