



Immunohistochemical Expression of Vitamin D receptor in Breast Cancer in Erbil city.

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Abstract:

Background & objectives: Vitamin D receptor is a nuclear receptor that modulates the activity of genes. The purpose of this study was to evaluate the frequency of vitamin D receptor expression in breast carcinoma by immunohistochemistry and to investigate the association between vitamin D receptor expression with some clinicopathologic factors, like tumor grading, staging, histologic type (ductal and non-ductal) and the hormonal status.

Methods: One hundred formalin-fixed paraffin embedded tissue blocks of mastectomy and core biopsy specimens diagnosed as carcinoma, were obtained from a private laboratory in Erbil city/Iraq from October 2020-October 2022. In this study, vitamin D antibody (a mouse monoclonal antibody) was applied to a breast cancer tissue samples and its expression was assessed.

Results: Nuclear vitamin D receptor was expressed in 32% of the cases, and a strong relation was noted between vitamin D receptor expression and low-grade tumor ($p = 0.034$), estrogen receptor ($p\text{-value} = 0.007$), and progesterone receptor ($p = 0.045$) positivity, while no significant association was noted between vitamin D receptor expression and other clinicopathologic parameters like age, lymph node status, tumor stage, the type of the cancer and human epidermal growth factor receptor 2 status.

Conclusion: increased Vitamin D receptor expression in breast tumors appears to be associated with a better prognosis. Vitamin D receptor expressed in one third of cases in which there was significant association with tumor grade and hormonal receptors.

Keywords: Breast cancer, Immunohistochemistry, Vitamin D receptor.

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Introduction:

Breast cancer is currently the most common cancer diagnosed in females.¹ It's the fourth leading reason for cancer death.^{2, 3} and the leading cause of cancer fatality in women.⁴ A survey done in 2021. Breast cancer has been the most often diagnosed kind of cancer in both Erbil and Duhok governorates, with an incidence of 39.4/100000 and 18/100000 population in Erbil and Duhok governorates respectively.⁵ In another survey in Sulaymaniyah, Kurdistan revealed that breast cancer is the most frequent cancer in Sulaymaniyah Kurdistan with an incidence rate of 17.1%.⁶ Breast cancer onset and progress have been linked to both hereditary and environmental causes. Age, lifestyle, reproductive status, radiation exposure, and the use of estrogen drugs are all known significant risk factors for the development of breast cancer.⁷ Vitamin D is a steroid hormone with the metabolic active substance 1,25 (OH)2D3. which is required in calcium and phosphorus metabolism.⁸ In premenopausal women, elevated serum 25-hydroxyvitamin D (25OHD) concentrations were related with a lower incidence of breast cancer subtypes with poor prognosis, including estrogen receptor (ER)-negative and triple-negative breast cancer.⁹ Vitamin D receptor VDR is a nuclear receptor,¹⁰ that mediates the activation of target genes during transcription. The binding of 1,25(OH)2D to VDR can modulate many genes.¹¹ (VDR) is known to have effects in the tissues of the body vitamin D signaling sites include the central nervous system, skin and hair follicles, immunological system, and endocrine glands. growth, division, and death of both normal and modified cells.¹² This study aimed to assess the frequency of VDR expression in breast carcinoma by

immunohistochemistry and to investigate the association between VDR expression with some clinicopathologic parameters, such as tumor grading, staging, histologic type (ductal and non-ductal) and hormonal status.

Materials and methods:

One hundred formalin fixed, paraffin embedded blocks of breast specimens of mastectomy or core biopsy specimens diagnosed as carcinoma obtained non-randomly. We selected the samples in which hormonal receptors ER, PR and HER-2 have been done for them from a private laboratory in Erbil city during October 2020-October 2022. Two sections were prepared from each block. One stained with Hematoxylin & Eosin for the purpose of histological analysis, while the other was used for immunohistochemical evaluation of VDR expression. The pathological staging was performed according to the eighth edition of the American Joint Committee on Cancer (AJCC).¹³ Histological grading was provided by the modified Bloom Richardson system.¹⁴ The inclusion criteria involve the invasive ductal and other types of breast carcinoma. The exclusion criteria involve other malignant tumors except carcinoma and carcinoma in situ. We subdivided the cases in age groups above and below 50 in correlation with other studies. All cases were approved by Ethics Committee of the Kurdistan Higher Council of Medical Specialties. Four μm thick sections were cut and mounted on charged slides. After drying at 60 °C for one hour, slides were deparaffinized and rehydrated at room temperature (20-25 °C). They were placed in a xylene bath and after 5 minutes incubation they were put in ethanol for the next 3 minutes. Lastly, immersion in distilled water was done for 30 seconds, Epitope retrieval was carried out by using a specific method in 10 mmol/L citrate buffer 1:10 ratio with distilled water. IHC staining was



performed using (DakoEnVision FLEX+) system, vitamin D antibody, a mouse monoclonal antibody (sc-13133; Santa Cruz Biotechnology, Dallas, USA) at 1:100 dilution antibody that bound to an antigen in tissue sections, the reactivity for VDR was considered as positive when nuclear staining was observed, and scoring of VDR immunoreactivity was done and reviewed by two pathologists. Positive and negative control slides were involved with each run of staining. Negative controls were created by removing the main antibody and utilizing N-Universal negative control, and normal breast was used as positive control for VDR expression. For scoring of the VDR expression, it was considered negative when VDR expression less than 10%, it was considered moderately positive when VDR expression 11-50%, and strongly positive if VDR expression was more than 51%.¹⁵Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 26). Chi square test of association was used to compare proportions of two or more groups, statistical significance was defined as a p value of ≤ 0.05 .

Results:

One hundred cases of breast carcinoma were included in the study. Patients’ age ranged from 30-82 years; the mean age (SD) was 51.57(11.631) years. Out of the 100 cases, 60 cases were in grade 2, 55% of cases were in stage 2, and 94 cases were of the ductal carcinoma type, Table (1).VDR was not or expressed at low level in 68% of tumors (n = 68), moderately expressed in 20% (n = 20), and strongly expressed in the other 12% (n = 12), as shown in Table (2).

Table (1): The frequency and percentage for pathological parameters of the breast cancer patients.

		Frequency	Percentage
Tumor Grade	1	7	7
	2	60	60
	3	33	33
Tumor Stage	Unknown	25	25.0
	1	15	15.0
	2	55	55.0
	3	5	5.0
Types of Carcinomas	Ductal	94	94.0
	Non-ductal	6	6.0
Axillary lymph nodes	Negative	44	44.0
	Positive	33	33.0
	Unknown	23	23.0
Estrogen receptor	Negative	27	27.0
	Positive	73	73.0
Progesterone receptor	Negative	39	39.0
	Positive	61	61.0
HER-2	Negative	72	72.0
	Positive	28	28.0
VDR expression	Negative	68	68.0
	Moderately positive	20	20.0
	Strongly positive	12	12.0
	Total	100	100.0

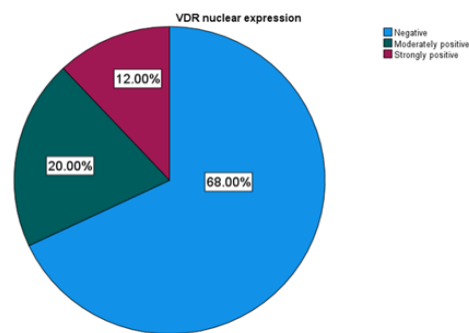


Figure (1): A pie chart showing the percentage of VDR nuclear expression.

Expression of VDR with low grade tumor grading was significant (p = 0.012), ER & PR receptor positivity (p-value =0.007 & 0.045) respectively. There is no notable relationship between VDR expression with age (p-value=



0.243), tumor stage (p = 0.786), axillary lymph node status (p = 0.660). type of breast carcinoma (p=0.610) and Her-2 receptors (p=0.056). 31% of ductal type breast cancer

cases were positive for VDR expression, while half of the non-ductal types of breast cancer cases (3) were positive for VDR expression.

Table (2): Association between VDR nuclear expression and Age (years)

		VDR nuclear expression			Total	p-value
		Negative	Moderately positive	Strongly positive		
Age (years)	< 50 years	34	8	3	45	
		75.6%	17.8%	6.7%	100.0%	
	≥ 50 years	34	12	9	55	0.243
		61.8%	21.8%	16.4%	100.0%	
Total		68	20	12	100	
		68.0%	20.0%	12.0%	100.0%	

Table (3): Association between VDR nuclear expression and the breast cancer patients' pathological parameters.

	Negative	Moderately positive	Strongly positive	Total	p-value
Tumor Grade					
1	5 (71.4%)	1 (14.3%)	1 (14.3%)	7 (100%)	0.012*
2	33 (55.0%)	17 (28.3%)	10 (16.7%)	60 (100%)	
3	30 (90.9%)	2 (6.1%)	1 (3.0%)	33 (100%)	
Total	68 (68.0%)	20 (20.0%)	12 (12.0%)	100 (100%)	
Tumor staging					
Unknown	18 (72.0%)	4 (16.0%)	3 (12.0%)	25 (100%)	0.786
1	10 (66.7%)	3 (20.0%)	2 (13.3%)	15 (100%)	
2	35 (63.6%)	13 (23.6%)	7 (12.7%)	55 (100%)	
3	5 (100.0%)	0 (0.0%)	0 (0.0%)	5 (100%)	
Total	68 (68.0%)	20 (20.0%)	12 (12.0%)	100 (100%)	
Axillary lymph nodes					
Negative	32 (72.7%)	6 (13.6%)	6 (13.6%)	44 (100%)	0.660
Positive	21 (63.6%)	9 (27.3%)	3 (9.1%)	33 (100%)	
Unknown	15 (65.2%)	5 (21.7%)	3 (13.0%)	23 (100%)	
Total	68 (68%)	20 (20%)	12 (12%)	100 (100%)	
Type of carcinoma					
Ductal carcinoma	65 (69.1%)	18 (19.1%)	11(11.7%)	94 (100%)	0.610
Non-ductal carcinoma	3 (50%)	2 (33.3%)	1 (16.7%)	6 (100%)	
Total	68 (68%)	20 (20%)	12 (12%)	100 (100%)	



Table (4): Association of VDR nuclear expression and Hormonal status.

		VDR nuclear expression			Total	p-value
		Negative	Moderately positive	Strongly positive		
Estrogen receptor ^a	Negative	24 (88.9%)	0 (0.0%)	3 (11.1%)	27 (100%)	0.007*
	Positive	44 (60.3%)	20 (27.4%)	9 (12.3%)	73 (100%)	
Total		68 (68%)	20 (20%)	12 (12%)	100 (100%)	
Progesterone receptor ^b	Negative	30 (76.9%)	3 (7.7%)	6 (15.4%)	39 (100%)	0.045*
	Positive	38 (62.3%)	17 (27.9%)	6 (9.8%)	61 (100%)	
Total		68 (68%)	20 (20%)	12 (12%)	100 (100%)	
HER-2 ^c	Negative	44 (61.1%)	17 (23.6%)	11 (15.3%)	72 (100%)	0.056
	Positive	24 (85.7%)	3 (10.7%)	1 (3.6%)	28 (100%)	
Total		68 (68%)	20 (20%)	12 (12.0%)	100 (100%)	

* Means significant correlation

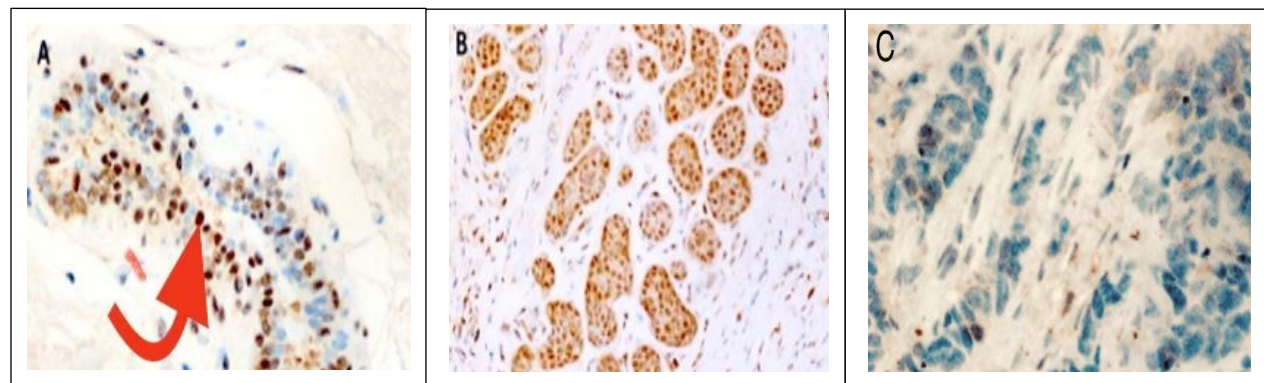


Figure (2): Nuclear VDR expression in breast tissue microarray
 A and B - VDR expression (IHCx400), C-Negative VDR expression (IHCx400).

Discussion:

Increased VDR expression in tumors appears to be associated with a better prognosis in breast cancer cases.¹⁶ In other studies, variable expressions of VDR for breast cancer was reported. In the present study. Vitamin D receptor was identified throughout all cancer cell compartments. VDR expression was expressed in 32% of cases. Comparable results were attained by other studies as in Heublein et al¹⁷ and in Al-Azhri et al¹¹ VDR expression was about 50% while in Huss et al¹⁵ and in Ditsch et al¹⁶ the VDR expression was about 90% and in Zulkeflee et al VDR expression was only 21%.¹⁸This

discrepancy in results can be accounted for the variation in the size of samples and the properties of antibodies used in those studies, in Al-Azhri et al, validated monoclonal antibody 9A7 (Thermo Fisher catalog #MA1-710) was used,¹¹ in Huss et al the mouse monoclonal D-6 antibody (sc-13133, Santa Cruz Biotechnology) was used,¹⁵ in Heublein et al (mouse anti human VDR, monoclonal, AbD Serotec, Oxford, UK) was used,¹⁷ while in this study mouse monoclonal antibody (sc-13133; Santa Cruz Biotechnology, Dallas, USA) was used, and every antibody has different properties, different clones and different productions. The study revealed that



the mean age of the patients (SD) was 51.57 (11.631) years, 45% of cases were aged less than 50 years and 55% were aged more than 50 years, age of the patients had no significant association with VDR expression, this result was in alignment to that observed by Al-Azhri et al,¹¹ in the present study 10% of the grade 3 cases showed VDR expression, this result was in alignment with that observed by Huss et al.¹⁵ The association between VDR expression and low tumor grade was extremely significant, similar to what was obtained by other studies like Huss et al in which there was a statistically relevant correlation between VDR negativity and high tumor grading.¹⁵ Also in Zati et al¹⁹ and Heublein et al,¹⁷ there was significant association between VDR expression and low-grade tumors, 31% of ductal type of breast cancer cases were positive for VDR expression, while 69% of them were negative for VDR expression, this means that there was no statistically significant relationship between VDR and the histological kind of the patients; this result was similar to what was obtained by Friedrich et al in which there was no link found between VDR expression and cancer type.²⁰ When it comes to VDR expression and nodal metastasis, 28% of the cases with negative nodal metastasis showed VDR expression, This result was not found to be statistically significant, similar to what was obtained by Huss et al,¹⁵ Al-Azhri et al¹¹ and Heublein et al.¹⁷ Regarding the hormonal receptor positivity, 11% of the ER negative cases (3) showed VDR expression while 40% of the ER positive cases (29) showed VDR expression. In the PR negative cases, 23% of them showed VDR expression (30) and it was 38% in the PR positive cases (23), this was significant and similar results were obtained by other studies.^{11,15} As for the Her-2 status, 40% of the negative Her-2 receptor cases (28) showed VDR Expression, while only 15% of the positive Her-2 receptor cases (4) showed

VDR expression, hence no association between VDR expression and Her-2 receptor, this finding was in concordance with those obtained by others.^{11, 15, 17}

Conclusion:

Increased VDR expression in breast tumors appears to be associated with a better prognosis. Vitamin D receptor expressed in one third of cases in which there was significant association with tumor grade and hormonal receptors.

Competing interests:

The authors declare no competing interests.

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