EJMI

DOI: 10.14744/ejmi.2021.99947
EJMI 2021;5(3):388–393

Research Article

Predictive Importance of Vitamin D on Response to Neoadjuvant Chemotherapy in Turkish Patients with Locally Advanced Hormone Receptor Positive Breast Cancer

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Abstract

Objectives: To investigate the relationship between the level of vitamin D with pathological response rates to neoadjuvant chemotherapy in breast cancer patients.

Methods: The pathological responses of patients treated with neoadjuvant chemotherapy for locally advanced breast cancer regarding Miller-Payne grading system were noted as ≤90% and >90% response. Vitamin D deficiency was defined as <20 ng/mL.

Results: Eighty-nine patients with a mean age of 49.7±12.1 years old were included. Forty-two (47.2%) patients were postmenopausal. Vitamin D deficiency was present in 38 (42.7%) patients. Forty-eight (53.9%) and 41 (46.1%) patients had a pathological response rate of ≤90 and >90 to neoadjuvant chemotherapy, respectively. In univariate analysis, tumor size, menopause status, and histologic grade were found to be statistically significant for pathological response. In multivariate analysis, progesteron receptor positivity, premenopause, and higher tumor diameter were associated with decreasing pathologic response. The level of vitamin D was not statistically significant on pathological response to neoadjuvant chemotherapy.

Conclusion: The level of vitamin D level was not associated with pathologic response rates to neoadjuvant chemotherapy in Turkish population. This may be explained with vitamin D receptor polymorphism in different ethnicities which should be evaluated in future studies in terms of pathologic response to neoadjuvant chemotherapy.

Keywords: Breast cancer, neoadjuvant chemotherapy, pathologic response, vitamin D

Cite This Article: *Atci MM, Cekin R. Predictive Importance of Vitamin D on Response to Neoadjuvant Chemotherapy in Turkish Patients with Locally Advanced Hormone Receptor Positive Breast Cancer. EJMI 2021;5(3):388–393.*

Breast cancer is the most frequently diagnosed malignancy and also the leading cause of cancer death in women worldwide.[1] Most patients with locally advanced breast cancer should receive neoadjuvant chemotherapy (NACT); mostly anthracycline-based regimens, to obtain a tumor response before surgery which enables breast conservation at the time of resection of primary tumors. In a meta-analysis assessing the effectiveness of preoperative chemotherapy in women with operable breast cancer when compared to postoperative, it was found that there was a reduced risk of radical mastectomy, an increased risk of locoregional recurrence, and an equivalent chance of overall survival (OS) and disease-free survival (DFS) with preoperative chemotherapy.[2]

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Submitted Date: March 13, 2021 **Accepted Date:** August 02, 2021 **Available Online Date:** September 22, 2021

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The Miller-Payne grading system (MPG) which is based on the degree of tumor cell loss, is commonly used to assess pathological response of breast cancer which occurs within the tumor during primary chemotherapy.[3] Previous studies have examined the markers predicting pathologic complete response (pCR), such as tumor subtype, grade, androgen receptor status or other conditions.[4-7] Additionally, in several neoadjuvant studies with breast cancer patients, it was shown that pCR is one of the most important markers for a favorable DFS and OS.[4,8]

The low vitamin D levels were found to be associated with increased breast cancer risk.[9] Many studies have found that vitamin D deficiency at the time of breast cancer diagnosis is associated with poorer outcomes and with an increased risk of breast cancer recurrence and mortality.[10-13] Also, some studies have shown that vitamin D replacement improves the outcome of breast cancer.[13,14] However, the effect of vitamin D on response to NACT in patients with breast cancer is still conflicting.

In this study, we aimed to investigate the effects of level of 25(OH) vitamin D at diagnosis on response to NACT in Turkish patients with locally advanced breast cancer.

Methods

Study Population and Treatment

This was a retrospective single center study. Medical details were obtained from the archived files of patients who had been treated with NACT between 2014 and 2019 for locally advanced invasive ductal breast cancer in the medical oncology clinic of, University of Health Sciences, Prof. Dr. Cemil Tascioglu Istanbul City Hospital. In the neoadjuvant setting, patients had received doxorubicin 60 mg/m^2 IV plus cyclophosphamide 600 mg/m2 IV every three weeks for four cycles followed by paclitaxel 80 mg/m^2 weekly for 12 cycles (AC-T). Patients with HER-2-positive breast cancer had received trastuzumab in addition to paclitaxel (4 mg/kg loading dose and 2 mg/kg weekly) for 12 weeks.

Demographic and histopathological information of the patients such as tumor size, histological type, lymph node status, grade, hormonal status (estrogen receptor [ER] and progesterone receptor [PR], human epidermal growth factor receptor 2 [HER-2]) were recorded and 25(OH) vitamin D levels were obtained from the archived files of patients. Patients without a pathology report and archived files and patients with hormone receptor negative breast cancer were excluded (Fig. 1). Disease staging was performed according to the Tumor, Node, Metastasis (TNM) staging 8th edition.[15] The histopathological response of the breast and axilla was assessed according to the MPG system.[3] Tumors were grouped into molecular phenotypes and classified as response rates according to the MPG system after completion of NACT. Patients were grouped according to MPG system response as >90% response and ≤90 % response.

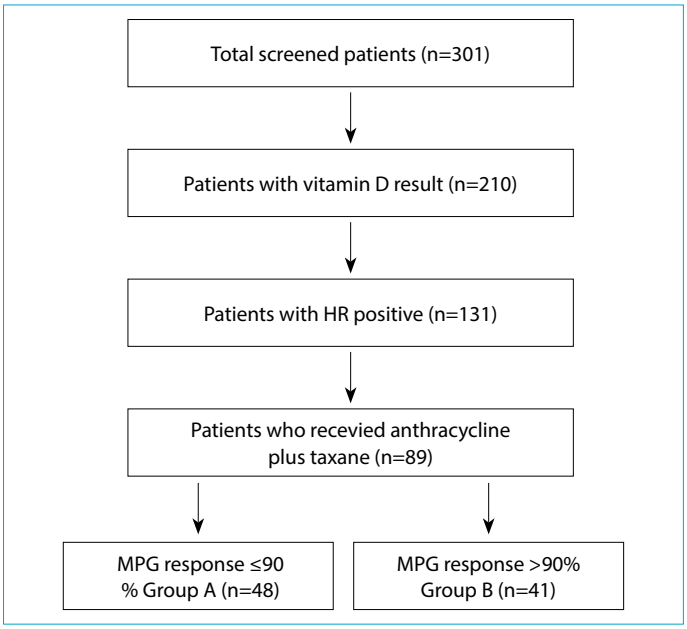

Figure 1. Consort diagram.

HR: Hormone receptor, MPG: Miller-Payne Grade.

Immunohistochemistry

The following antibodies were used: rabbit monoclonal antibody against human ERa (ER; SP1, Biocare, USA); rabbit monoclonal antibody against human progesterone PgR (SP2, Biocare, USA); rabbit monoclonal antibody against human HER2 (SP3, Cell Marque, USA); and rabbit monoclonal antibody against Ki 67 (SP6, Biocare, USA). ER and PgR immunohistochemistry was scored positive if at least 10% of tumor cell nuclei showed a staining signal. Hormone receptor (HR) positive was defined as ER and/or PgR positive.[16] HER-2 overexpression required either immunohistochemical staining of 3+ or positivity by fluorescence in situ hybridization (FISH) technique in the case of a 2+ IHC score. In the evaluation of Ki-67, nuclear staining was considered positive. The percentage of Ki67-positive cells in the total number of evaluated cells was calculated.[17]

25 (OH) Vitamin D analysis

Serum samples were collected at baseline of chemotherapy. Serum concentrations of 25 (OH) vitamin D3 was measured by electrochemiluminescence immunassay (ECLIA) method. (Elecsys assay on Roche Cobas E601 analyzer, Roche Diagnostics, Germany). Vitamin D deficiency was defined as <20 ng/mL.

Statistical Methods

SPSS 15.0 for Windows was used for statistical analysis. Descriptive statistics were given as a number and as a percentage for categorical variables, average, standard deviation, and minimum and maximum for numeric variables. The numerical variables did not meet the normal distribution condition, and comparisons of more than two independent groups were made using the Kruskal–Wallis test and comparisons of two independent groups were made using the Mann–Whitney U test. Comparisons of the ratios in the groups were made using the chi-square test. The determinant factors were examined by logistic regression analysis, and a statistical significance level of alpha was accepted as $p<0.05$.

Results

In total, 89 Turkish women with locally advanced breast cancer stage III disease who were treated with NACT were included in the study. All patients had invasive ductal carcinoma histology. The mean age was 49.7±12.1 years-old and 42 (47.2%) patients were postmenopausal. The mean 25 (OH) D vitamin level was 17.8±9.7 ng/ml and the number of patients with vitamin D deficiency was 38. The mean tumor diameter was calculated as 20.8±16.6 mm, and the mean Ki-67 level was calculated as 38.1% (Table 1). The percentages of patients with histologic grade I, II, and III were 3.6%, 63.1%, and 33.3%, respectively. The number of ER positive patients was 62 (69.7%) and PR positive patients was 44 (49.4%). Human epidermal growth factor receptor

Table 1. Patients characteristics and univariate analysis for determining factors associated with non-pCR

All patients		**Miller-payne response**		
Variables	**(Mean±SD)**	**≤%90**	**>%90**	**p**
Age (years)	49.7±12.1	48.4±12.5/22-81	51.4±11.4/24-70	0.097
Tumor size (mm)	20.8±16.6	23.9±14.8/5-70	17.7±17.9/1-85	0.019
Ki-67 (%)	38.1±25.9	34.6±26.6/2-90	41.8±25.1/5-90	0.125
Vitamin D (ng/ml)	17.8±9.7	18.7±10.5/3.9-52.9	16.7±8.7/3.8-39	0.361
Variables	**n (%)**	**≤%90**	**>%90**	**p**
ER				
Negative	27 (30.3)	11 (22.9)	16 (39.0)	0.099
Positive	62 (69.7)	37 (77.1)	25 (61.0)	
PR				
Negative	45 (50.6)	21 (43.8)	24 (58.5)	0.164
Positive	44 (49.4)	27 (56.3)	17 (41.5)	
HER-2/NEU				
Negative	51 (57.3)	31 (64.6)	20 (48.8)	0.133
Positive	38 (42.7)	17 (35.4)	21 (51.2)	
Menopause				
Post-	42 (47.2)	18 (37.5)	24 (58.5)	0.048
Pre-	47 (52.8)	30 (62.5)	17 (41.5)	
Grade				
1	3 (3.6)	2 (4.4)	1 (2.6)	0.049
2	53 (63.1)	33 (73.3)	20 (51.3)	
3	28 (33.3)	10 (22.2)	18 (46.2)	
Miller response				
0%	8 (9.0)			
%1-30	20 (22.5)			
%31-90	20 (22.5)			
%91-99	14 (15.7)			
100%	27 (30.3)			
Vitamin D				
<20 ng/ml	38 (42.7)	18 (37.5)	20 (48.8)	0.284
≥20 ng/ml	51 (57.3)	30 (62.5)	21 (51.2)	

ER: Estrogen Receptor, PR: Progesterone Receptor, HER-2/NEU: Human Epidermal Growth Factor Receptor Type 2.

Table 2. Backward method for multivariate analysis to determine factors associated with non-pCR

Variables	p	OR	95.0% CI	
PR positivity	0.042	0.211	0.047	0.944
Pre-menopause	0.033	0.199	0.045	0.875
Tumor size	0.009	0.918	0.862	0.979

PR: Progesterone Receptor.

2 positivity was observed in 38 (42.7%) patients. According to the MPG system, 8 (9.0%) patients showed 0% response, 20 (22.5%) patients showed 1–30% response, 20 (22.5%) patients showed 31–90% response, 14 (15.7%) patients showed 91–99% response, and 27 (30.3%) showed 100% response (Table 1). In total, 48 (53.9%) and 41 (46.1%) patients had a pathological response rate of ≤90 and >90, respectively.

In the univariate analysis, age, menopause status, tumor diameter, histologic grade, Ki-67, ER, PR, HER-2 status, and 25 (OH) D vitamin levels were assessed to determine the factors associated with a >90% response. Tumor size, menopause status, and histologic grade were found to be the statistically significant factors (p=0.019, p=0.048, and p=0.049, respectively; Table 1). The mean 25 (OH) D vitamin levels were 18.7±10.5 ng/ml and 16.7± ng/ml in patients with a pathological response rate of ≤90 and >90, respectively (p=0.361). Also, the number of patients with a deficient vitamin D level were similar in both group (p=0.284) (Table 1).

In the multivariate analysis, which included variables that showed p<0.250 in the univariate analysis, PR positivity, pre-menopause, and higher tumor diameter were associated with decreasing pathologic response in patients receiving NACT for breast cancer (Table 2). The level of 25 (OH) vitamin D was not statistically significant on response to NACT in either univariate and multivariate analysis.

Discussion

This was a retrospective study aiming to detect the relationship between vitamin D levels and pathologic response rates in patients receiving NACT for stage III breast cancer in Turkish population. We found that vitamin D level was not a predictor for pathologic response rates in patients with breast cancer. Also, we found that PR positivity, pre-menopause, and higher tumor diameter were associated with decreasing pathologic response.

In the neoadjuvant chemotherapy setting, some trials have evaluated the effects of vitamin D on pathologic response to NACT in patients with breast cancer. In a study, a total of 327 non-metastatic breast cancer patients who had received NACT were evaluated to assess the predictive effects of vitamin D status on pCR. Vitamin D deficiency was found to be a predictor for non-pCR.[18] Similarly, in another study published in 2018 evaluating a total of 144 patients who had received NACT for non-metastatic breast cancer, vitamin D deficiency was observed as a predictive marker of non-pCR.[19] However, there are also some studies showing that there is no relationship between vitamin D deficiency and pathologic response. The data from the NEOZOTAC trial reported a significant decrease in vitamin D post-neoadjuvant chemotherapy but at baseline and end of therapy vitamin D levels were not related to pathological response. Also, there has not been association between pCR and vitamin D level changes.[20] In a Korean study of 374 patients who received NACT for breast cancer, vitamin D deficiency was not observed as an important independent prognostic factor that has an adverse effect on pCR.[21] In retrospective cohort analysis of I-SPY TRIAL has been showed that vitamin D levels had no impact on tumor response to NACT in patients with HER-2 negative breast cancer.[22] The conflicting results may be explained with different ethnicities in these studies. There are some studies focusing on genetic variations of vitamin D receptor (VDR) in the literature to support this explanation.[23-26] In a study, it was found that VDR haplotypes are associated with breast cancer in African-Americans, but not in Hispanic/Latinas.[27] Also, some studies showed that changes in inflammatory biomarkers associated with breast cancer risk and survival in breast cancer survivors with low plasma 25(OH)D levels, supplemented with vitamin D3 depends on VDR polimorphism and haplotypes.[28,29] Our study showed that there was no association between vitamin D levels and pathologic response rates in Turkish breast cancer patients.

Our study has some limitations. Firstly, it was planned retrospectively, which might lead to several biases. Also, the absence of vitamin D levels at end of the NACT may result a conflicting but we excluded the patients who received vitamin D supplementation during NACT to reduce this bias. Previously, the effects of vitamin D on predicting pathologic response to NACT have been shown to be different in various ethnicities. Although our study which includes a small number of patient, is important because it is the first

study showing that there is no predictive role of vitamin D for pathologic response to NACT in Turkish breast cancer patients.

Conclusion

In conclusion, our results showed that vitamin D level is not associated with a favorable pathologic response to neoadjuvant chemotherapy in Turkish breast cancer patients. The findings presented in this study may guide new studies to examine vitamin D levels in breast cancer patients with different VDR polymorphism in terms of pathologic response to NACT.

Disclosures

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Review Committee of University of Health Sciences, Professor Doctor Cemil Tascioglu, Istanbul City Hospital.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – M.M.A., R.C.; Design – M.M.A., R.C.; Supervision – M.M.A., R.C.; Materials – M.M.A., R.C.; Data collection &/or processing – M.M.A., R.C.; Analysis and/or interpretation – M.M.A., R.C.; Literature search – M.M.A., R.C.; Writing – M.M.A., R.C.; Critical review – M.M.A., R.C.

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