Research Article

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Better survival associated with successful vitamin D supplementation in non-metastatic breast cancer survivors

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Abstract

Background: We aimed to clarify whether successful vitamin D supplementation could predict improved survival in breast cancer (BC) survivors after completion of adjuvant treatment.

Materials and Methods: Patients were classified into four groups based on changes of 25(OH)D level during the treatment follow-up. Log-rank statistics were used to compare survival distributions among groups. ORs and 95% CIs were given for mortality ratios.

Results: The risk of death in group II with low 25(OH)D levels was 4.2 times higher than in group I with high 25(OH)D levels. (OR = 4.17 (95% CI = 1.46–11.91), P = 0.008) and the risk of death in group IV whose 25(OH)D levels never increased was 4.3 times higher (OR = 4.29 (95% CI = 1.13–16.3)). According to the log-rank test, life expectancy was significantly higher in group II compared to group I (P = 0.017) and group III (P = 0.001). Group IV had significantly lower survival times than group III (P = 0.021).

Conclusions: Vitamin D supplementation may play an important role in the response of the received treatments and provide a lower mortality rate and better overall -free survival (OFS) and disease-free survival (DFS) to BC

patients. However, we observed a sign of poorer BC survival still after sufficient vitamin D supplementation.

Keywords: breast cancer; mortality; prognosis; vitamin D; vitamin D supplementation.

Introduction

Calcitriol is the activated form of vitamin D hormone that act to binds the nuclear vitamin D receptor (VDR) resulting in activation of gene transcription [1]. VDR expression in malignant cells suggests that it plays a considerable role in cancer growth and progression [2, 3]. Along with genomic effects, vitamin D acts by non-genomic mechanisms characterized by rapid activation of intracellular signalling mechanisms [4]. Recently, vitamin D has gained much more interest for its biologic effects such as inhibition of cancer cell progression, induction of apoptosis and autophagic cell death, suppression of angiogenesis, and immunomodulatory effects [5, 6].

Several observational studies indicated that low 25(OH)D levels are related with an increased cancer risk, including, colorectal, breast, and prostate cancer [7–14]. Therefore, many vitamin d replacement studies have been conducted to decrease the cancer risk [15]. Accordingly, the data provided clear results for vitamin D intake associated with its protective effects against colorectal cancer, but the data were unclear for breast cancer (BC) [16–18]. A recent meta-analysis indicated that there was a significant inverse association between vitamin D intake and BC risk [16]. On the other hand, the other meta-analysis indicated weak protective effect of vitamin D supplementation (pooled OR 0.89; 0.84–0.95) for premenopausal women, but no protective effect for postmenopausal population (OR: 0.82 95% CI: 0.49–1.35) [17].

Besides the importance of vitamin D in cancer risk, the possible role of 25(OH)D levels in cancer prognosis and tumour progression has also been further investigated. Better survival was achieved with adequate 25(OH)D levels

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in advanced/metastatic stage colorectal cancer [19] and improved overall survival was shown in patients who had high vitamin D after curative intent surgery [20, 21]. Additionally, the beneficial outcome of vitamin D supplementation on survival in both the early and metastatic stages of colorectal cancer has also been demonstrated [22–24]. While the impact of 25(OH)D level and supplementation on prognosis provides more consistent data in colorectal cancer studies, mixed data are available for BC and the benefit of vitamin D supplementation in BC has not yet been fully elucidated [25–27]. This may be related to the difficulty in evaluating the isolated effect of vitamin D in BC due to the prognostic impact of many determinant parameters. In addition, due to possible differences in VDR, the desired 25(OH)D level may not be achieved despite vitamin D supplementation. Therefore, we aimed to evaluate the prognostic effect of vitamin D replacement in BC, considering the follow-up 25(OH)D values after replacement, together with all clinicopathological prognostic factors.

The aim of this study was to investigate the effect of vitamin D supplementation on disease recurrence and overall survival of BC patients after primary treatment.

Materials and methods

Early-stage BC patients who received treatment in Gaziantep University Hospital, Division of Medical Oncology between November 2012 and November 2019 were retrospectively enrolled in the study. All early-stage BC patients in stage I, II, and III regarding the 8th edition of American Joint Committee on Cancer (AJCC) criteria were scanned.

Patient with initial vitamin and patients with consecutive follow-up 25(OH)D levels after vitamin D replacement were evaluated. In total, 1136 patients were screened, then patients with metastatic stage at beginning and without 25(OH)D follow-up were excluded. 634 BC survivors who completed primary treatment for early-stage BC were enrolled in the study. The Eastern Cooperative Oncology Group (ECOG) performance status of all included patients was 0–1.

Patients' clinical features were recorded as follows: age, menopausal status, tumour pathological characteristics. Our study was single centred, and our adjuvant treatment recommendations were performed according to the patient's stage, hormone receptor level and HER-2 positivity. All patients who have a tumour size of 2 cm and above or positive lymph nodes were treated with anthracycline-based chemotherapy $\pm$ taxane $\pm$ 5-fluorouracil (5-FU) $\pm$ carboplatin. Chemotherapy decision was made for patients with tumour size smaller than 2 cm, taking into account the poor prognostic risk factors (nonluminal, HER2+, triple-negative group, high grade). All patients with HER-2-positive tumours were treated with 1 year treatment with anti-HER2 treatments.

Patients who have positive over 1% estrogen or progesterone receptor staining were treated with adjuvant endocrine therapy for at least 5 years. Patient follow-up was done every three months for the first 2 years, then every 6 months for up to 5 years, then once a year. The development of a new primary BC, contralateral BC, local regional

cancer recurrence, or distant metastasis was accepted as recurrence. Overall survival was defined as the date from BC diagnosis to death.

Vitamin D levels were measured as circulating 25-hydroxyvitamin D (25(OH)D) levels and recorded after completion of primary treatments. The serum total 25-hydroxyvitamin D [25(OH)D] levels were measured using chemiluminescence immunoassay on the UniCel DxI 800 analyser (Beckman Coulter, Inc., Brea, CA, USA). 25(OH)D levels demonstrated acceptable linearity on the analytical measuring range of 2.00–210 ng/mL. The intra assay CV < 4.7%, inter assay CV < 8.1% of the (25-OHD) measurements.

We accepted 25(OH)D levels according to the Endocrine Society guidelines; respectively the sufficiency of 25(OH)D levels greater than 30 ng/mL, the insufficiency of 25(OH) D levels at 20–29.9 ng/mL, and the failure of 25(OH) D levels below 20 ng/mL.

First, the patients divided into two groups; sufficient and insufficient 25(OH)D levels according to the 20 mg/mL cut off value. Then, vitamin D supplementation was given to patients whose 25(OH)D levels were less than 20 ng/mL. Then, cholecalciferol drops per oral 5000–8000 IU/day were suggested for 6–8 weeks. The median value of 25(OH)D was noted in post-supplementation follow ups.

According to the baseline value and median value after supplementation, patients were divided into as four groups; (1) adequate 25(OH)D at baseline and follow-up; (2) insufficient levels of 25(OH)D initially and insufficient after supplements; (3) insufficient initially, but adequate 25(OH)D levels after supplementation; (4) Adequate 25(OH)D levels in the beginning but insufficient in follow-up. Patients who were inadequate 25(OH)D users were also included in the group 2.

First, the association of 25(OH)D levels at baseline and survival was analysed, then the association of 25(OH)D according to follow up values and disease-free survival (DFS) and overall survival (OS) was analysed together with other clinic-pathologic/treatment related parameters.

Statistics

Clinicopathological characteristics were compared among the four groups with the chi-square test and/or Fischer-exact test. Quantitative variables were defined as means with standard deviation, while qualitative variables were described as proportional frequencies. Median observation time for all patients was considered as median follow-up. Survival distributions were determined by the Kaplan–Meier method and compared between groups with log-rank statistic for groups and all clinicopathological factors and post-hoc test was performed to determine subgroup differences. Then, adjustment for age, menopausal status, tumour stage, luminal type was performed. All potential prognostic factors with a probability value less than or equal to 0.05 were considered statistically significant and statistical analyses were performed with the statistical software package SPSS 22.0.

Results

Characteristics of the breast cancer patients

Totally, 634 patients were enrolled in the retrospective study. The median follow-up time was 75.7 (9.4–332.9) months. The mean age was 50.5 ± 10.9 years. Among all, 54

patients (8.5%) were stage I, 355 patients were stage II (55.9%), and 226 (35.6%) were stage III BC. Of the patients, 81% of patients had luminal A or B disease, 8% of the patients had her2 positive BC, and the remaining had triple negative disease.

The clinical and pathological features of the participants are shown in Table 1.

Definition of the groups based on 25(OH)D levels at initially and after vitamin D supplement treatment were given below.

- **Group 1;** adequate levels of 25(OH)D (32.27 ± 19.94) initially and adequate 25(OH)D level (29.67 ± 8.06) also after vitamin D supplement treatment,
- **Group 2;** insufficient levels of 25(OH)D (9.9 ± 4.1) initially and insufficient 25(OH)D level (11.19 ± 3.39) still after vitamin D supplement treatment,
- **Group 3;** insufficient levels of 25(OH)D (11.83 ± 4.61) initially, but adequate 25(OH)D level (25.89 ± 7.59) after vitamin D supplement treatment,

- **Group 4;** adequate levels of 25(OH)D (27.17 ± 6.84) in the beginning, but insufficient 25(OH)D level (14.37 ± 3.16) after vitamin D supplement treatment.

Accordingly, insufficient vitamin D values at baseline and follow-up (group 2) were more common in patients under 35 years of age, preperimenopausal and triple negative (<0.05 for all). On the other hand, blood sampling season and disease stage were well balanced among the four groups (Table 1).

Relationship between 25(OH)D and survival based on baseline level of 25(OH)D

While 122 of the patients (19.4%) had sufficient vitamin D levels at baseline, 512 of them (80.6%) had insufficient. According to baseline vitamin D levels, disease free survival

Table 1: Clinicopathological features of the patients according to 25(OH)D levels at baseline and during the follow-up.

Baseline characteristics	Group 1 (n = 87) n, %	Group 2 (n = 287) n, %	Group 3 (n = 225) n, %	Group 4 (n = 35) n, %	p-Value
Age					
<35	4 (7)	37 (64.9)	12 (21.1)	4 (7.0)	<i>0.009</i>
≥35	84 (14.5)	250 (43.3)	213 (36.9)	31 (5.4)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
25(OH)D levels after primary treatment	32.27 ± 19.94	9.9 ± 4.1	11.83 ± 4.61	27.17 ± 6.84	<i>0.001</i>
25(OH)D levels after vitamin D supplement treatment	29.67 ± 8.06	11.19 ± 3.39	25.89 ± 7.59	14.37 ± 3.16	<i>0.001</i>
	n (%)	n (%)	n (%)	n (%)	
Menopausal status					
Pre/peri-menopausal	48 (11.7)	207 (50.5)	137 (33.4)	18 (33.4)	<i>0.002</i>
Postmenopausal	40 (17.8)	80 (35.6)	88 (39.1)	17 (7.6)	
Stage					
I	10 (18.5)	19 (35.2)	21 (38.9)	4 (7.4)	<i>0.14</i>
II	56 (15.8)	156 (43.9)	128 (36.1)	15 (4.2)	
III	22 (9.7)	112 (49.6)	76 (33.6)	16 (7.1)	
Pathological types					
IDC	82 (14.4)	256 (44.8)	202 (35.4)	31 (5.4)	<i>0.79</i>
ILC	2 (6.5)	16 (51.6)	10 (32.3)	3 (9.7)	
Others	4 (12.1)	15 (45.5)	13 (39.4)	1 (3.0)	
Luminal types					
Luminal A or B	80 (15.6)	222 (43.4)	179 (35.0)	31 (6.1)	<i>0.008</i>
Her-2 enriched	3 (5.8)	22 (42.3)	25 (48.1)	2 (3.8)	
Triple negative	3 (4.3)	43 (62.3)	21 (30.4)	2 (2.9)	
Season of blood draw [n, %]					
Dec to Apr (higher)	48 (14.5)	149 (45.2)	120 (36.4)	13 (3.9)	<i>0.32</i>
May to Nov (lower)	40 (13.1)	138 (45.2)	105 (34.4)	22 (7.2)	

SD: standard deviation, Group 1: adequate 25(OH)D at baseline and follow-up; Group 2: insufficient levels of 25(OH)D initially and insufficient after supplements; Group 3: insufficient initially, but adequate 25(OH)D levels after supplementation; Group 4: adequate 25(OH)D levels in the beginning but insufficient in follow-up. IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma. Values in italic are $p < 0.05$ statistically significant values.

(HR: 1.746; 95%CI: 0.927–3.289, $p = 0.085$) and overall survival (HR: 1.396; 95%CI: 0.710–2.743, $p = 0.334$) were not different between the two groups.

in patients who had insufficient 25(OH)D level at baseline, better DFS was obtained, as in patients who had initial sufficient 25(OH)D level (Table 2).

Disease free survival according to clinicopathological factors and 25(OH)D groups

Among clinicopathological factors, stage and vitamin D groups were associated with worse DFS in univariate analysis (Table 2). In multivariate analysis included age, menopausal status, luminal type and stage, vitamin D groups had statistically independent prognostic parameter for DFS. Among the four groups, only group 2 defined as initial insufficient 25(OH)D levels and insufficient after supplementation, showed worse DFS than group 1 (OR = 3.30 95% CI = 1.19–7.65, $P = 0.020$). If sufficient 25(OH)D levels were achieved after vitamin D replacement

Overall survival according to clinicopathological factors and vitamin D groups

Among clinicopathological factors, pre-perimenopausal status, luminal types, and four groups were associated with worse OS in univariate analysis (Table 3). In the multivariate analysis, four groups were adjusted for age, because there was an imbalance between the four groups. Accordingly, pre-perimenopausal status, triple negative luminal types and 25(OH) D groups had a statistically independent effect on OS. Among the four groups, only group 2, defined as initial insufficient 25(OH)D level and insufficient after supplementation, showed worse OS than

Table 2: Disease free survivals according to clinicopathologic characteristics and 25(OH)D levels.

	Disease free survival					
	Univariate analysis			Multivariate analysis		
	OR	95%CI	p-Values	OR	95%CI	p-Value
Age						
<35	1.48	0.76–2.87	0.24	–		
≥35	1 (ref)					
Menopausal status						
Pre/peri-menopausal	1.09	0.71–1.69	0.69	–		
Postmenopausal	1 (ref)					
Stage						
I	1 (ref)			1 (ref)		
II	2.59	0.62–10.8	0.19	2.63	0.63–10.98	0.18
III	6.04	1.47–24.9	0.013	5.63	1.36–23.3	0.017
Pathological types						
IDC	0.66	0.20–2.01	0.48	–		
ILC	0.65	0.21–2.06	0.46			
Others						
Luminal types						
Luminal A or B	1 (ref)			1 (ref)		
Her-2 enriched	0.27	0.07–1.12	0.07	0.28	0.07–1.16	0.08
Triple negative	1.34	0.72–2.47	0.35	1.21	0.65–2.26	0.54
Vitamin D						
Group 1	1 (ref)			1 (ref)		
Group 2	3.46	1.38–8.66	0.008	3.02	1.19–7.65	0.020
Group 3	1.69	0.63–4.49	0.29	1.63	0.61–4.36	0.33
Group 4	2.82	0.86–9.25	0.087	2.34	0.71–7.73	0.16

Group 1: adequate 25(OH)D level at baseline and follow-up; Group 2: insufficient levels of 25(OH)D initially and insufficient after supplements; Group 3: insufficient initially, but adequate 25(OH)D levels after supplementation; Group 4: adequate 25(OH)D levels in the beginning but insufficient in follow-up. IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma. OR: odds ratio, CI: confidence interval. Multivariate analysis was performed for 25(OH)D level groups, stage, luminal types, age, and menopausal status. Menopausal status and age were included because there was imbalance among 25(OH)D level groups as was shown in Table 1. Values in italic are $p < 0.05$ statistically significant values.

Table 3: Overall survivals according to clinicopathologic characteristics and 25(OH)D levels.

	Overall survival					
	Univariate analysis			Multivariate analysis		
	OR	95%CI	p-Values	OR	95%CI	p-Value
Age						
<35	1.11	0.48–2.58	0.81	1.46	0.59–3.60	0.40
≥35	1 (ref)			1 (ref)		
Menopausal status						
Pre/peri-menopausal	1.79	1.10–2.93	0.019	2.09	1.24–3.52	0.005
Postmenopausal	1 (ref)			1 (ref)		
Stage						
I	1 (ref)					
II	0.69	0.26–1.77	0.43			
III	1.49	0.58–3.84	0.40			
Pathological types						
IDC	1 (ref)					
ILC	0.63	0.15–2.59	0.52			
Others	0.97	0.30–3.09	0.95			
Luminal types						
Luminal A or B	1 (ref)			1 (ref)		
Her-2 enriched	0.46	0.11–1.88	0.28	0.52	0.16–2.14	0.36
Triple negative	2.44	1.32–4.50	0.007	2.14	1.14–4.01	0.018
Vitamin D						
Group 1	1 (ref)			1 (ref)		
Group 2	3.31	1.19–9.21	0.022	3.26	1.16–9.19	0.025
Group 3	0.96	0.29–3.12	0.29	0.96	0.29–3.13	0.95
Group 4	3.06	0.86–10.9	0.084	2.99	0.84–10.6	0.091

Group 1: adequate 25(OH)D level at baseline and follow-up; Group 2: insufficient levels of 25(OH)D initially and insufficient after supplements; Group 3: insufficient initially, but adequate 25(OH)D levels after supplementation; Group 4: adequate 25(OH)D levels in the beginning but insufficient in follow-up. IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma. OR: odds ratio, CI: confidence interval. Multivariate analysis was performed for 25(OH)D level groups, luminal types, age, and menopausal status. Menopausal status and age were included because there was imbalance among 25(OH)D level groups as was shown in Table 1. Values in italic are $p < 0.05$ statistically significant values.

group I (OR = 3.26, 95% CI = 1.16–9.19, $p = 0.025$). If sufficient 25(OH)D levels were achieved after vitamin D replacement in patients who had insufficient 25(OH)D levels at baseline, better OS was obtained, as in patients who had sufficient 25(OH)D level at baseline (Table 3).

Life expectancy relationship with 25(OH)D levels at baseline and during the follow-up

According to log-rank test results, there was a significant difference between groups in terms of survival times ($P = 0.001$). Post-hoc analysis was showed that live expectancy was significantly higher in group II compared to group I ($P = 0.017$) and group III ($P = 0.001$). Group IV had significantly lower survival times than group III ($P = 0.021$) (Table 4). Median survival is defined as long as at least half of the observed patients die before the end of follow-up. In our study because mortality rate was lower than 50%,

median survival time cannot be not calculated. So, we used mean survival time to show the life expectancy.

Mortality relationship with 25(OH)D levels at baseline and during the follow-up

The risk of death in group II with low 25(OH)D levels was 4.2 times higher than in group I with high 25(OH)D levels. (OR = 4.17 (95% CI = 1.46–11.91), $P = 0.008$) and the risk of death in group IV whose 25(OH)D levels never increased was 4.3 times higher (OR = 4.29 (95% CI = 1.13–16.3)).

Discussion

In the present study, a positive relationship was observed between vitamin D supplementation after primary

Table 4: Life expectancy and mortality relationship with 25(OH)D levels at baseline and during the follow-up.

Variables	Group 1 (n = 87) n (%)	Group 2 (n = 287) n (%)	Group 3 (n = 225) n (%)	Group 4 (n = 35) n (%)	p-Value
Mortality [n, %]	4 (4.6)	48 (16.7)	10 (4.4)	6 (17.1)	<i>0.001</i>
OR [95%CI]	1 (reference)	4.17 [1.46–11.91]	0.97 [0.3–3.16]	4.29 [1.13–16.3]	
P		<i>0.008</i>	0.953	<i>0.032</i>	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
Mean survival times (year)	15.75 ± 2.17	19.00 ± 2.17	16.52 ± 0.3	14.40 ± 1.03	<i>0.001</i>

SE: standard error, Group 1: adequate 25(OH)D level at baseline and follow-up; Group 2: insufficient levels of 25(OH)D initially and insufficient after supplements; Group 3: insufficient initially, but adequate 25(OH)D levels after supplementation; Group 4: adequate 25(OH)D levels in the beginning but insufficient in follow-up. IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma. Values in italic are $p < 0.05$ statistically significant values.

treatment of BC. The recent studies have produced inconsistent result regarding the effect of 25(OH)D levels or supplementations on survival in BC [28–32]. While the intervention of some key determinant clinicopathologic prognostic factors in BC prognosis may play a role in this inconsistent result, 25(OH)D levels may not have reached the desired level even after supplementation due to VDR differences. Therefore, although initial 25(OH)D level is important, it should also be evaluated whether the post-supplementation level reaches optimal 25(OH)D levels. Therefore, we evaluated the impact of 25(OH)D levels on survival by also considering follow up values of 25(OH)D for the first time, accordingly patients were assessed under four groups. The most striking result about the study was that while initial insufficient 25(OH)D levels did not have a worse effect on DFS and OS, patients who had insufficient 25(OH)D even after vitamin D replacement showed worse DFS and OS compared to sufficient 25(OH)D levels after supplementation (Tables 2 and 3).

In our study, we confirmed that low 25(OH)D levels are associated with mortality, but similar to the Kanstrup et al. [33], we also found that women with high 25(OH)D levels were also associated with poor breast cancer survival (Table 4).

A prospective study conducted by Goodwin et al. evaluated the relationship between 25(OH)D level and BC prognosis in 512 Canadian survivors for the 11 years follow up after primary treatment. Among them, initial insufficient 25(OH)D concentration was related with higher distant recurrence and poor survival [28]. Although approximately a quarter of patients received vitamin D replacement, the effect of the vitamin D supplementation on survival was not clarified. In the present study, initial 25(OH)D levels was not prognostic, because some patients received vitamin D supplementation and after normalization of 25(OH)D levels, the prognosis was equivalent to those in patients with sufficient 25(OH)D levels at baseline.

The WHEL study evaluated the associations between 25(OH)D levels and BC recurrence. Some of the patients used vitamin D through diet or supplementation and 25(OH)D levels were assessed about 2 years after diagnosis. No relationship was indicated between 25(OH)D level and recurrence [29]. Therefore, it is crucial to evaluate 25(OH)D concentrations at diagnosis after primary therapy, as well as whether adequate 25(OH)D concentrations are achieved after supplementation.

Associations between risk of BC prognosis and supplement use (Vitamins A, B, C, D, E, and multivitamins) after primary treatment were evaluated in the After Breast Cancer Pooling Project (ABCP). The study assessed the impact of age at diagnosis, menopausal status, AJCC pathologic stage, estrogen/progesterone status, cancer therapy, and lifestyle habits, including smoking history, body mass index, exercise participation on survival. Among them, an extremely high percentage of 60.6% of patients reported an additional supplement use 1–5 years after diagnosis, of these, 27.5% of whom were multivitamin users. Accordingly, the use of any antioxidant supplement was not related with BC recurrence, while vitamin D replacement was associated with a reduced risk of recurrence in estrogen positive tumour, but it was unrelated in estrogen negative tumours [34]. In our study, luminal sub-type of the patients was not evaluated only according to ER/PR status, but also, we assessed under three groups; luminal A-B (ER-PR positive; her-2 negative); Her2-enriched; and triple negative sub-groups, which is a better prognostic classification in BC. Additionally, we adjusted for the four groups with other main prognostic parameters, including menopausal status, age, and stage. Then, better DFS and OS were obtained with sufficient 25(OH)D level at baseline or during the follow-up after vitamin D supplementation, independent of menopausal status age, stage, and luminal types. Thanks to this single centred study, treatment approaches including chemotherapy, radiotherapy, and endocrine therapies, that have an extremely high effect on

BC prognosis, have been standardized. Therefore, we can assess the prognostic effect of 25(OH)D level alone through treatment standardization and exact pathological classification, which are the dominant prognostic parameters in BC. Additionally, although vitamin D supplementation provided survival benefit according to the ABCPP study, we showed that although vitamin D supplementation is necessary in those with insufficient 25(OH)D concentration initially, patients with already sufficient 25(OH)D levels do not require supplementation as they show good prognosis. Thus, measurement of initial 25(OH)D concentration is an important tool to guide the clinicians as to which patients need vitamin D supplementations.

The recent retrospective study involving her-2 enriched early stage BC showed that vitamin D supplementation significantly increased DFS, and a trend towards OS benefit in multivariate analysis in patients treated with trastuzumab-based chemotherapy ($p = 0.026$) [35]. While 69.2% of patients presented 5-year DFS in patients who received vitamin D supplements, 48.3% of patients survived without recurrence in those patients did not use vitamin D supplement ($p = 0.02$). Addition to effect on the promotion of cancer cell apoptosis, decrease in cancer cell invasion and angiogenesis, immune modulation, and decrease in inflammatory mediators, vitamin D induces the VDR with disruption of downstream signalling and HER-2 signalling through the ErbB2/AKT/ERK pathway [36]. Therefore, its contribution appears to be more intense in her-2 enriched BC sub-type, however, we showed that vitamin D supplementation in all BC sub-types for DFS and OS are independent prognostic parameters.

The following sentences were revised in the limitations part of discussion section; the main limitation of the study is retrospective design. Due to retrospective design, we have no information about patients' lifestyles of the patients that could affect BC recurrence and survival, such as participants' diet, physical activity, and body mass index during follow up. On the other hand, we evaluated the four groups with multivariate analysis together with disease stage, pathological subtype, age, and menopausal status, which are main prognostic parameters. Although we have obtained valuable clues about the importance of vitamin D supplementation, our results need to be confirmed by prospectively designed studies together with the patients' lifestyle features. Additionally, the time of the blood sample taken was not determined by strict rules for initial and follow up measurements. However, after completion of primary treatment and follow-up evaluations to separate the four groups, the blood sample was selected based on consecutive median 25(OH)D values. Although, inadequate 25(OH)D levels at baseline and follow-up (group 2) was more common in patients

who were under 35 years old, in pre-perimenopausal, and with triple negative disease, which are poor prognostic clinicopathological features, we showed that initial and follow-up 25(OH)D levels were independently predictor tool for BC mortality. Additionally, prognosis was improving after successful vitamin D supplementation, for those patients who had initial insufficient 25(OH)D levels.

Conclusions

Vitamin D supplementation may play an important role in the response of the received treatments and provide lower mortality rate and better OFS and DFS to BC patients. However, we observed a sign of poorer BC survival still after sufficient vitamin D supplementation.

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Competing interests: The authors declare that they have no competing interests.

Informed consent: All participated in the study were informed about the study and inscribed permission forms were obtained.

Ethical approval: This study was accepted by the Gaziantep University Faculty of Medicine Ethics Committee with the decision dated 05.02.2020 and reference numbered 61 as non-pharmaceutical clinical research and it was worked in suitability with the Helsinki Declaration.

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