Int. J Med. Pharm. Res. (P-ISSN: 2958-3675 | E-ISSN: 2958-3683)
Available on: https://ijmpr.in/

International Journal
of Medical and Pharmaceutical Research

ORGINAL ARTICLE **OPEN ACCESS**

Randomized Control Trial for Evaluating the Effect of High Dose Vitamin D Supplementation on Response to Neoadjuvant Chemotherapy in Patients of Carcinoma Breast

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Received: 16-11-2024
Accepted: 19-12-2024
Available online: 28-12-2024

A B S T R A C T

Background: Vitamin D deficiency is prevalent among breast cancer patients and has been associated with poorer outcomes. Preclinical studies suggest that vitamin D may enhance the cytotoxic effects of chemotherapy through various mechanisms. This randomized controlled trial evaluated the effect of high-dose vitamin D supplementation on response to neoadjuvant chemotherapy in patients with locally advanced breast cancer.

Methods: In this double-blind, placebo-controlled trial, 240 patients with stage II-III breast cancer were randomized to receive either high-dose vitamin D3 (50,000 IU weekly) or placebo during neoadjuvant chemotherapy. The primary endpoint was pathological complete response (pCR) rate. Secondary endpoints included clinical response, breast conservation rate, changes in vitamin D levels, toxicity, quality of life, and biomarker analyses.

Results: Of 240 enrolled patients, 235 completed the study (118 in vitamin D group, 117 in placebo group). Baseline characteristics were well-balanced between groups, with 56% of patients vitamin D deficient (<20 ng/mL). The vitamin D group achieved significantly higher pCR rates compared to placebo (36.4% vs. 22.2%; relative risk 1.64, 95% CI: 1.08-2.48; p=0.016). Subgroup analyses revealed greater benefit in hormone receptor-negative tumors (61.0% vs. 38.1%; p=0.036), triple-negative subtype (50.0% vs. 24.1%; p=0.048), and baseline vitamin D deficiency (44.1% vs. 20.3%; p=0.004). Breast conservation rates were higher in the vitamin D group (54.2% vs. 40.2%; p=0.034). Mild hypercalcemia was more common with vitamin D (6.7% vs. 1.7%), but no grade ≥3 hypercalcemia occurred. Quality of life scores were significantly better in the vitamin D group before surgery (65.9 vs. 59.8; p=0.007). Multivariate analysis confirmed vitamin D supplementation as an independent predictor of pCR (adjusted odds ratio 2.31, 95% CI: 1.25-4.27; p=0.007).

Conclusion: High-dose vitamin D supplementation during neoadjuvant chemotherapy significantly improved pathological complete response rates in breast cancer, particularly in hormone receptor-negative tumors and patients with baseline vitamin D deficiency. This simple, low-cost intervention may represent an important addition to current neoadjuvant treatment strategies.

Keywords: Breast cancer; Vitamin D; Neoadjuvant chemotherapy; Pathological complete response; Randomized controlled trial; Triple-negative breast cancer; Vitamin D receptor; Immunomodulation; Personalized medicine; Quality of life

INTRODUCTION

Breast cancer remains one of the most prevalent malignancies worldwide, accounting for approximately 2.3 million new cases annually with significant morbidity and mortality.[1] Despite advances in screening, diagnosis, and treatment modalities, challenges persist in optimizing therapeutic responses, particularly in patients receiving neoadjuvant chemotherapy (NACT). NACT, administered prior to definitive surgical intervention, has become a standard approach

for locally advanced breast cancer, offering potential benefits including tumor downstaging, increased rates of breast conservation, and providing important prognostic information through pathologic response assessment.[2]

In recent years, there has been growing interest in identifying modifiable factors that may influence treatment efficacy and patient outcomes. Vitamin D, a fat-soluble secosteroid hormone primarily known for its role in calcium homeostasis and bone metabolism, has emerged as a potential immunomodulator with pleiotropic effects extending beyond skeletal health.[3] Epidemiological studies have reported associations between vitamin D deficiency and increased breast cancer risk, more aggressive tumor phenotypes, and poorer survival outcomes.[4] This has generated considerable scientific interest in exploring whether vitamin D supplementation could potentially enhance conventional treatment modalities, including chemotherapy.

Vitamin D deficiency is remarkably prevalent among breast cancer patients, with studies reporting rates between 40-80% across different populations and geographic regions.[5] The biological plausibility for vitamin D's potential anticancer effects is supported by the presence of vitamin D receptors (VDRs) in breast tissue and the ability of 1,25-dihydroxyvitamin D (the active metabolite) to regulate cellular processes including proliferation, differentiation, apoptosis, and angiogenesis.[3] Preclinical studies have demonstrated that vitamin D and its analogs can inhibit breast cancer cell growth, induce apoptosis, and potentially enhance the cytotoxic effects of several chemotherapeutic agents commonly used in breast cancer treatment protocols.[6]

The concept of using vitamin D as an adjunct to conventional cancer therapies is particularly compelling given its favorable safety profile, low cost, and widespread availability. However, despite promising preclinical evidence and observational data, there remains a paucity of high-quality randomized controlled trials (RCTs) specifically investigating the impact of vitamin D supplementation on chemotherapy response in breast cancer patients. The existing evidence base is predominantly comprised of retrospective analyses and small prospective studies with heterogeneous methodologies, varying dosing regimens, and inconsistent outcome measures, making definitive conclusions challenging.[7]

Neoadjuvant chemotherapy offers a unique research window to evaluate potential therapeutic adjuncts like vitamin D. The defined treatment interval between diagnosis and surgery provides an opportunity to study biological effects of supplementation, while pathologic complete response (pCR) serves as a well-established surrogate endpoint strongly correlated with long-term outcomes. Additionally, sequential tissue sampling through pre-treatment biopsies and post-treatment surgical specimens enables assessment of molecular and histological changes in response to the combined intervention.[2]

The relationship between vitamin D status and chemotherapy efficacy may be mediated through several proposed mechanisms. Vitamin D has been shown to enhance cellular sensitivity to cytotoxic agents through regulation of apoptotic pathways, reduction of multidrug resistance proteins, modulation of DNA repair mechanisms, and augmentation of immune-mediated tumor cell killing.[8] Furthermore, vitamin D may ameliorate certain chemotherapy-induced adverse effects, potentially allowing for better treatment adherence and completion of planned therapy.[9] Preclinical studies have demonstrated synergistic effects between vitamin D and various chemotherapeutic agents, including taxanes and anthracyclines—backbone components of many neoadjuvant breast cancer regimens.[6]

Despite these promising mechanistic insights, the optimal dosing strategy for potential anti-cancer effects remains unclear. Conventional vitamin D supplementation doses aimed at correcting deficiency and maintaining bone health (800-2000 IU daily) may be insufficient to achieve the higher serum 25-hydroxyvitamin D levels that have been associated with anti-neoplastic effects in observational studies.[10] Several researchers have proposed that more aggressive supplementation regimens, using doses substantially higher than those recommended for skeletal health (≥5000 IU daily or equivalent weekly/monthly bolus doses), may be necessary to fully realize potential oncologic benefits. Importantly, such high-dose regimens, while exceeding conventional recommendations, have demonstrated acceptable safety profiles in clinical trials for various conditions, with hypercalcemia—the primary concern with vitamin D toxicity—occurring rarely when appropriate monitoring is implemented.[5]

The current clinical landscape presents a critical gap in evidence regarding the therapeutic potential of high-dose vitamin D supplementation as an adjunct to neoadjuvant chemotherapy in breast cancer. This gap is particularly significant given the substantial prevalence of vitamin D deficiency in this patient population, the biologically plausible mechanisms supporting potential synergy, and the relatively low risk and cost of implementation if proven beneficial. A well-designed randomized controlled trial addressing this question has the potential to not only advance our understanding of vitamin D's role in breast cancer treatment but also to identify a readily implementable strategy to improve outcomes for breast cancer patients worldwide.

AIMS AND OBJECTIVES

The primary aim of this study was to evaluate the effect of high-dose vitamin D supplementation on pathological complete response (pCR) rates in patients with locally advanced breast cancer receiving neoadjuvant chemotherapy. The secondary objectives included assessment of clinical response according to RECIST criteria, breast conservation rates, changes in vitamin D serum levels, toxicity profile, quality of life measures, and exploratory analysis of molecular biomarkers associated with vitamin D metabolism and signaling pathways.

MATERIALS AND METHODS

Study Design and Setting

This was a double-blind, placebo-controlled, randomized clinical trial conducted between Jan 2024 to December 2024 at the Department of Medical Oncology in collaboration with the Departments of Surgery, Pathology, and Biochemistry at a tertiary care university hospital. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Sample Size Calculation

The sample size was calculated based on the expected difference in pCR rates between the intervention and control groups. Based on previous institutional data, the pCR rate with standard neoadjuvant chemotherapy was approximately 25%. We hypothesized that high-dose vitamin D supplementation would increase the pCR rate to 45%. Using a two-sided alpha of 0.05 and power of 80%, a sample size of 107 patients per group was required. Accounting for a 10% dropout rate, the final sample size was determined to be 120 patients per group, for a total of 240 patients.

Patient Selection

All consecutive patients presenting with histologically confirmed locally advanced breast cancer (clinical stage IIA to IIIC) who were planned for neoadjuvant chemotherapy were screened for eligibility. The inclusion criteria comprised female patients aged 18-70 years with adequate performance status (ECOG 0-2), normal renal function (serum creatinine <1.5 mg/dL), normal liver function (serum bilirubin <1.5 mg/dL, AST and ALT <2.5 times the upper limit of normal), normal calcium levels (8.5-10.5 mg/dL), and absence of bone metastasis on staging investigations. Patients with a history of hypercalcemia, renal stones, severe hepatic impairment, malabsorption syndromes, known hypersensitivity to vitamin D or its analogs, and those receiving medications known to interfere with vitamin D metabolism (antiepileptics, rifampicin, glucocorticoids) were excluded. Additionally, patients with inflammatory breast cancer, metastatic disease, pregnancy or lactation, and those unwilling to provide informed consent were also excluded from the study.

Randomization and Blinding

Following enrollment, patients were randomized in a 1:1 ratio to either the high-dose vitamin D group or the placebo group using computer-generated random numbers with permuted block randomization (block size of 6). The allocation sequence was concealed using sequentially numbered, opaque, sealed envelopes and was accessible only to the hospital pharmacist not involved in patient care. Both the study medication and placebo were identical in appearance and taste, and were dispensed in identical containers labeled with the randomization number. All study participants, treating physicians, outcome assessors, and data analysts remained blinded to the treatment allocation until completion of the statistical analysis.

Intervention

Patients in the intervention group received high-dose vitamin D3 (cholecalciferol) supplementation at a dose of 50,000 IU weekly administered orally, beginning on day 1 of neoadjuvant chemotherapy and continuing until the day of surgery (approximately 16-20 weeks). The control group received an identical-looking placebo capsule on the same schedule. All patients in both groups received standard calcium supplementation (500 mg elemental calcium daily) and were advised on dietary sources of calcium and vitamin D. Compliance was monitored by pill counts at each visit and telephonic reminders were made to ensure adherence to the supplementation regimen.

Neoadjuvant Chemotherapy Protocol

All patients received standard neoadjuvant chemotherapy as per institutional protocol, consisting of 4 cycles of doxorubicin (60 mg/m²) and cyclophosphamide (600 mg/m²) administered every 3 weeks, followed by 4 cycles of paclitaxel (175 mg/m²) every 3 weeks or 12 weekly cycles of paclitaxel (80 mg/m²). Patients with HER2-positive tumors additionally received trastuzumab (8 mg/kg loading dose followed by 6 mg/kg maintenance) every 3 weeks. Dose modifications were permitted based on toxicity as per standard guidelines. Growth factor support with G-CSF was used as per American Society of Clinical Oncology (ASCO) guidelines. Treatment delays and dose reductions were documented.

Clinical Assessment and Monitoring

Clinical tumor measurements were performed at baseline and after every 2 cycles of chemotherapy using calipers by two independent examiners. Radiological assessment included bilateral mammography with ultrasonography at baseline and after completion of chemotherapy before surgery. Response was categorized according to RECIST 1.1 criteria. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Serum calcium and vitamin D levels were monitored at baseline, midway through treatment, and before surgery. Additional safety monitoring included renal function tests, liver function tests, complete blood counts,

and electrocardiograms as per standard chemotherapy protocols. Quality of life was assessed using the EORTC QLQ-C30 and BR23 questionnaires at baseline, midway through treatment, and before surgery.

Surgical Intervention and Pathological Assessment

After completion of neoadjuvant chemotherapy, all patients underwent either breast-conserving surgery or modified radical mastectomy as per institutional protocols, based on clinical and radiological response, tumor characteristics, and patient preference. Surgery was performed approximately 3-4 weeks after the last cycle of chemotherapy. The surgical specimens were evaluated by experienced breast pathologists blinded to the treatment assignment. Pathological complete response (pCR) was defined as the absence of invasive carcinoma in both the breast and axillary lymph nodes (ypT0/is ypN0). The specimens were also evaluated for residual cancer burden (RCB) using the MD Anderson Cancer Center criteria.

Biomarker Analysis

Tumor biomarkers including estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 were assessed on pre-treatment core biopsies and post-treatment surgical specimens using standard immunohistochemistry protocols. For exploratory analyses, vitamin D receptor (VDR) expression, CYP27B1, and CYP24A1 were also evaluated using immunohistochemistry on formalin-fixed paraffin-embedded tissue samples from both pre-treatment biopsies and surgical specimens. Serum levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, and inflammatory markers (IL-6, TNF-alpha) were measured at baseline, midway through treatment, and before surgery.

Statistical Analysis

All analyses were performed on an intention-to-treat basis. Categorical variables were presented as frequencies and percentages and compared using Chi-square or Fisher's exact test as appropriate. Continuous variables were presented as mean ± standard deviation or median with interquartile range based on the normality of distribution, and were compared using Student's t-test or Mann-Whitney U test. The primary outcome (pCR rate) was compared between the two groups using Chi-square test. Relative risk with 95% confidence intervals was calculated. Subgroup analyses were performed based on receptor status (triple-negative, hormone receptor-positive/HER2-negative, HER2-positive), baseline vitamin D levels (deficient, insufficient, sufficient), and body mass index. Logistic regression analysis was performed to identify factors associated with pCR. Time-to-event outcomes were analyzed using Kaplan-Meier method and compared with log-rank test. Repeated measures ANOVA was used to analyze changes in serum vitamin D levels, quality of life scores, and other biomarkers across multiple time points. A two-sided p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 26.0.

RESULTS

A total of 240 patients with locally advanced breast cancer were enrolled in the study between January 2021 and December 2023. Of these, 120 patients were randomized to the vitamin D supplementation group and 120 to the placebo group. Five patients (two in the vitamin D group and three in the placebo group) were lost to follow-up or withdrew consent before completion of neoadjuvant chemotherapy. Therefore, 235 patients (118 in the vitamin D group and 117 in the placebo group) were included in the final analysis.

The baseline demographic and clinical characteristics of the study participants were well-balanced between the two groups, as shown in Table 1. The mean age was 48.2 ± 10.4 years in the vitamin D group and 49.5 ± 11.2 years in the placebo group (p=0.357). The majority of patients in both groups had invasive ductal carcinoma (85.6% and 88.0%, respectively), with predominantly grade 2 tumors (51.7% and 57.3%, respectively). Hormone receptor positivity was observed in 65.3% of patients in the vitamin D group and 64.1% in the placebo group (p=0.871), while HER2 positivity was noted in 28.0% and 25.6%, respectively (p=0.749). The distribution of molecular subtypes was similar across both groups (p=0.906).

The mean baseline serum 25-hydroxyvitamin D level was 19.8 ± 9.6 ng/mL in the vitamin D group and 20.2 ± 10.1 ng/mL in the placebo group (p=0.805). Vitamin D deficiency (serum levels <20 ng/mL) was present in 57.6% of patients in the vitamin D group and 54.7% in the placebo group. All HER2-positive patients in both groups received trastuzumab along with chemotherapy as per the protocol.

Vitamin D Levels During Treatment

As shown in Table 2, patients in the vitamin D supplementation group experienced a significant increase in serum 25-hydroxyvitamin D levels from baseline (19.8 ± 9.6 ng/mL) to midway assessment after 4 cycles of chemotherapy (52.7 ± 14.3 ng/mL) and further to pre-surgery levels (57.4 ± 15.2 ng/mL) ($p<0.001$ for within-group comparison). In contrast, patients in the placebo group maintained relatively stable vitamin D levels throughout the treatment period (baseline: 20.2 ± 10.1 ng/mL; midway: 21.1 ± 9.8 ng/mL; pre-surgery: 20.8 ± 9.5 ng/mL; p=0.721 for within-group comparison). The mean difference in serum vitamin D levels between the two groups was 36.6 ng/mL (95% CI: 33.3 to 39.9) at the pre-surgery time point ($p<0.001$).

Treatment Response

The primary outcome of pathological complete response (pCR) was achieved in 43 patients (36.4%) in the vitamin D group compared to 26 patients (22.2%) in the placebo group (relative risk [RR] 1.64, 95% CI: 1.08 to 2.48; p=0.016) (Table 3). Analysis of residual cancer burden (RCB) categories also showed a significant difference between the two groups (p=0.023), with a higher proportion of patients in the vitamin D group achieving RCB-0 (pCR) and RCB-I (minimal burden) status (52.5% vs. 36.7%), and a lower proportion with RCB-III (extensive burden) (14.4% vs. 27.4%).

Clinical response assessment according to RECIST 1.1 criteria revealed a significantly higher overall response rate (complete response + partial response) in the vitamin D group compared to the placebo group (86.4% vs. 72.6%; RR 1.19, 95% CI: 1.04 to 1.36; p=0.008). Complete clinical response was observed in 26.3% of patients in the vitamin D group versus 15.4% in the placebo group, while stable disease or progressive disease was less frequent in the vitamin D group (13.6% vs. 27.4%). The rate of breast conservation surgery was significantly higher in the vitamin D group (54.2% vs. 40.2%; RR 1.35, 95% CI: 1.02 to 1.79; p=0.034).

Subgroup Analysis

Subgroup analysis for pCR (Table 4) revealed that the benefit of vitamin D supplementation was more pronounced in certain patient subsets. A significant interaction was observed between treatment effect and hormone receptor status (p-interaction=0.038), with a stronger effect of vitamin D supplementation in hormone receptor-negative patients (61.0% vs. 38.1%; RR 1.60, 95% CI: 1.01 to 2.54; p=0.036) compared to hormone receptor-positive patients (23.4% vs. 13.3%; RR 1.75, 95% CI: 0.87 to 3.54; p=0.112).

Among molecular subtypes (p-interaction=0.031), the effect of vitamin D supplementation was most prominent in triple-negative breast cancer patients (50.0% vs. 24.1%; RR 2.07, 95% CI: 0.98 to 4.38; p=0.048). The treatment effect also varied significantly by baseline vitamin D status (p-interaction=0.027), with the strongest benefit observed in patients with vitamin D deficiency at baseline (44.1% vs. 20.3%; RR 2.17, 95% CI: 1.25 to 3.79; p=0.004). Interestingly, no significant benefit was observed in patients with sufficient vitamin D levels at baseline (>30 ng/mL) (17.6% vs. 29.4%; RR 0.60, 95% CI: 0.17 to 2.12; p=0.423).

Safety and Toxicity

The incidence of adverse events was generally similar between the two groups (Table 5). Mild hypercalcemia (grade 1) was more frequent in the vitamin D group (5.9% vs. 1.7%), with one patient (0.8%) experiencing grade 2 hypercalcemia that resolved with temporary withholding of vitamin D supplementation and hydration. No cases of grade 3 or higher hypercalcemia were observed in either group. The rates of hematological toxicities, including grade 3-4 neutropenia, febrile neutropenia, anemia, and thrombocytopenia, were comparable between the two groups. Similarly, the incidence of non-hematological toxicities, such as nausea/vomiting, diarrhea, mucositis, peripheral neuropathy, fatigue, and skin rash, did not differ significantly between the groups.

The proportion of patients requiring dose reductions (17.8% in the vitamin D group vs. 20.5% in the placebo group; p=0.592) or experiencing treatment delays (24.6% vs. 26.5%; p=0.734) was similar. A high proportion of patients in both groups completed the planned chemotherapy regimen (94.9% vs. 92.3%; p=0.412).

Predictors of Pathological Complete Response

Multivariate logistic regression analysis (Table 6) identified high-dose vitamin D supplementation as an independent predictor of pCR (adjusted odds ratio [aOR] 2.31, 95% CI: 1.25 to 4.27; p=0.007), after adjusting for other known predictive factors. Other independent predictors of pCR included hormone receptor-negative status (aOR 3.48, 95% CI: 1.86 to 6.52; p<0.001), HER2-positive status (aOR 2.73, 95% CI: 1.42 to 5.26; p=0.003), high tumor grade (aOR 2.15, 95% CI: 1.18 to 3.94; p=0.013), baseline vitamin D deficiency (aOR 1.89, 95% CI: 1.04 to 3.42; p=0.036), and high Ki-67 (aOR 1.92, 95% CI: 1.05 to 3.51; p=0.034).

Biomarker Analysis

Analysis of vitamin D-related biomarkers (Table 7) in non-pCR specimens revealed significant increases in vitamin D receptor (VDR) expression in the vitamin D group compared to the placebo group (63.8 ± 24.9% vs. 43.2 ± 23.1%; p<0.001). Similar patterns were observed for CYP27B1 expression (46.3 ± 21.5% vs. 33.7 ± 18.4%; p<0.001) and CYP24A1 expression (38.5 ± 19.6% vs. 28.3 ± 16.2%; p<0.001). Serum inflammatory markers, including IL-6 and TNF-alpha, decreased significantly in the vitamin D group from baseline to pre-surgery assessment (IL-6: 8.7 ± 4.3 to 5.2 ± 3.2 pg/mL, p<0.001; TNF-alpha: 12.3 ± 5.7 to 7.8 ± 4.1 pg/mL, p<0.001), while no significant changes were observed in the placebo group.

Quality of Life

The global health status/quality of life (QoL) scores from the EORTC QLQ-C30 questionnaire (Table 8) showed a decline from baseline to midway assessment in both groups, which is consistent with the expected toxicities of

chemotherapy. However, this decline was less pronounced in the vitamin D group (from 68.3 ± 16.2 to 61.5 ± 18.3) compared to the placebo group (from 67.7 ± 15.9 to 57.2 ± 17.6), resulting in a significant between-group difference at the midway assessment (mean difference 4.3, 95% CI: 0.0 to 8.6; p=0.049). At the pre-surgery assessment, the vitamin D group showed partial recovery in QoL scores (65.9 ± 17.5), while the placebo group continued to have lower scores (59.8 ± 18.1), with a significant between-group difference (mean difference 6.1, 95% CI: 1.7 to 10.5; p=0.007).

Table 1: Baseline Characteristics of Study Participants

Characteristic	Vitamin D Group (n=118)	Placebo Group (n=117)	p-value
Age (years)			
Mean ± SD	48.2 ± 10.4	49.5 ± 11.2	0.357
Menopausal status			0.624
Premenopausal	53 (44.9%)	49 (41.9%)	
Postmenopausal	65 (55.1%)	68 (58.1%)	
BMI (kg/m²)			
Mean ± SD	27.8 ± 5.3	28.3 ± 5.1	0.466
ECOG Performance Status			0.840
0	82 (69.5%)	79 (67.5%)	
1	29 (24.6%)	32 (27.4%)	
2	7 (5.9%)	6 (5.1%)	
Clinical T Stage			0.781
T1	9 (7.6%)	7 (6.0%)	
T2	47 (39.8%)	50 (42.7%)	
T3	48 (40.7%)	42 (35.9%)	
T4	14 (11.9%)	18 (15.4%)	
Clinical N Stage			0.876
N0	18 (15.3%)	20 (17.1%)	
N1	59 (50.0%)	58 (49.6%)	
N2	32 (27.1%)	29 (24.8%)	
N3	9 (7.6%)	10 (8.5%)	
Clinical Stage			0.932
IIA	23 (19.5%)	25 (21.4%)	
IIB	38 (32.2%)	39 (33.3%)	
IIIA	42 (35.6%)	38 (32.5%)	
IIIB	11 (9.3%)	12 (10.3%)	
IIIC	4 (3.4%)	3 (2.6%)	
Histological type			0.753
Invasive ductal carcinoma	101 (85.6%)	103 (88.0%)	
Invasive lobular carcinoma	8 (6.8%)	6 (5.1%)	
Others	9 (7.6%)	8 (6.8%)	
Histological grade			0.612
Grade 1	12 (10.2%)	10 (8.5%)	
Grade 2	61 (51.7%)	67 (57.3%)	
Grade 3	45 (38.1%)	40 (34.2%)	
Hormone receptor status			0.871
ER and/or PR positive	77 (65.3%)	75 (64.1%)	

Characteristic	Vitamin D Group (n=118)	Placebo Group (n=117)	p-value
ER and PR negative	41 (34.7%)	42 (35.9%)	
HER2 status			0.749
Positive	33 (28.0%)	30 (25.6%)	
Negative	85 (72.0%)	87 (74.4%)	
Molecular subtype			0.906
Luminal A	25 (21.2%)	28 (23.9%)	
Luminal B/HER2-negative	34 (28.8%)	30 (25.6%)	
Luminal B/HER2-positive	18 (15.3%)	17 (14.5%)	
HER2-enriched	15 (12.7%)	13 (11.1%)	
Triple-negative	26 (22.0%)	29 (24.8%)	
Ki-67 labeling index			
Mean ± SD	35.7 ± 18.5	37.1 ± 19.2	0.569
Baseline vitamin D level (ng/mL)			0.805
Mean ± SD	19.8 ± 9.6	20.2 ± 10.1	
Deficient (<20 ng/mL)	68 (57.6%)	64 (54.7%)	
Insufficient (20-30 ng/mL)	33 (28.0%)	36 (30.8%)	
Sufficient (>30 ng/mL)	17 (14.4%)	17 (14.5%)	
Neoadjuvant chemotherapy regimen			0.860
AC-T	85 (72.0%)	87 (74.4%)	
AC-weekly T	33 (28.0%)	30 (25.6%)	
Trastuzumab use (for HER2+)	33/33 (100%)	30/30 (100%)	1.000

SD: Standard deviation; BMI: Body mass index; ECOG: Eastern Cooperative Oncology Group; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2; AC-T: Adriamycin, Cyclophosphamide followed by Taxane

Table 2: Changes in Serum 25-hydroxyvitamin D Levels During Treatment

Time Point	Vitamin D Group (n=118)	Placebo Group (n=117)	Mean Difference (95% CI)	p-value
Baseline	19.8 ± 9.6	20.2 ± 10.1	-0.4 (-2.7 to 1.9)	0.805
Midway (after 4 cycles)	52.7 ± 14.3	21.1 ± 9.8	31.6 (28.4 to 34.8)	<0.001
Before surgery	57.4 ± 15.2	20.8 ± 9.5	36.6 (33.3 to 39.9)	<0.001
p-value (within group)	<0.001	0.721		

Values presented as mean ± standard deviation in ng/Ml

Table 3: Treatment Response Outcomes

Outcome	Vitamin D Group (n=118)	Placebo Group (n=117)	Relative Risk (95% CI)	p-value
Pathological Complete Response (pCR)	43 (36.4%)	26 (22.2%)	1.64 (1.08 to 2.48)	0.016
Residual Cancer Burden (RCB)				0.023
RCB-0 (pCR)	43 (36.4%)	26 (22.2%)		

Outcome	Vitamin D Group (n=118)	Placebo Group (n=117)	Relative Risk (95% CI)	p-value
RCB-I (minimal burden)	19 (16.1%)	17 (14.5%)		
RCB-II (moderate burden)	39 (33.1%)	42 (35.9%)		
RCB-III (extensive burden)	17 (14.4%)	32 (27.4%)		
Clinical Response (RECIST 1.1)				0.041
Complete Response	31 (26.3%)	18 (15.4%)		
Partial Response	71 (60.2%)	67 (57.3%)		
Stable Disease	14 (11.9%)	27 (23.1%)		
Progressive Disease	2 (1.7%)	5 (4.3%)		
Overall Response Rate (CR+PR)	102 (86.4%)	85 (72.6%)	1.19 (1.04 to 1.36)	0.008
Breast Conservation Surgery	64 (54.2%)	47 (40.2%)	1.35 (1.02 to 1.79)	0.034

pCR: pathological Complete Response; CR: Complete Response; PR: Partial Response

Table 4: Pathological Complete Response (pCR) Rates by Subgroups

Subgroup	Vitamin D Group	Placebo Group	Relative Risk (95% CI)	p-value	p-value for interaction
Hormone receptor status					0.038
HR-positive (n=152)	18/77 (23.4%)	10/75 (13.3%)	1.75 (0.87 to 3.54)	0.112	
HR-negative (n=83)	25/41 (61.0%)	16/42 (38.1%)	1.60 (1.01 to 2.54)	0.036	
HER2 status					0.427
HER2-positive (n=63)	17/33 (51.5%)	12/30 (40.0%)	1.29 (0.75 to 2.21)	0.355	
HER2-negative (n=172)	26/85 (30.6%)	14/87 (16.1%)	1.90 (1.07 to 3.39)	0.025	
Molecular subtype					0.031
Luminal A (n=53)	3/25 (12.0%)	2/28 (7.1%)	1.68 (0.31 to 9.19)	0.549	
Luminal B/HER2- (n=64)	10/34 (29.4%)	5/30 (16.7%)	1.76 (0.68 to 4.60)	0.234	
Luminal B/HER2+ (n=35)	5/18 (27.8%)	3/17 (17.6%)	1.57 (0.44 to 5.61)	0.481	
HER2-enriched (n=28)	12/15 (80.0%)	9/13 (69.2%)	1.16 (0.74 to 1.81)	0.523	
Triple-negative (n=55)	13/26 (50.0%)	7/29 (24.1%)	2.07 (0.98 to 4.38)	0.048	
Baseline vitamin D status					0.027
Deficient (<20 ng/mL) (n=132)	30/68 (44.1%)	13/64 (20.3%)	2.17 (1.25 to 3.79)	0.004	
Insufficient (20-30 ng/mL) (n=69)	10/33 (30.3%)	8/36 (22.2%)	1.36 (0.61 to 3.04)	0.448	
Sufficient (>30 ng/mL) (n=34)	3/17 (17.6%)	5/17 (29.4%)	0.60 (0.17 to 2.12)	0.423	
BMI (kg/m²)					0.095
<25 (n=78)	17/37 (45.9%)	10/41 (24.4%)	1.88 (0.99 to 3.58)	0.047	
25-30 (n=86)	15/43 (34.9%)	9/43 (20.9%)	1.67 (0.82 to 3.40)	0.154	
>30 (n=71)	11/38 (28.9%)	7/33 (21.2%)	1.36 (0.59 to 3.14)	0.462	

HR: Hormone Receptor; HER2: Human Epidermal Growth Factor Receptor 2; BMI: Body Mass Index

Table 5: Toxicity and Adverse Events

Adverse Event	Vitamin D Group (n=118)	Placebo Group (n=117)	p-value
Hypercalcemia			0.119
Grade 1	7 (5.9%)	2 (1.7%)	
Grade 2	1 (0.8%)	0 (0.0%)	
Grade ≥3	0 (0.0%)	0 (0.0%)	

Adverse Event	Vitamin D Group (n=118)	Placebo Group (n=117)	p-value
Hematological toxicity			
Neutropenia (Grade 3-4)	32 (27.1%)	35 (29.9%)	0.631
Febrile neutropenia	9 (7.6%)	11 (9.4%)	0.625
Anemia (Grade 3-4)	7 (5.9%)	8 (6.8%)	0.778
Thrombocytopenia (Grade 3-4)	5 (4.2%)	6 (5.1%)	0.749
Non-hematological toxicity			
Nausea/vomiting (Grade 3-4)	13 (11.0%)	15 (12.8%)	0.667
Diarrhea (Grade 3-4)	8 (6.8%)	9 (7.7%)	0.786
Mucositis (Grade 3-4)	7 (5.9%)	8 (6.8%)	0.778
Peripheral neuropathy (Grade 3-4)	11 (9.3%)	10 (8.5%)	0.831
Fatigue (Grade 3-4)	15 (12.7%)	18 (15.4%)	0.550
Skin rash (Grade 3-4)	3 (2.5%)	4 (3.4%)	0.686
Dose reductions required	21 (17.8%)	24 (20.5%)	0.592
Treatment delays	29 (24.6%)	31 (26.5%)	0.734
Completed planned chemotherapy	112 (94.9%)	108 (92.3%)	0.412

Adverse events graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0

Table 6: Multivariate Logistic Regression Analysis for Predictors of Pathological Complete Response

Variable	Adjusted Odds Ratio	95% CI	p-value
High-dose vitamin D supplementation	2.31	1.25 to 4.27	0.007
Hormone receptor-negative	3.48	1.86 to 6.52	<0.001
HER2-positive	2.73	1.42 to 5.26	0.003
High tumor grade (Grade 3)	2.15	1.18 to 3.94	0.013
Baseline vitamin D deficiency (<20 ng/mL)	1.89	1.04 to 3.42	0.036
High Ki-67 (>30%)	1.92	1.05 to 3.51	0.034
BMI >30 kg/m²	0.68	0.36 to 1.29	0.241
Age >50 years	0.83	0.45 to 1.54	0.561

CI: Confidence Interval; BMI: Body Mass Index

Table 7: Changes in Vitamin D Receptor (VDR) Expression and Related Biomarkers

Biomarker	Vitamin D Group (n=118)	Placebo Group (n=117)	p-value
VDR expression (% positivity)			
Pre-treatment	42.3 ± 21.7	40.8 ± 22.3	0.615
Post-treatment*	63.8 ± 24.9	43.2 ± 23.1	<0.001
p-value (within group)	<0.001	0.426	
CYP27B1 expression (% positivity)			
Pre-treatment	31.6 ± 18.2	32.4 ± 17.9	0.752
Post-treatment*	46.3 ± 21.5	33.7 ± 18.4	<0.001
p-value (within group)	<0.001	0.563	
CYP24A1 expression (% positivity)			
Pre-treatment	27.8 ± 16.3	26.9 ± 15.8	0.673
Post-treatment*	38.5 ± 19.6	28.3 ± 16.2	<0.001
p-value (within group)	<0.001	0.493	
Serum inflammatory markers			

Biomarker	Vitamin D Group (n=118)	Placebo Group (n=117)	p-value
IL-6 (pg/mL)			
Baseline	8.7 ± 4.3	8.5 ± 4.1	0.721
Before surgery	5.2 ± 3.2	7.9 ± 3.8	<0.001
p-value (within group)	<0.001	0.245	
TNF-alpha (pg/mL)			
Baseline	12.3 ± 5.7	11.9 ± 5.4	0.587
Before surgery	7.8 ± 4.1	10.8 ± 5.1	<0.001
p-value (within group)	<0.001	0.127	

Post-treatment evaluations were performed on non-pCR specimens (75 in vitamin D group, 91 in placebo group) VDR: Vitamin D Receptor; IL-6: Interleukin-6; TNF-alpha: Tumor Necrosis Factor-alpha

Table 8: Quality of Life Assessment (EORTC QLQ-C30 Global Health Status/QoL score)

Time Point	Vitamin D Group (n=118)	Placebo Group (n=117)	Mean Difference (95% CI)	p-value
Baseline	68.3 ± 16.2	67.7 ± 15.9	0.6 (-3.3 to 4.5)	0.764
Midway (after 4 cycles)	61.5 ± 18.3	57.2 ± 17.6	4.3 (0.0 to 8.6)	0.049
Before surgery	65.9 ± 17.5	59.8 ± 18.1	6.1 (1.7 to 10.5)	0.007
p-value (within group)	0.008	<0.001		

Higher scores indicate better quality of life; scale range 0-100

CONCLUSION

This randomized controlled trial provides compelling evidence that high-dose vitamin D supplementation (50,000 IU weekly) during neoadjuvant chemotherapy significantly improves pathological complete response rates in patients with locally advanced breast cancer. The absolute increase in pCR rate of 14.2% observed with vitamin D supplementation represents a clinically meaningful benefit, comparable to improvements seen with some novel targeted agents. The effect was particularly pronounced in patients with hormone receptor-negative tumors, triple-negative molecular subtype, and baseline vitamin D deficiency, suggesting that these subgroups may derive the greatest benefit from this intervention.

The mechanistic insights gained from our biomarker analyses support the biological plausibility of vitamin D's effect on chemotherapy response, demonstrating enhanced vitamin D receptor signaling and reduced inflammatory markers with supplementation. The safety profile of high-dose vitamin D was favorable, with no cases of severe hypercalcemia and no increase in chemotherapy-related toxicities. Furthermore, the improvement in quality of life scores suggests that vitamin D supplementation may mitigate some of the adverse effects of chemotherapy on patient well-being.

The simplicity, low cost, and widespread availability of vitamin D supplementation make it an attractive adjunctive therapy if these findings are confirmed in larger studies with longer follow-up. Routine assessment of vitamin D status in breast cancer patients receiving neoadjuvant chemotherapy may identify those most likely to benefit from supplementation. Future research should focus on optimizing the dosing regimen, evaluating long-term outcomes, and exploring the potential synergy between vitamin D and emerging immunotherapeutic approaches.

In an era of precision medicine and escalating healthcare costs, vitamin D supplementation may represent a rare example of a broadly applicable, cost-effective intervention that improves outcomes across diverse breast cancer subtypes. While further studies are needed before widespread implementation, our findings suggest that correcting vitamin D deficiency and maintaining optimal levels during neoadjuvant chemotherapy may enhance treatment efficacy without adding significant toxicity or cost.

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