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Research Article

Negative Impact of Vitamin D Deficiency at Diagnosis on Breast Cancer Survival: A Prospective Cohort Study

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Objective. We prospectively evaluated the association between vitamin D concentration at diagnosis and overall survival (OS), disease-free survival (DFS), and cancer-specific survival (CSS) in postmenopausal women treated for breast cancer. *Methods*. This study included 192 patients newly diagnosed with invasive breast cancer, aged ≥45 years, and serum 25-hydroxy vitamin D (25 (OH)D) concentration assessment at diagnosis. Patients were classified into groups according to 25 (OH)D concentrations: sufficient (≥30 ng/mL), insufficient (between 20 and 29 ng/mL), and deficient (<20 ng/mL). The primary outcome was OS, and the secondary outcomes were DFS and CSS. The Kaplan–Meier curve and Cox regression model were used to assess the association between 25 (OH)D concentrations and survival rates. Differences in survival were evaluated by hazard ratios (HRs). *Results*. The mean age was 61.3 ± 9.6 years, 25 (OH)D concentration was 26.9 ± 7.5 ng/mL (range 12.0–59.2 ng/mL), and the follow-up period was between 54 and 78 months. Sufficient 25 (OH)D was detected in 33.9% of patients, insufficient in 47.9%, and deficient in 18.2%. A total of 51 patients (26.6%) died during the study period, with a mean OS time of 54.4 ± 20.2 months (range 9–78 months). Patients with 25 (OH)D deficiency and insufficiency at diagnosis had a significantly lower OS, DFS, and CSS compared with patients with sufficient values ($p < 0.001$). After adjustment for clinical and tumoral prognostic factors, patients with 25 (OH) D concentrations considered deficient at diagnosis had a significantly higher risk of global death (HR, 4.65; 95% CI, 1.65–13.12), higher risk of disease recurrence (HR, 6.87; 95% CI, 2.35–21.18), and higher risk of death from the disease (HR, 5.91; 95% CI, 1.98–17.60) than the group with sufficient 25(OH)D concentrations. *Conclusion*. In postmenopausal women treated for breast cancer, vitamin D deficiency and insufficiency at diagnosis were independently associated with lower OS, DFS, and CSS compared with patients with sufficient 25(OH)D concentrations.

1. Introduction

Breast cancer is the type of cancer that mostly affects women in the world, in both developing and developed countries, with about 2.3 million new cases in 2020, comprising 25% of all cancers diagnosed in women [1]. In Brazil, the National Cancer Institute (INCA) estimates 66,280 new cases of breast cancer, for each year of the 2020–2022 triennium [2]. According to data from the American Cancer Society, the 5- and 10-year relative survival rates for women with invasive breast cancer are 90% and 84%, respectively [1]. Despite being considered a relatively good prognosis cancer if diagnosed and treated in a timely manner, the AMAZONA study demonstrated that Brazilian women have a higher risk

of being diagnosed with late-stage breast cancer and at a younger age than women in high-income countries [3].

Vitamin D concentration can be considered a prognostic factor in women with breast cancer [4, 5]. Vitamin D is a steroid hormone that has various physiologic effects on several tissues [6]. The major source of vitamin D is endogenous synthesis in the skin (dermis and epidermis). When the skin is exposed to sunlight, 7-dehydrocholesterol absorbs UVB radiation, leading to chemical bonds within the 7-dehydrocholesterol molecule to break and rearrange, resulting in the formation of previtamin D_3. In the skin, previtamin D_3 undergoes rapid thermally induced transformation to vitamin D_3. Cutaneously synthesized vitamin D_3 is released from the plasma membrane and is transported to the liver, where it is hydroxylated at carbon 25 to 25-hydroxy vitamin D (25 (OH)D). The activation of vitamin D requires hydroxylation of 25 (OH)D at position 1 in the proximal renal tubules. This step is catalyzed by the enzyme 1α-hydroxylase, which converts 25 (OH)D to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the biologically active form of vitamin D [7, 8].

The main organs where vitamin D acts are those involved in calcium homeostasis, including bones, intestine, and kidneys, but most tissues of the body express vitamin D receptor (VDR), including the mammary gland [6]. These receptors were also present in a breast cancer cell line, suggesting a possible association between vitamin D and cancer [9]. The effects of the active form of vitamin D ($1,25(OH)_2D$) on the breast are mediated by VDR, which controls the expression of genes that regulate antineoplastic actions, such as cell proliferation, differentiation, and apoptosis, which is in accordance with scientific evidence linking hypovitaminosis D to breast cancer incidence and mortality [10, 11]. The antiproliferative effect of $1,25(OH)_2D$ cancer cells has since been confirmed in most normal and cancerous cells whereby $1,25(OH)_2D$ especially inhibits cell cycle progression at the G1 stage. The VDR was not a marker for malignancy but might play a role in the pathogenesis or evolution of cancer [9]. Demonstrating the role of vitamin D in cancer mortality, an updated meta-analysis of randomized controlled trials (RCTs) found that vitamin D supplementation was associated with 13% reduced cancer mortality over 3–10 years of follow-up [12].

Vitamin D deficiency is common in postmenopausal breast cancer women, and some evidence suggests that low vitamin D status increases the risk of disease development or progression [5, 8]. A recent systematic review and meta-analysis of studies reporting data on the role of vitamin D in patients with newly diagnosed breast cancer found that 67.4% of patients with breast cancer had a baseline 25 (OH) D concentration below 30 ng/mL, whereas this percentage was 33.7% in the control group. A high prevalence of vitamin D insufficiency observed in patients with newly diagnosed breast cancer may have a physiopathological link to breast cancer development and progression [6].

The impact of vitamin D deficiency at the time of diagnosis on the outcome of patients with breast cancer is less well understood. A meta-analysis based on five studies assessing the relation between vitamin D and breast cancer mortality revealed that high serum 25 (OH)D concentrations were associated with a lower mortality rate. Patients with concentrations in the highest quartile had approximately half of the breast cancer mortality rate when compared to those with the lowest quartile. However, more clinical trials should be designed to confirm this association [13]. Another systematic review identified six studies that evaluated the association of circulating 25 (OH)D with the prognosis in patients with breast cancer. Elevated 25 (OH)D concentrations were associated with a significantly better survival in two studies, a borderline better survival in two studies, and no impact on survival in the other two studies [14]. In view of the increasing number of breast cancer survivors and the high prevalence of vitamin D deficiency among patients with breast cancer, an evaluation of the role of vitamin D in prognosis and survival among patients with breast cancer is essential. Therefore, in our study, we aimed at evaluating the association between serum vitamin D concentrations at diagnosis and overall survival, disease-free survival, and cancer-specific survival in postmenopausal women treated for breast cancer.

2. Patients and Methods

2.1. Study Design and Sample Selection. This study is a single-center prospective cohort. The population group comprised 192 postmenopausal women diagnosed with breast cancer who attended the Breast Disease Assessment Center of the University Hospital in Southeastern Brazil during 2014–2016. Women with the following characteristics were included: histological diagnosis of breast cancer, last menstruation at least 12 months prior to presentation, age of 45 years or older, and serum vitamin D measurement at the time of breast cancer diagnosis, before any cancer treatment. Exclusion criteria included pharmacological use of any dose of vitamin D; *in situ* or bilateral breast cancer; personal history of other cancers; patients in neoadjuvant chemotherapy; renal failure (creatinine >1.4 mg/dL); liver diseases; abusive alcohol consumption; and grade III obesity. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki. Informed consent was obtained from all individual participants included in this study. Ethical approval was awarded by the Research Ethics Committee of the Botucatu Medical School, Sao Paulo State University (UNESP).

2.2. Clinical Assessments. All participants underwent individual interviews in which the following data were collected: age, menopausal age, time since menopause, parity, current smoking, previous use of hormone therapy, and history of chronic diseases. Smokers were defined as patients who reported smoking, regardless of the number of cigarettes smoked. Anthropometric data included weight, height, and body mass index (BMI; weight/height2). BMI was classified according to the system used by the World Health Organization in 2002: less than 24.9 kg/m2 as normal, from 25 to 29.9 kg/m2 as overweight, from 30 to 34.9 kg/m2 as grade I

obesity, from 35 to 39.9 kg/m2 as grade II obesity, and 40 kg/m2 or greater as grade III obesity.

2.3. Vitamin D Measurement. Serum 25 (OH)D assessment was collected immediately after diagnosis of breast cancer prior to any proposed treatment, approximately 20 to 30 days post-diagnosis. For serum 25 (OH)D dosage, we used the Architect 25-OH Vitamin D Assay Kit by the ARCHITECT® i2000 Analyzer (Abbott®, Santa Clara, California, USA), which is based on the chemiluminescent microparticle immunoassay (CMIA). The detection limit was 1.9 ng/mL and the intra- and inter-assay coefficient of variation was <10%, as described in the assay kit. The reference range was 0.0–160.0 ng/mL, according to the method. Serum concentrations ≥30 ng/mL were considered sufficient, that between 20 and 29 ng/mL were considered insufficient, and that <20 ng/mL were considered deficient [15]. All laboratory tests were processed by the Clinical Analysis Laboratory of Botucatu Medical School.

2.4. Tumor Features. Breast cancer tumors were classified according to established prognostic characteristics: tumor size, type and histological staging, grade, axillary lymph node status, hormone receptor status (estrogen receptor, ER; progesterone receptor, PR), human epidermal growth factor receptor 2 (HER2), and epithelial proliferative activity (Ki-67). Tumor diameter was obtained from histopathological reports and histologically graded as grade 1 (well-differentiated), grade 2 (moderately differentiated), and grade 3 (undifferentiated) according to the method proposed by Elston and Ellis (1993), which uses architectural aspects, nuclear differentiation levels, and mitotic index as criteria. Axillary lymph node status was classified as positive lymph node involvement if there was at least one positive lymph node according to clinical and/or histopathological examination. Tumors were categorized according to the 8th Edition of the American Joint Committee on Cancer (AJCC) TNM system (tumor size and extension, lymph node status, metastasis) [16], grouped into stages (I-IV): stage I (T1N0M0), stage II (IIA—T0N1M0, T1N1M0, and T2N0M0 and IIB—T2N1M0 and T3N0M0), stage III (IIIA—T0N2M0, T1N2M0, T2N2M0, T3N1M0, and T3N2M0, stage IIIB—T4N0M0, T4N1M0, and T4N2M0, and stage IIIC—any TN3M0), and stage IV (any *T,* any N, and M1) [16].

Hormone receptor status and epithelial proliferative activity were assessed by standard methods, using immunohistochemistry (IHC) for ER, PR, HER2, and Ki-67 status performed according to the streptavidin-biotin-peroxidase complex methods (DakoCytomation®, Glostrup, Denmark). ER and PR were considered positive if >1% of cells were stained positive on IHC [17]. Ki-67 was considered high if > 20% of the tumor cells were labeled [18]. HER2 was considered positive if the fluorescence in situ hybridization (FISH) test, systematically performed in all IHC 2+ tumors, showed HER2 genomic amplification or, in the absence of FISH, if IHC was 3+ [19]. All histopathological and immunohistochemical analyses were performed by the Department of Pathology of Botucatu Medical School.

Breast cancer was then categorized into four molecular subtypes based on surrogate immunohistochemical profiles: 1—luminal HER-negative (ER-positive and/or PR-positive, HER2-negative); 2—luminal HER2-positive (ER-positive and/or PR-positive and HER2-positive); 3—HER2-enriched (ER- and PR-negative and HER2-positive); and 4—triple-negative (ER-, PR-, and HER2-negative) [20].

2.5. Statistical Analysis. The estimation of sample size was based on the study by Kermani et al. [21] who found a difference in the HER2 + between women with and without vitamin deficiency (12 and 2 cases, respectively). Considering the difference between the values and correcting the estimate for type I (5%) and type II (20%) errors attributed to the study, the estimated sample size was at least 153 women with breast cancer. From all data, tables of clinical variables and parameters in each group were created according to serum 25 (OH)D concentrations in sufficient ($n = 65$), insufficient ($n = 92$), and deficient ($n = 35$). For data analysis, mean and standard deviation were calculated for quantitative variables and frequency and percentage for qualitative variables. The following variables were assessed: 1—clinical (age, parity, age and time since menopause, past use of menopausal hormone therapy, hypertension, diabetes, smoking, and BMI); 2—serum dosage of 25 (OH)D; and 3—anatomopathological and immunohistochemistry data of breast cancer (type and histopathological grade, tumor size, tumor stage, axillary lymph node status, ER, PR, HER2, and Ki-67). For comparison between groups in terms of quantitative characteristics, ANOVA and gamma distribution (asymmetric variables) were used. In the association between frequencies of categorical characteristics, the chi-square test was used.

The primary outcome was overall survival (OS), and the secondary outcomes were disease-free survival (DFS) and cancer-specific survival (CSS). Overall survival was defined as the time interval between date of diagnosis and date of death (related to breast cancer or death from any cause). Disease-free survival was defined as the time interval between date of diagnosis and date of the first recurrence or death, whichever came first. Recurrence was considered as any local, regional, or distant tumor recurrence. Cancer-specific survival was defined as the time interval between date of diagnosis and date of death related to breast cancer. Follow-up data were collected until December 31, 2020.

To calculate survival outcomes, the Kaplan–Meier method and log-rank test were performed for all patients. A Cox proportional hazard model was used to analyze the association between 25 (OH)D and survival. Univariate and multivariate analyses were used to estimate hazard ratios (HRs) and 95% confidence intervals (95% CI). Clinical, anatomopathological, and immunohistochemistry variables (ER, PR, HER2, and Ki-67) were assessed using univariate analysis. Multivariate analysis was adjusted for age, BMI, size, type and tumor grade, tumor stage, axillary status, and immunohistochemistry data. A level of significance of 5% or the corresponding p value was adopted in all tests. The analyses were performed using the Statistical Analysis System (SAS) 9.2 program.

3. Results

A total of 192 postmenopausal women were included in the prospective cohort, with a mean age at the time of breast cancer diagnosis of 61.3 ± 9.6 years, a mean serum 25 (OH)D concentration of 26.9 ± 7.5 ng/mL (ranging from 12.0 to 59.2 ng/mL), and a follow-up period of between 54 and 78 months. Patients were classified according to serum 25 (OH) D concentrations at the time of diagnosis into three groups: sufficient (≥30 ng/mL), insufficient (20–29 ng/mL), and deficient (<20 ng/mL). Sufficient serum concentrations were detected in 65 patients (33.9%), insufficient in 92 patients (47.9%), and deficient in 35 patients (18.2%).

Clinical features according to serum 25 (OH)D concentrations are shown in Table 1. There were no differences in age, time since menopause, parity, BMI, smoking, use of menopausal hormone therapy, and history of hypertension or diabetes. The associations between 25 (OH)D concentration and tumor characteristics are shown in Table 2. Patients with insufficient and deficient 25 (OH)D concentrations had a significantly higher proportion of high-grade tumors, positive axillary lymph nodes, advanced-stage tumors and distant metastasis, lower proportion of positive ER and PR, higher Ki-67 indices, and higher rate of triple-negative tumors (Table 2).

During the proposed follow-up period, overall and cancer-specific survivals (OS and CSS) were 73.4% and 78.1%, respectively. A total of 51 patients (26.6%) died during the study period, with a mean OS time of 54.4 ± 20.2 months (range 9–78 months). Of these patients, 44 (21.9%) died from cancer and 7 (3.6%) from other causes: 4 due to CVD (coronary disease, stroke, or venous thromboembolism) and 3 due to infection/sepsis. Overall, 47 patients had distant metastasis, 22 of whom had bone metastasis, 20 lung/pleura metastasis, 3 liver metastasis, and 2 central nervous system metastasis. Of 168 patients (87.5%) who were not diagnosed with distant metastasis at the time of the initial diagnosis of breast cancer (metastasis *de novo*), 33 (19.6%) developed distant metastases with an average time from the diagnosis of 24.8 ± 12.8 months. In 9 cases in which locoregional recurrence was identified, the patients also had distant disease.

Figures 1–3 show the Kaplan–Meier graphs of overall survival, recurrence-free survival, and cancer-specific survival according to serum 25 (OH)D concentrations. Patients with vitamin D deficiency and insufficiency at diagnosis had significantly inferior OS, DFS, and CSS compared with patients with sufficient values ($p < 0.001$). Multivariate analysis was performed to determine the risk factors that affected survival curves for breast cancer. Among all factors analyzed, those that significantly influenced overall survival were age, tumor size and stage, lymph node status, hormone receptors (ER and PR), and serum 25 (OH)D concentrations. After adjusting these variables, patients with serum 25 (OH)D levels considered deficient at the time of diagnosis had a significantly higher risk of global death (HR, 4.65; 95% CI, 1.65–13.12), higher risk of disease recurrence (HR, 6.87; 95% CI, 2.35–21.18), and higher risk of death from the disease (HR, 5.91; 95% CI, 1.98–17.60) than the group with sufficient 25 (OH)D concentrations.

4. Discussion

From our analysis, in postmenopausal women, vitamin D deficiency and insufficiency at the time of diagnosis were associated with lower overall survival (OS), disease-free survival (DFS), and cancer-specific survival (CSS) compared with patients with sufficient vitamin D concentration, regardless of prognostic factors of breast cancer (age, stage and size of the tumor, lymph node status, hormone receptors, and breast cancer subtypes). It is well established that prognostic and predictive factors are of paramount importance when dealing with breast cancer and it has been in accordance with the improving knowledge on tumor biology and options for proper management [22]. The evidence of a relationship between serum 25 (OH)D concentrations of patients with breast cancer and their subsequent survival has been an important subject in many recent researches and debates. In fact, several previous preclinical and clinical studies assessing the role of vitamin D in breast cancer have revealed that serum 25 (OH)D concentrations might be independently associated with breast cancer features and prognosis [4, 8, 10, 23].

In this study based on 192 Brazilian postmenopausal women with primary invasive breast cancer, low serum 25 (OH)D concentrations at diagnosis were observed in 66.1% of patients, in which 47.9% had insufficient and 18.2% had deficient vitamin D. This finding is consistent with previous trials, which report 50–74% of vitamin D insufficiency and deficiency in newly diagnosed breast cancer patients [24–29]. Our analysis also showed that low serum 25 (OH)D concentrations were significantly associated with higher-grade tumors, positive axillary status, negative ER and PR, higher Ki-67 index, and higher rates of the most aggressive intrinsic subtype, triple-negative tumors. During follow-up, OS, DFS, and CSS were also independently affected by vitamin D deficiency.

As increasing tumor grade is classically noted to lead to decreased breast cancer-specific survival and axillary lymph node status reflects actual end result data on the interaction between tumor aggressiveness and host defense mechanisms, the fact that low concentrations of vitamin D were observed in 76.9% of all grade 3 tumors and in 75% of the patients with positive axilla (N1+) suggests a prognostic effect of vitamin D related to a more aggressive behavior, consistent with a potential role of vitamin D in breast carcinogenesis and regulation of breast cancer cell phenotypes. In the study by Hatse et al. [30], serum 25 (OH)D concentrations at the time of diagnosis were shown to be linked to larger tumor size and higher malignancy grade, and two other studies by Villasenor et al. [31] and Vrieling et al. [25] found that lower 25 (OH)D concentrations were associated with more lymph node involvement and distant metastases.

An important finding in our study was the relation between vitamin D insufficiency and deficiency and a higher proportion of locally advanced and metastatic disease at diagnosis, which is in accordance with literature data showing that locally advanced breast cancer patients have more severe vitamin D deficiency than those with early-stage diseases. Thanasitthichai et al. [32] conducted a cross-sectional analysis of 25 (OH)D concentrations and clinicopathological

Table 1: Comparison between clinical features according to serum 25-hydroxy vitamin D concentrations, sufficient (≥30 ng/mL), insufficient (20–30 ng/mL), and deficient (<20 ng/mL).

Variables	Sufficient ($n = 65$)	Insufficient ($n = 92$)	Deficient ($n = 35$)	p value*
Age (years)	60.7 ± 9.6a	61.2 ± 9.2a	61.9 ± 10.3a	0.71
Menarche age (years)	13.1 ± 1.6a	12.8 ± 1.6a	12.8 ± 1.2a	0.53
Menopause age (years)	48.4 ± 3.8a	48.8 ± 3.5a	48.1 ± 3.1a	0.61
Postmenopausal time (years)	12.3 ± 10.3a	12.5 ± 9.1a	13.3 ± 10.5a	0.66[§]
Parity (number of children)	2.9 ± 2.2a	2.7 ± 1.9a	2.8 ± 1.9a	0.85[§]
BMI (kg/m^2)	31.0 ± 5.1a	31.0 ± 5.5a	30.0 ± 3.9a	0.58
25 (OH)D (ng/mL)	35.0 ± 6.0a	24.8 ± 2.8 b	17.1 ± 2.5c	<0.0001
Smoking, n (%)	11 (16.9)a	14 (15.2)a	6 (17.1)a	0.81[#]
Past use of MHT, n (%)	10 (15.4)a	8 (8.7)a	4 (11.4)a	0.31[#]
Hypertension, n (%)	30 (46.2)a	52 (56.5)a	18 (51.4)a	0.44[#]
Diabetes, n (%)	9 (13.9)a	20 (21.7)a	8 (22.8)a	0.30[#]

Values are presented as mean ± standard deviation (SD) or frequency (number) and percentage. 25 (OH)D, 25-hydroxyvitamin D; BMI, body mass index; MHT, menopausal hormone therapy. *p value: (a, b) significant difference between groups and (a, a) without difference ($p > 0.05$) (ANOVA, gamma distribution[§] or chi-square test[#]).

Table 2: Comparison between anatomopathological characteristics according to serum 25-hydroxyvitamin D concentrations, sufficient (≥30 ng/mL), insufficient (20–30 ng/mL), and deficient (<20 ng/mL).

Variables	Normal ($n = 65$)	Insufficient ($n = 92$)	Deficient ($n = 35$)	p^* value
Tumor size (cm), n (%)				0.69
≤2	19 (29.2)	25 (27.2)	13 (37.1)	
2–5	40 (61.6)	57 (62.0)	17 (48.6)	
≥5	6 (9.2)	10 (10.9)	5 (14.3)	
Histological type, n (%)				0.38
Ductal	62 (95.4)	88 (95.7)	34 (97.1)	
Lobular	3 (4.6)	4 (4.3)	1 (2.9)	
Histological grade, n (%)				0.02
Low (grade 1)	9 (13.8)	6 (6.5)	1 (2.8)	
Intermediate (grade 2)	38 (58.5)	47 (51.1)	13 (37.1)	
High (grade 3)	18 (27.7)	39 (42.4)	21 (60.1)	
AJCC clinical stage, n (%)				0.01
I	19 (33.3)	32 (33.3)	8 (20.5)	
II	23 (40.4)	38 (39.6)	8 (20.5)	
III	10 (17.5)	19 (19.7)	13 (33.3)	
IV	5 (8.8)	7 (7.3)	10 (25.6)	
Lymph node status, n (%)				0.004
Negative	39 (60.0)	31 (33.7)	14 (40.0)	
Positive	26 (40.0)	61 (66.3)	21 (60.0)	
Estrogen receptor, n (%)				<0.0001
Positive	61 (93.8)	77 (83.4)	21 (60.0)	
Negative	4 (6.2)	15 (16.6)	14 (40.0)	
Progesterone receptor, n (%)				0.047
Positive	52 (80.0)	63 (68.5)	20 (57.1)	
Negative	13 (20.0)	29 (31.5)	15 (42.9)	
HER2 status, n (%)				0.91
Positive	13 (20.0)	19 (20.6)	6 (17.1)	
Negative	52 (80.0)	73 (79.4)	29 (82.9)	
Ki-67 index, n (%)				0.001
<20%	32 (49.2)	25 (27.2)	5 (14.3)	
≥20%	33 (50.8)	67 (72.8)	30 (85.7)	
Intrinsic subtype, n (%)				0.0003
Luminal HER2-	40 (72.7)	79 (76.7)	16 (47.1)	
Luminal HER2+	12 (21.8)	10 (9.7)	5 (14.7)	
HER2-enriched	1 (1.8)	8 (7.8)	2 (5.9)	
Triple-negative	2 (3.7)	6 (5.8)	11 (33.3)	

Values are presented in frequency and percentage. AJCC, American Joint Committee on Cancer; HER2, human epidermal growth factor receptor type 2.
* Significant difference if $p < 0.05$ (chi-square test).

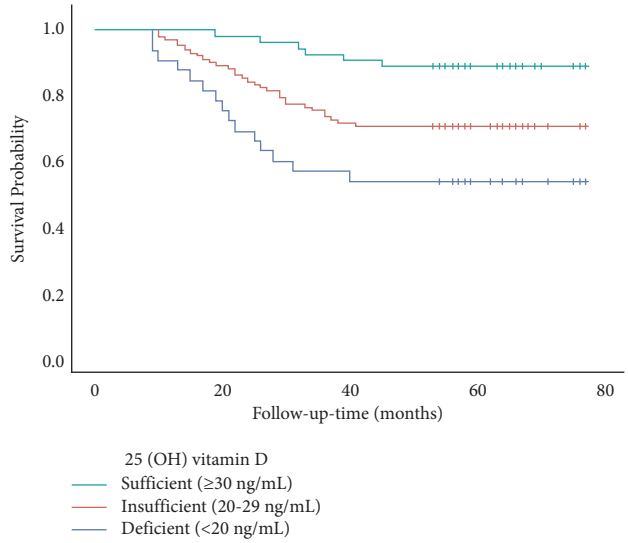

Figure 1: Kaplan–Meier graph for overall survival in patients with breast cancer according to serum 25-hydroxy vitamin D concentrations, sufficient (≥30 ng/mL), insufficient (20–30 ng/mL), and deficient (<20 ng/mL). * Significance between sufficient x insufficient $p = 0.004$ and between sufficient x deficient $p < 0.0001$.

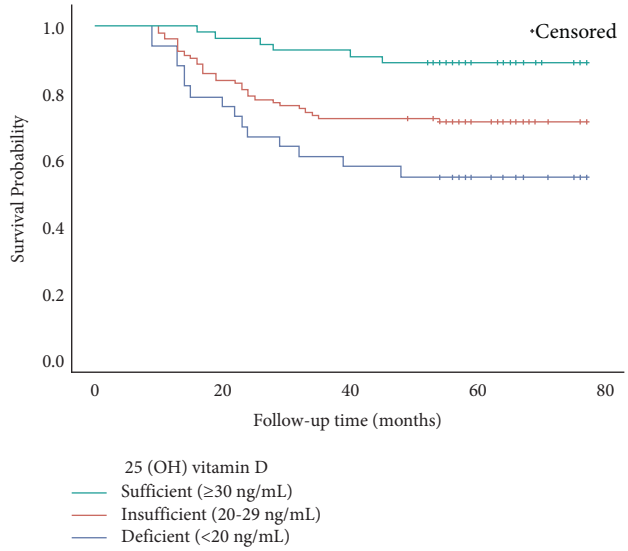

Figure 2: Kaplan–Meier graph for disease-free survival in patients with breast cancer according to serum 25-hydroxyvitamin D concentrations, sufficient (≥30 ng/mL), insufficient (20–30 ng/mL), and deficient (<20 ng/mL). * Significance between sufficient x insufficient $p = 0.001$ and between sufficient x deficient $p < 0.0001$.

characteristics in 200 cases of breast cancer. Low 25 (OH)D concentration (<32 ng/mL) was significantly found in the majority of cases with advanced stage of the disease, positive node involvement, and large tumors. [32] In view of the increasing number of cancer survivors and the high prevalence of vitamin D deficiency among patients with cancer, an evaluation of the role of circulating 25-OHD in prognosis among patients with cancer is essential. A previous study of Canadian women with breast cancer found a similar association; patients who had very low 25 (OH)D concentration at

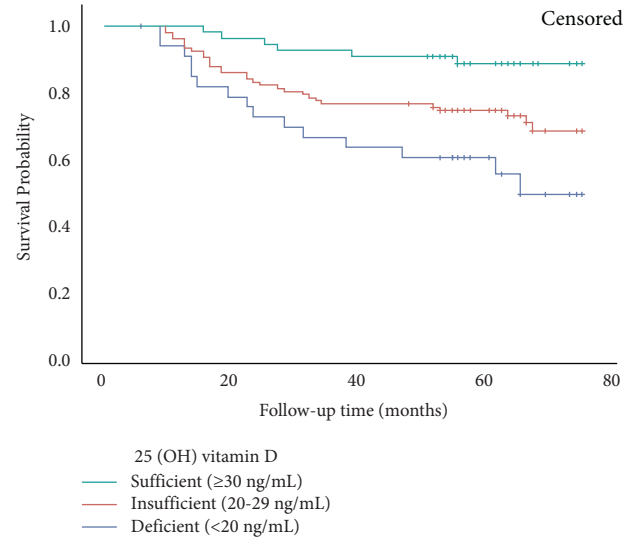

Figure 3: Kaplan–Meier graph for cancer-specific survival in patients with breast cancer according to serum 25-hydroxy vitamin D concentrations, sufficient (≥30 ng/mL), insufficient (20–30 ng/mL), and deficient (<20 ng/mL). * Significance between sufficient x insufficient $p = 0.002$ and between sufficient x deficient $p < 0.0001$.

diagnosis were more likely to have aggressive and metastatic disease. In fact, women with very low concentrations of vitamin D were 94% more likely to develop metastases than women with normal concentrations and were 73% more likely to die from breast cancer [28]. In our study, of 47 patients who had distant metastasis at diagnosis or during follow-up, only 6 (12%) had sufficient 25 (OH)D concentrations at breast cancer diagnosis.

As for biological and intrinsic prognostic factors, the measurement of ERs, PRs, and HER2 is known to be a standard practice in the evaluation of patients with primary breast cancer because it provides phenotypical features related to tumor molecular subtype. Our finding that insufficient and deficient concentrations of vitamin D were associated with a lower proportion of positive ER and PR tumors mainly characterizes higher-risk tumors. A significantly reduced rate of Ki-67 expression in patients with low vitamin D was also observed, and despite the controversies related to the reproducibility of its expression, several metanalyses point to an association between Ki-67 with the risk of both relapse and overall survival and its role as an independent prognostic factor for both disease-free survival and overall survival [22, 33–35].

Of all 162 patients with luminal phenotype tumors in this study, only 21 (12.9%) had deficient 25 (OH)D concentrations, whereas among patients with triple-negative tumors (ER-, PR-, and HER2-), 11 of 19 patients (57.8%) had vitamin D deficiency; this is in accordance with other studies assessing the relation of vitamin D with receptor expression and tumor subtypes [4, 8, 13, 36, 37]. A case-control study comprising women with breast cancer and the same number of women in the control group revealed that lower concentrations of vitamin D were associated with poorly differentiated cell cancers and triple-negative tumors [36].

Another study, a large prospective cohort of 1666 breast cancer survivors by Yao et al. [38], showed that low 25 (OH) D concentrations were associated with poor prognostic characteristics and a higher risk of triple-negative breast cancer [38]. Similar results were observed in studies evaluating the expression of VDR. In the recent study by Huss et al. [39], vitamin D receptor (VDR) expression was evaluated in a tissue microarray of invasive breast tumors and it was found to be associated with favorable prognostic characteristics, such as low grade, ER positivity, PR positivity, low Ki-67 expression, and luminal-like subtypes [39].

In terms of mortality from breast cancer or all-cause mortality, we found significant inverse associations between vitamin D status and cancer survival. Patients with 25 (OH) D concentrations of deficiency or insufficiency at the time of diagnosis had a significantly worse OS (HR, 4.65; 95% CI, 1.65–13.12), DFS (HR, 6.87; 95% CI, 2.35–21.18), and CSS (HR, 5.91; 95% CI, 1.98–17.60) compared with patients with sufficient 25 (OH)D concentrations, which has been observed in most previous reports [28–30]. In a Norwegian study, patients with breast cancer and higher serum 25 (OH) D concentrations (>35 ng/mL) had a significantly decreased risk of breast cancer-specific mortality compared with those with lower serum 25 (OH)D concentrations (<20 ng/mL) [27]. In a pooled analysis of two randomized studies and a prospective cohort, 25 (OH)D concentration was significantly inversely associated with breast cancer risk. All three analyses showed that women with 25 (OH)D concentrations ≥60 ng/mL had a significantly lower risk of breast cancer (~80%) compared with women with concentrations <20 ng/mL [40].

Three different metanalyses assessing the impact of vitamin D on breast cancer survival showed similar results [13, 41, 42]. Two meta-analyses by Mohr et al. [13] and Maalmi et al. [41] assessed the relationship between vitamin D in breast cancer diagnosis and case fatality rates. The analyses found that women in the highest serum 25 (OH)D concentration group were more likely to have a better prognosis than women in the lowest serum 25 (OH)D concentration group [13, 41]. A larger meta-analysis by Kim et al. [42] including 30 studies revealed that among 6092 patients with breast cancer, high 25 (OH)D status was weakly associated with low breast cancer risk, but strongly associated with better breast cancer survival. They observed that high blood concentration of vitamin D was significantly associated with lower breast cancer mortality (RR, 0.58; 95% CI, 0.40–0.85) and overall mortality (RR, 0.61; 95% CI, 0.48–0.79) [42]. More recently, a study by Kanstrup et al. [43] with 2510 women with primary invasive breast cancer and patients with the lowest 25(OH)D concentrations (≤20 ng/mL) had an inferior event-free survival with a HR of 1.63 (95% CI, 1.21–2.19) compared with women in the third quartile (30–40 ng/mL) [43].

In accordance with these robust data, several mechanisms through which sufficient concentrations of 25 (OH)D may improve survival among breast cancer patients have been studied. Vitamin D has been associated with the control of a variety of cellular mechanisms through interaction with a number of different genes with respect to cancer development such as differentiation, cell proliferation, apoptosis, angiogenesis, and metastatic potential [5, 11, 44]. The net effect is characterized by slowing tumor cell growth and progression and maintaining a more differentiated and, subsequently, less aggressive state [5, 10]. In addition, vitamin D sufficiency seems to suppress the downregulation of E-cadherin, a glycoprotein that helps cells to keep in close contact and consequently a well-differentiated state, thus improving breast cancer prognosis [45, 46]. It is well known that breast cancer is complex and that risk factors and etiologies differ on the basis of endogenous and exogenous exposures, underlying biology and genetic concepts [11, 47]. Furthermore, differing and multifactor patterns impact distant disease-free and overall survival [47]. Therefore, although our findings may indicate an association between vitamin D and breast cancer outcome, it is unlikely that these prognostic effects of vitamin D are casual or due to chance.

As a strength of this study, we used a longitudinal cohort and it is the first prospective analysis assessing the impact of serum 25 (OH)D concentrations on prognostic factors and long-term survival in Brazilian women with breast cancer, which provides a direct glance evidence that vitamin D may be a leading host factor influencing breast cancer prognosis to our patients. This is encouraging, but it is very clear that more research is needed. Other strengths of our data include no patients lost during follow-up and homogeneity of clinical features in the three groups categorized by 25 (OH) D. This is important considering that factors related to comorbidities and lifestyle are associated with both circulating 25 (OH)D and risk of breast cancer and could interfere with data analysis.

As limitation, it was a single-center study with a relatively low number of patients and only a single measurement of serum 25 (OH)D concentrations made at diagnosis. The significant associations between 25 (OH)D concentrations and breast cancer survival rates in our analyses had relatively wide confidence intervals, making the level of uncertainty greater, although there may still be enough precision to make decisions about the utility of the findings. Considering that the width of the confidence interval for an individual study depends to a large extent on the sample size, larger studies would certainly give more precise estimates of the effects observed.

5. Conclusion

In postmenopausal women treated for breast cancer, vitamin D deficiency and insufficiency at the time of diagnosis were associated with lower overall survival (OS), disease-free survival (DFS), and cancer-specific survival (CSS) compared with patients with sufficient vitamin D concentration, regardless of prognostic factors of breast cancer (age, stage and size of the tumor, lymph node status, hormone receptors, and breast cancer subtypes).

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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