Vitamin D levels in Mediterranean breast cancer patients, compared to healthy women

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Abstract

Objectives: To evaluate the vitamin D status of postmenopausal women with early estrogen-receptor-positive breast cancer compared to healthy postmenopausal women from the same Mediterranean region.

Study design and outcome measures: Data from 691 breast cancer patients (BC) in the B-ABLE cohort were analyzed after recent cancer intervention (recent-BC) or after a minimum of two years from this intervention (long-term-BC). Patients were also stratified by previous chemotherapy exposure (ChT+ and ChT-). Plasma levels of 25-hydroxyvitamin D [25(OH)D] (25(OH)D) were compared to data from 294 healthy women (non-BC) by linear regression to estimate β -coefficients using non-BC participants as reference group. Age, body mass index and season of blood extraction data were selected as confounders.

Results: A 23.7% of recent-BC patients had 25(OH)D deficiency, followed by 17.7% in long-term-BC group, and just 1.4% of non-BC participants. Most of the women were located in the insufficient 25(OH)D category regardless of study group. BC patients had significantly lower 25(OH)D levels than non-BC participants (adjusted β -coefficients: -4.84 [95%CI -6.56 to -3.12] in recent-BC, and -2.05 [95%CI -4.96 to -0.14] in long-term-BC). Among BC patients, the lowest 25(OH)D levels were found in recent-BC (ChT+) ($p<0.001$). No differences were found between long-term-BC (ChT-), long-term-BC (ChT+) and recent-BC (ChT-). Considering only BC patients ChT+, results showed significant reduced 25(OH)D levels in recent-BC compared to long-term-BC ($p<0.001$).

Conclusion: Severely reduced 25(OH)D levels were detected in patients with breast cancer, particularly after recent chemotherapy. These 25(OH)D levels would be partially recovered at long-term, but remained much lower than in the healthy population.

Keywords: vitamin D, breast cancer, chemotherapy, B-ABLE cohort study.

Abbreviations: VDR, vitamin D receptor; 25(OH)D, vitamin D; B-ABLE, Barcelona–Aromatase induced Bone Loss in Early breast cancer; BC, breast cancer patients; BMI, body mass index; PCA, principal component analysis; ChT, chemotherapy; ChT+, patients with chemotherapy; ChT-, patients without chemotherapy.

1. Introduction

Many genes are regulated by the active form of vitamin D (1,25-dihydroxyvitamin D) in tissues such as the immune system, bone, muscles, lungs, heart, and kidney, among others. Although its primary biological action, which is mediated by vitamin D receptor (VDR), is to regulate serum calcium levels and promote bone mineralization, several studies have described vitamin D as a potent non-proliferation, pro-differentiation and immunomodulation factor [1]. Its precursor, 25-hydroxyvitamin D (25(OH)D) is the circulating form of vitamin D which is considered the best marker to assess vitamin D levels [2-5].

The role of vitamin D in several physiological processes has been widely studied, but its role in breast cancer has been under discussion for years. Preclinical studies suggest a protective effect of vitamin D on cancer risk and progression [6, 7], but clinical and epidemiologic studies have reported controversial results [8-12]. However, the available evidences that vitamin D levels may affect breast cancer survival [13, 14] are sufficient to suggest that vitamin D status should be monitored. Previous studies in the B-ABLE cohort -a prospective, clinical cohort study of women diagnosed with early breast cancer and candidates for aromatase inhibitor treatment- showed that low levels of serum 25(OH)D are associated with worsening joint pain and increased loss of bone mass [15-17]. Exploring the behavior of vitamin D in these patients could be important for preventing musculoskeletal disorders as well as other events affecting quality of life.

In the present study, 25(OH)D status has been exhaustively explored in the B-ABLE cohort and compared to healthy postmenopausal women from the same Mediterranean region.

2. Methods

2.1 Study design and participants

This observational cohort study compared serum vitamin D levels (25(OH)D) in Mediterranean postmenopausal women diagnosed with early estrogen-receptor-positive breast cancer and a control group of healthy postmenopausal women. Postmenopausal status was defined as >55 years old with amenorrhea

for >12 months, or ≤55 years with levels of luteinizing hormone >30 mIU/mL or follicle-stimulating hormone values >40 mIU/mL.

Data from 691 breast cancer patients (BC) were collected in the B-ABLE cohort (Barcelona–Aromatase induced Bone Loss in Early breast cancer) [17] from January 2006 to Jun 2017 in Hospital del Mar (Barcelona, Spain), of which 460 were postmenopausal women with breast cancer included at 6 weeks post-surgery or 1 month after the last cycle of chemotherapy (recent-BC); the remaining 231 women were included once menopause started after taking tamoxifen for a minimum of 2 years and up to 5 years (long-term-BC). Data were collected for a large number of demographic and clinical variables, including age at recruitment, body mass index (BMI), and serum levels of 25(OH)D, among others. Exclusion criteria included previous history of any metabolic or endocrine diseases, alcoholism, rheumatoid arthritis, and oral corticosteroids.

Healthy postmenopausal women were recruited as a control group (non-BC, n=294) in the Hospital Clínico Universitario de Valencia from January 2004 to October 2009. Registered variables included age, BMI, and serum levels of 25(OH)D, among others. Exclusion criteria were the same as for the B-ABLE cohort.

2.2 Variables

The main outcome of the study was the serum level of 25(OH)D, obtained from peripheral blood using competitive immunoluminometric direct assay with direct-coated magnetic microparticles (coefficient of variation<10%) (Elecsys Vitamin D total II, model 07028148190; Cobas e801 system, Roche Diagnostics GmbH, Mannheim, Germany). Season of blood extraction was registered and 25(OH)D levels were classified as optimal (≥30 ng/ml), insufficient (<30 ng/ml and ≥10 ng/ml), or deficient (<10 ng/ml) [18]. Age, BMI and chemotherapy (ChT) status were also used in the statistical analysis.

2.3 Statistical methods

Differences between selected populations were assessed by Kruskal–Wallis test and Chi-square test. Differences in percentages of patient distribution in 25(OH)D categories were evaluated using the Chi-square test.

Linear regression models were performed to estimate the β -coefficients in 25(OH)D levels between study groups, using non-BC participants as reference group. Age, BMI, and season of blood extraction data were selected as confounders. Linearity, interaction, and lack of perfect multicollinearity of independent variables were tested.

A sub-analysis was conducted, stratifying the study groups according to chemotherapy (ChT) status: patients treated (ChT+) or non-treated (ChT-) with chemotherapy. No non-BC participants had been exposed to ChT.

All statistical analyses were performed with R for Windows version 3.3.3 using foreign, Hmisc, compareGroups, pgirmess, fifer, pca3d, plyr, boot, ggplot2, car, and stats packages. $P < 0.05$ was considered significant, and all statistical contrasts were corrected by Bonferroni test per multiple comparisons.

3. Results

3.1 Patient characteristics

A total of 985 participants were included, 691 patients from the B-ABLE cohort and 294 healthy post-menopausal women. Their characteristics are reported in Table 1. Recent-BC patients were significantly older ($p < 0.001$) and had higher BMI ($p < 0.01$) than long-term-BC and non-BC participants. Different exposure to previous ChT treatment was observed between BC groups ($p < 0.001$). Differences in serum levels of 25(OH)D were detected between the three groups ($p < 0.001$), but no significant differences were found in season of blood sample collection.

3.2 Participants distribution by vitamin D categories

Subject distribution by categories of 25(OH)D showed significant differences between the three groups ($p < 0.05$) (Figure 1). The recent-BC patients had the

higher percentage of 25(OH)D deficiency (23.7%), followed by 17.7% in long-term-BC group, and just 1.4% of non-BC participants. Most of the women were located in the insufficient 25(OH)D category regardless of study group.

3.3 Linear regression analysis of vitamin D

In the adjusted linear regression analysis using non-BC group as reference, significant differences in 25(OH)D levels were found between three groups: non-BC vs recent-BC, $p<0.001$; non-BC vs long-term-BC, $p<0.05$; and recent-BC vs long-term-BC patients, $p<0.001$ (Table 2). Additionally, a significant interaction between recent-BC patients and ChT status ($p<0.01$) suggested differences in 25(OH)D levels according to previous ChT exposure.

3.3 Subanalysis stratifying by chemotherapy exposure

In the subanalysis stratified by ChT status (Table 3), all patients with breast cancer had significantly lower 25(OH)D levels than non-BC participants. Among BC patients, the lowest 25(OH)D levels were found in recent-BC with previous ChT ($p<0.001$). No differences were found between recent-BC (ChT-), long-term-BC (ChT-) and long-term-BC (ChT+) groups.

Patient distribution by categories of 25(OH)D, stratified by ChT status, corroborated previous regression results (Figure 2): 25(OH)D levels of recent-BC group were significantly lower in patients with previous ChT than in those without ChT ($p<0.001$). However, in long-term-BC patients, no 25(OH)D differences according to previous ChT were observed. Hence, considering only the 25(OH)D levels of patients with previous ChT, there were significant differences ($p<0.001$) between patients just finalizing ChT [recent-BC (CT+)] and those after 2 to 5 years from intervention [long-term-BC (CT+)] (Figure 2).

4. Discussion

Breast cancer patients in the B-ABLE cohort exhibited significantly lower 25(OH)D levels compared to a control group of healthy postmenopausal women from the same geographical region. The lowest 25(OH)D levels were observed in patients with recent chemotherapy.

Although our Mediterranean population could be expected to have relatively optimal 25(OH)D levels due to the region's solar patterns, a large majority of the women had insufficient 25(OH)D levels regardless of study group. Even though healthy women had higher 25(OH)D levels than BC patients, normal levels were found only in 21.4%. Vitamin D3 insufficiency and deficiency could be related to cultural factors limiting exposure to sunlight, including indoor occupations, population aging, or consistent use of protective clothing or sunscreen when outdoors, reducing the individual's UVR exposure [19]. Matsuoka et al. (1987) reported a 97.5% decrease of previtamin D3 production after the application of a sunscreen with a sun protection factor of only 8 [20]. Webb et al. (1988) described a severe reduction of 25(OH)D levels in latitudes above and below 35°N and 35°S in winter [21]. These could also explain the lower 25(OH)D values observed in our populations (latitudes: 41.38°N in Barcelona, and 39.45°N in Valencia).

Although cases and controls were recruited from different places, Barcelona and Valencia are very similar cities, geographically close to one another, with very similar climate, cultural behaviors and clinical health care systems. The same exclusion criteria were used for both cohorts and all analyses were adjusted by age, season of blood draw, and BMI as confounding variables.

Among women with BC, long-term participants had 25(OH)D levels significantly higher than the rest of breast cancer women; nonetheless, 17.7% of the long-term-BC group had 25(OH)D deficiency, compared to 1.4% of healthy women. This places the long-term-BC group in an intermediate 25(OH)D status between the recent-BC and non-BC groups. The overall prevalence of suboptimal levels of 25(OH)D in our study was similar to other studies of baseline 25(OH)D levels in breast cancer patients [22, 23], suggesting that women with breast cancer tend to have inferior 25(OH)D levels.

Interestingly, we found an interaction between chemotherapy and 25(OH)D levels. When breast cancer women were stratified according to chemotherapy status, the lowest 25(OH)D levels were observed in patients who underwent recent chemotherapy. These results could be attributed to the photosensitivity effect of chemotherapy, affecting patients' sunlight exposure. This effect on

25(OH)D levels disappeared at long-term, when no differences according to previous chemotherapy were observed in these patients. Even though 25(OH)D levels seemed to partially recover in long-term patients, it is unclear why values comparable to the healthy population were not achieved. More time may be required to recover 25(OH)D levels, or perhaps these women have lower 25(OH)D levels due to genetic or physiological factors. Indeed, recent studies have suggested an association between 25(OH)D levels and breast cancer risk [12, 24, 25], overall survival [26, 27], and cancer prognosis [7, 28].

One limitation of our study is the time point when 25(OH)D levels were assessed. Blood analysis was done at 6 weeks post-surgery or 1 month after the last cycle of chemotherapy, not at the time of cancer diagnosis; therefore, some small variation of 25(OH)D levels could occur during this period. We cannot know the levels of 25(OH)D before breast cancer detection or before any intervention related to breast cancer treatment. Hence, we cannot analyze either the associations between 25(OH)D levels and breast cancer incidence or the effect of cancer disease *per se* on 25(OH)D levels. However, the main purpose of the present study was to report the 25(OH)D status of early breast cancer patients compared to healthy women with similar features. Additionally, our cohorts were very well-characterized and our analysis controlled for most potential confounders.

A strength of this study is that 25(OH)D deficiency in breast cancer patients was observed both in early and long-term follow-up, underlining the consistence of this finding over time. This observation adds another evidence on the association between 25(OH)D and breast cancer as is consistently supported by numerous case-control studies and randomized controlled trials [29, 30].

5. Conclusion

In summary, severely reduced 25(OH)D levels were detected in patients with breast cancer, particularly after recent chemotherapy. These 25(OH)D levels seemed to be partially recovered at long-term but remained much lower than the healthy population. Considering that 25(OH)D levels have been related to

better prognosis and survival, it may be speculated that 25(OH)D supplementation might impact prognosis in all patients diagnosed of breast cancer, and especially in patients receiving chemotherapy. Intervention studies might provide an answer to that question.

Contributors

Marta Pineda-Moncusí, Natalia Garcia-Giralt and Xavier Nogues contributed to study concept and design.

Miguel Angel Garcia-Perez, Abora Rial, Guillem Casamayor, Maria Lourdes Cos, Sonia Servitja, Ignasi Tusquets, Antonio Cano and Xavier Nogues recruited participants and contributed to data collection.

Marta Pineda-Moncusí and Natalia Garcia-Giralt performed data analysis.

Marta Pineda-Moncusí, Sonia Servitja, Ignasi Tusquets, Adolfo Diez-Perez, Natalia Garcia-Giralt and Xavier Nogues contributed to result interpretation and discussion.

All authors helped with manuscript drafting and critically reviewed the manuscript. All authors approved the final version of the manuscript.

Ethical statement

The study protocol was approved by the ethics committee of Parc de Salut Mar (2016/6803/I) and it was carried out in accordance with the Declaration of Helsinki. All participants gave their written informed consent to participate in this research once they had read the study information sheet and any questions had been answered. The privacy rights of human subjects must always be observed.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Table 1. Patient characteristics

Variable	recent-BC (N=460)	long-term-BC (N=231)	non-BC (N=294)
Age (median [Q1;Q3])	63.0 [57.0;67.0]	57.0 [52.0;66.0]	58.0 [53.0;62.0]
BMI (median [Q1;Q3])	28.5 [26.0;32.4]	26.8 [23.7;30.9]	26.2 [23.6;29.2]
Patients exposed to ChT (n;%)	253 (55.0%)	157 (67.7%)	0 (0%)
Serum levels of 25(OH)D (median [Q1;Q3])	15.8 [10.3;21.6]	17.5 [12.0;24.6]	22.6 [18.1;28.7]
Sorted by season of baseline blood sample collection (median [Q1;Q3](n;%)			
January–March	13.4 [8.64;20.5] (124;27.0%)	14.1 [8.90;22.0] (67;29.0%)	22.6 [18.1;28.7] (118;40.1%)
April–June	15.6 [11.9;21.7] (85;18.5%)	18.1 [12.0;23.9] (58;25.1%)	23.2 [18.8;28.2] (87;29.6%)
July–September	19.0 [11.8;24.0] (120;26.1%)	20.6 [16.4;28.8] (42;18.2%)	20.4 [17.2;28.2] (27;9.18%)
October–December	14.7 [9.11;19.7] (131;28.5%)	18.2 [13.2;27.1] (64;27.7%)	25.3 [18.0;32.2] (62;21.1%)
Abbreviations: BC, breast cancer; BMI, body mass index; ChT, chemotherapy; Q, quartile.			

Table 2. Values of constant α and β coefficients of 25(OH)D serum levels in recent-BC and long-term-BC patients compared with non-BC women.

Group	Unadjusted α value [95%CI]	Unadjusted β coefficient [95%CI]	Adjusted α value [95%CI]	Adjusted β coefficient [95%CI] ^a
non-BC (ref)	23.76 [22.67 to 24.85]		33.27 [27.91 to 38.63]	
recent-BC		-6.88 [-8.27 to -5.49]		-4.84 [-6.56 to -3.12]
long-term-BC		-4.27 [-5.91 to -2.64]		-2.05 [-4.96 to -0.14]
Abbreviations: BC, breast cancer patients; CI, confidence interval; ref, reference category. In 95%CI, reference group is non-BC patients. ^a Adjusted by age, body mass index, season of blood extraction and chemotherapy status.				

Table 3. Subanalysis of vitamin D serum levels according to patient exposure to chemotherapy and compared with non-BC women: constant α value and β coefficients in recent-BC and long-term-BC patients.

Group	Unadjusted α value [95%CI]	Unadjusted β coefficient [95%CI]	Adjusted α value [95%CI] ^a	Adjusted β coefficient [95%CI] ^a
non-BC (ref)	23.76 [22.69 to 24.83]		31.62 [26.18 to 37.06]	
recent-BC (ChT-)		-4.06 [-5.72 to -2.40]		-4.21 [-5.96 to -2.45]
recent-BC (ChT+)		-9.19 [-10.76 to -7.62]		-9.24 [-10.91 to -7.58]
long-term-BC (ChT-)		-4.72 [-7.11 to -2.34]		-4.46 [-6.90 to -2.03]
long-term-BC (ChT+)		-4.06 [-5.88 to -2.25]		-4.59 [-6.40 to -2.77]
Abbreviations: BC, breast cancer patients; (ChT-), non-chemotherapy exposure; (ChT+), chemotherapy exposure; CI, confidence interval; ref, reference category. In 95%CI, reference group is non-BC patients. ^a Adjusted by age, body mass index and season of vitamin D blood.				

Figure legends

Figure 1. Participant distribution in each study group according to vitamin D categories. Abbreviations: BC, breast cancer; Percentage, percentage of patients. * Bonferroni post hoc comparison of Chi-square test: $p < 0.001$.

Figure 2. Subanalysis stratifying by previous chemotherapy. Patient distribution in each study group according to vitamin D categories. Abbreviations: BC, breast cancer; ChT-, non-previous chemotherapy treatment; ChT+, previous chemotherapy treatment; Percentage, percentage of patients. * Bonferroni post hoc comparison of Chi-square test: $p < 0.05$.