Low Levels of Vitamin D in a Cohort of Women with Impalpable Breast Lesions from Rio de Janeiro/Brazil

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Abstract

Background: Low levels of vitamin D have been described as a risk factor for the development of breast cancer. The aim of this study was to evaluate the serum levels of vitamin D (25OHD) in patients with impalpable breast lesions comparing with a control group. Methods: Vitamin D quantification (25OHD) was assessed in the plasma of 65 patients with impalpable breast lesions and from 20 health controls using a chemiluminescent microparticle immunoassay. Pearson’s chi-square test and nonparametric t-Student were used to evaluate statistical significance between the clinical variables and the means of quantification of vitamin D. The receiver operating characteristic (ROC) curve was used to evaluate the correlation between age and vitamin sufficiency for the cases and the controls. Results: The prevalence of vitamin D deficiency and/or insufficiency in women with malignant lesions was 84% and 60% for the control group. Using the chi-square or Fisher’s exact test, the relationship between vitamin D levels and age presented significant association only for the control group (P=0.002). Using ROC curve, the plot area (0.778) for the control group defined a cut-off value of 45 years to age, with specificity and sensitivity of 60% and 50%, respectively. Thus, the odds ratio for vitamin D insufficiency in women over 45 years was 1.37 (P=0.011). For the case group, clinical characteristics, histological grade, and lymph node involvement did not show any significant association. Conclusion: The prevalence of vitamin D deficiency/insufficiency is high in women with impalpable breast lesions, as well as in the control group, even in a tropical city. According to the results the age advancement may be involved with the decrease in vitamin D levels in plasma, but there was no statistical association between low levels of Vitamin D and breast cancer.

Keywords: Impalpable breast lesions- 25OHD deficiency- hypovitaminosis D- aging

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Lucas Delmonico et al
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reduced the ability of metastasis in in vitro and in vivo assays (Wilmanski et al., 2016; Williams et al., 2016).

In Brazil, breast cancer is the most common tumor affecting women, comprising about 28% of all tumors diagnosed annually, excluding skin tumors (non-melanoma) (INCA, 2017). With the improvement of breast cancer screening, more impalpable breast lesions suspicious of cancer have been detected (Delmonico et al., 2015; Romeiro et al., 2016). The aim of this study was to evaluate the serum levels of vitamin D (25OHD) in patients with impalpable breast lesions comparing with a control group.

Materials and Methods

Study population

Patients were recruited from 2008 to 2016 at Hospital Gafrée-Guinle University Hospital (HUGG) and Americas Barra Medical City (Mauricio Magalhães Costa Clinic), in the city of Rio de Janeiro, Brazil. All patients provided written informed consent. The study was conducted according to the guidelines of the Declaration of Helsinki. This study was approved by the ethics committee of both institutions (HUGG-07/2007-80/2012 and Rio de Janeiro State University 43560115.5.0000.5259). All patients were diagnosed with impalpable lesions BIRADS (Breast imaging-reporting and data system) grade 4. Following biopsy or surgery, the lesion was classified as malignant (N=58) or benign (N=7). Data on age, tumor classification, grade, size, nodal involvement, and immunohistochemical profile were obtained from participating institutions. Histologic classification was graded according to current (2012) World Health Organization criteria (Lakhan et al., 2012), and nuclear grade was defined as grades I to III according to Elston and Ellis (Elston and Ellis, 1991). The histologic classification and the nuclear grading were performed by a medical pathologist. Peripheral blood of 20 eligible healthy women (controls) was collected in Rio de Janeiro.

Laboratory analysis

Four mL of blood was collected in EDTA and centrifuged at room temperature for 10 minutes at 2,000 g and plasma was stored at -80°C. We measured serum 25-hydroxyvitamin D (25OHD) using the Roche Elecsys Vitamin D Total assay (Roche Diagnostics, Mannheim, Germany). The measurements were performed on the Modular Cobas E601 Analyzer according to the manufacturer’s protocol. Serum concentrations below 20 ng/mL (50 nmol/L) were classified as deficiency, ≥20 ng/mL (50 to 74 nmol/L) as insufficiency, and ≥30 ng/mL (≥75 nmol/L) as sufficiency (Holick, 2006; Holick et al., 2011).

Statistical Analysis

Calculation of the sample size

The calculation of the sample size required to establish the chi-square test depends on the specification of the following parameters, described in the literature according to Cohen (1988): a) Level of significance is 5% (α = 0.05 two-sided); b) for the measurement of the effect, the value described in the literature, according to Cohen (1988), was considered the following reference value: medium effect = 0.30; c) test power (1-β): β = 0.25; d) degree of freedom equal to 1, since each variable has two categories. These parameters showed that the sample size is 77 individuals.

Analysis of vitamin D levels

Initially, an exploratory analysis was carried out to characterize the patients’ sample, using frequency distributions and descriptive measures for the clinical variables.

For a bivariate analysis, individuals with vitamin D deficiency and insufficiency were grouped into a single group (D) due to the number of cases evaluated here. The Fischer chi-square or Pearson test was used for the evaluation of differences between the benign/malignant lesions and control group regarding the vitamin D levels. It was calculated the odds ratio for significance chi-square test.

For the associations between deficient and insufficient in control group and cases, the parametric t-Student test was used to determine differences of 25(OH)D concentrations.

The optimal cut-off point for sensitivity and specificity to evaluate the correlation between vitamin sufficiency and the age was done using the receiver operating characteristic (ROC curve). A contingency table was used to associate the age variable with the presence of sufficient amount of vitamin D. The chi-square or Fisher’s Exact test was used to test the statistical significance of the association between these variables. The odds ratio for the occurrence of clinical events was calculated by comparing the age groups. The 95% confidence interval was obtained for the odds ratio.

The data were processed in the statistical program Predictive Analytics Software (PASW 18). A level of significance of 5% was considered in all the statistical tests used. Statistically significant data were considered as those whose P value was less than 0.05.

Results

Our study quantified vitamin D in 58 women with malignant breast lesions, which included 45/58 (77%) Luminal A/B, 8/58 (14%) HER positive, 5/58 (9%) triple negative, and 7 cases with benign breast lesions. The mean age was 59 years (SD 12.66), 62 (SD 12.58), 43 (SD 3.07), and 46 (SD 6.96) for the Luminals A/B, HER2, triple negative, and benign lesions, respectively. The mean age of the control group was 37 years (SD 11.31).

Clinical-pathological variables of cases are summarized in Table 1.

Regarding vitamin D dosage, 49/58 (84%) of patients with malignant tumors presented deficiency and/or insufficiency, of which 30/58 (65%) were Luminals A/B. All patients with HER2 (n=8) and triple negative (n=5) tumors had insufficient and/or deficiency as well.

Using the chi-square test, the vitamin D levels were compared between groups, malignant and benign lesions versus control, and between subtypes of breast cancer versus control. These associations are summarized in
Low Levels of Vitamin D in Women

The statistical relationship between sufficiency and deficiency/insufficiency of vitamin D for different types of histological grade (P=0.102), and lymph node involvement (P=1.00) did not show any significant association, according to the chi-square test.

Discussion

This study revealed low levels of vitamin D in a heterogeneous group of women with impalpable breast lesions and in the controls as well. Although vitamin D deficiency is a widespread health problem, it has been gaining prominence in regions where sunshine prevails all the year (Schoenmakers et al., 2008). Indoor lifestyles, urban living, and nutrition may explain this finding (Arabi et al., 2010). Nevertheless, low levels of vitamin D were positively associated with aging only in the control group.

Based on our previous study, the vitamin D-binding protein level was considered a candidate to differentiate patients with breast impalpable lesions from controls. A nano-liquid chromatography-quadrupole-time-of-flight mass spectrometry method was used to measure the vitamin D-binding protein level.

Table 1. Clinical Data of the Cases with Malignant Impalpable Lesion Evaluated in This Study

| Variable                  | Mean (years) |
|---------------------------|--------------|
| Luminal A/B               | 59 (SD 12.66) |
| HER2                      | 62 (SD 12.58) |
| Triple Negative           | 43 (SD 3.07)  |

| Variable                  | Patients N=58 |
|---------------------------|---------------|
| Histopathologic grade     |               |
| I                         | 13            |
| II                        | 28            |
| III                       | 17            |

| Variable                  |               |
|---------------------------|---------------|
| Molecular classification   |               |
| Luminal A/B               | 45            |
| HER2                      | 8             |
| Triple negative           | 5             |

| Variable                  |               |
|---------------------------|---------------|
| Histopathologic classification |         |
| IDC                       | 36            |
| DCIS                      | 5             |
| IDC-DCIS                  | 6             |
| ILC                       | 2             |
| Other malignant lesions   | 9             |

IDC, infiltrative ductal carcinoma; DCIS, ductal carcinoma in situ; ILC, infiltrative lobular carcinoma; LCIS, infiltrative carcinoma in situ.

Table 2. Association of the Control Group with the Benign and Malignant Lesions Regarding the Levels of Vitamin D

| Variable                  | Controls (N=20) | Benign lesions (N=7) | Malignant lesions (N=57) | Controls (N=20) | Luminals A/B (N=45) | Controls (N=20) | HER2 (N=8) | Controls (N=20) | Triple negative (N=5) |
|---------------------------|-----------------|----------------------|--------------------------|-----------------|---------------------|-----------------|------------|-----------------|-----------------------|
| I=D (%)                   | 12 (60%)        | 6 (86%)              | 49 (86%)                 | 12 (60%)        | 30 (67%)            | 12 (60%)        | 8 (100%)   | 12 (60%)        | 5 (100%)              |
| Vitamin D                 | 8 (40%)         | 1 (14%)              | 8 (14%)                  | 8 (40%)         | 15 (33%)            | 8 (40%)         | 0 (0%)     | 8 (40%)         | 0 (0%)                |
| P value                   | 0.363           | 0.255                | 0.779                    | 0.063           | 0.140               |                |            |                 |                       |

I, insufficient; D, deficient; S, sufficient. *The probability of significance (P-value) refers to the chi-square test.

Figure 1. ROC Curve of Association between Age and Vitamin D Quantification for the Control Group
flight (nLC-Q-TOF) technology was used, and vitamin D-binding protein was found down expressed in the plasma of cases with benign and malignant breast lesions in comparison with controls’ plasmas (Delmonico et al., 2016). In view of these findings, vitamin D-binding protein should be validated as a putative biomarker in a future study.

With regard to the Brazilian population, two reports evaluated breast cancer and vitamin D deficiency (de Lyra et al., 2006; Oliveira et al., 2016). Lyra (2006) studied the levels of 1,25(OH)2D3 and 25OHD in 88 women with breast cancer and 35 controls (submitted to mammoplasties or resection of benign lesions) in the city of São Paulo. These authors found no significant difference between the groups (P= 0.722). Further, when they evaluated the differences in vitamin D levels according to the histological grade of the tumor, no significant difference was found between grade tumors I/II (P=0.329) and III/IV (P=0.256). These results were similar to those of our study, in which no significant difference was detected for the different degrees of tumors (P=0.102) and lymph node involvement (P=1.00). On the other hand, the second Brazilian study, conducted in Belo Horizonte city, evaluating 181 women with breast cancer and 197 controls, determined that the levels of vitamin D sufficiency (P=0.012) and the moderate practice of physical activity (P=0.037) were protective variables for breast cancer (Oliveira et al., 2016).

Considering population studies from other countries, Acevedo (2016) reported that 98/105 (93.3%) Chilean women with breast cancer and 88/93 (94.6%) of cases with Luminal A/B tumors had vitamin D insufficiency/deficiency (≤29.9 ng / mL). Further, 51/53 (96%) of histological grade 3 tumors showed levels of vitamin D in the same range of insufficiency. In our study, these values were lower, with 30/45 (67%) of the Luminal tumors A/B and 15/17 (88%) from grade 3 tumors (see Table 3). In the study by Bilinski and Boyages (2013), 217 Australian women with breast cancer and 852 controls had vitamin D insufficiency with rates of 39.7% (85/214) and 32.3% (275/852), respectively. We found a higher value, being 49/65 (75%) and 12/20 (60%) for malignant tumors and controls, respectively.

The low levels of Vitamin D herein presented for the control group, as well as for the other studies (Oliveira et al., 2016; Bilinski et al., 2013; Unger et al., 2010), are alarming. These values become more evident in countries whose sun incidence is low or that have periods of rigorous winter (Nouri et al., 2017; Shirazi et al., 2016). Consequently, we believe that a part of the global population may be experiencing hypovitaminosis D, increasing the susceptibility to development of the carcinogenic process and other diseases. In relation to the carcinogenic process, the studies with larger numbers of evaluated cases have highlighted low levels of vitamin D as a risk factor for breast cancer (Rose et al., 2013), colorectal cancer (Maalmi et al., 2014), and haematological tumors (Wang et al., 2015). In addition, low levels of vitamin D were associated with the lower overall survival rate (OS) and the lower rate of disease-free survival (RFS) in these tumors.

An equally important finding here was the relationship between the low levels of vitamin D and aging. One reason why vitamin D tends to decrease with aging is

| Groups* | Number of cases | Mean (ng/mL) | SD | t-student test | P-value** |
|---------|----------------|--------------|----|---------------|-----------|
| Benign lesions | 6 | 17.83 | 7.60 | 0.336 | 0.741 |
| Controls | 12 | 18.88 | 5.84 | | |
| Luminal A/B | 30 | 20.29 | 5.66 | 0.751 | 0.457 |
| Controls | 12 | 18.88 | 5.84 | | |
| HER2 | 8 | 17.13 | 7.26 | 0.621 | 0.542 |
| Controls | 12 | 18.88 | 5.84 | | |
| Triple Negative | 5 | 17.60 | 5.03 | 0.433 | 0.670 |
| Controls | 12 | 18.88 | 5.84 | | |
| Luminal | 30 | 20.29 | 5.66 | 0.908 | 0.371 |
| Benign lesions | 6 | 17.83 | 7.60 | | |
| HER2 | 8 | 17.13 | 7.26 | 0.177 | 0.862 |
| Benign lesions | 6 | 17.83 | 7.60 | | |
| Triple Negative | 5 | 17.60 | 5.03 | 0.059 | 0.955 |
| Benign lesions | 6 | 17.83 | 7.60 | | |
| Luminal A/B | 30 | 20.29 | 5.66 | 0.990 | 0.330 |
| Triple Negative | 5 | 17.60 | 5.03 | | |
| Luminal A/B | 30 | 20.29 | 5.66 | 1.308 | 0.200 |
| HER2 | 8 | 17.13 | 7.26 | | |
| Triple Negative | 5 | 17.60 | 5.03 | 0.127 | 0.901 |
| HER2 | 8 | 17.13 | 7.26 | | |

SD, standard deviation, * Only deficiency and insufficiency cases of vitamin D, ** The probabilities of significance (P-value) refer to the t-Student test for independent samples.
directly related to the decreasing capacity to produce vitamin D3, due to degeneration in the cutaneous levels of 7-dehydrocholesterol (Bolland et al., 2006; Bolland et al., 2007). Two other reasons associated with decreasing vitamin D in the elderly are the increase of fat mass and the decrease of sun exposure. The first is related to the number of adipocytes that sequester vitamin D naturally, ingested or produced in the skin, before being transported to the liver to be converted to 25(OH)D3 (Bolland et al., 2007); and the second that elderly tend to wear more clothing and spend more time indoors due to reduced walking capacity (Unger et al., 2010).

This study is original in that it shows that vitamin D deficiency is prevalent lower in women with impalpable breast lesions and that vitamin levels are directly related to aging. We emphasize the need for monitoring vitamin D levels especially in the elderly, even in sunny countries, to prevent diseases such as cancer.

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Low Levels of Vitamin D in Women

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