Nutrición Hospitalaria

Trabajo Original

Otros

Lifestyle and vitamin D dosage in women with breast cancer
Relación de la vitamina D y el ejercicio físico en mujeres con cáncer de mama

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Abstract

Introduction: The prevention strategy of breast cancer is still the key factor for early diagnosis and the most effective method for tracking the disease.

Objective: This study aimed to evaluate the association vitamin D level with breast cancer in women.

Methods: This hospital case-control study was conducted with 181 women with breast cancer and 197 healthy controls. Vitamin D status, calcium, phosphorus and PTH serum dosage and data collection related to lifestyle and patient's history, besides anthropometric measurements were performed. Univariate analysis (Chi-square and raw odds ratio) and multivariate analysis were performed through multivariate logistic regression.

Results: This study shows a higher value of vitamin D in health controls (26.9 mg/dL) than in breast cancer women (24.8 mg/dL). Higher numbers of women with sufficient vitamin D status (34.85%) were found in control group than cancer group. Using the multiple logistic regression model, the family history of breast cancer (OR 36.37, 95%CI 4.75-278.50) and menopause (OR 5.17, 95% CI 2.72-9.80) had a direct association with breast cancer, while the level of vitamin D (OR 0.95, 95%CI 0.91-0.99) and moderate physical activity (OR 0.31, 95%CI 0.10-0.93) maintained the inverse associations with the disease.

Conclusion: Vitamin D status and the practice of moderate physical activity were considered protective factors for breast cancer. However, menopause and family history of breast cancer were considered a risk factor for breast cancer.

Key words:

Breast cancer. Vitamin D. Menopause. Physical activity. Case-control study.

Resumen

Introducción: la estrategia de prevención del cáncer de mama sigue siendo el factor clave para el diagnóstico precoz y el método más eficaz para el seguimiento de la enfermedad.

Objetivo: este estudio tuvo como objetivo evaluar el nivel de vitamina D asociado con el cáncer de mama en las mujeres.

Métodos: este estudio de casos y controles hospitalarios se llevó a cabo con 181 mujeres con cáncer de mama y 197 controles sanas. Se estudió el nivel de vitamina D, calcio, fósforo y la dosis de suero PTH; se recopilaron de datos relacionados con el estilo de vida y com la historia de las pacientes, además se realizaron mediciones antropométricas. El análisis univariante (Chi-cuadrado probabilidades y primas ratio) y el análisis multivariado se realizó mediante regresión logística multivariante.

Resultados: este estudio muestra un valor más alto de vitamina D en los controles de salud (26,9 mg/dl) que en las mujeres con cáncer de mama (24,8 mg/dl). Se encontraron más mujeres con suficiente vitamina D (34,85%) en el grupo control que en el grupo de cáncer. Usando el modelo de regresión logística múltiple, la historia familiar de cáncer de mama (OR 36,37; IC del 95%: 4,75 a 278,50) y la menopausia (OR 5,17; IC del 95%: 2,72 a 9,80) se halló una relación directa con el cáncer de mama, mientras que el nivel de vitamina D (OR 0,95; IC del 95%: 0,91 a 0,99) y la actividad física moderada (OR 0,31; IC del 95%: 0,10 a 0,93) mantienen las asociaciones inversas con la enfermedad.

Conclusión: el estado de vitamina D y la práctica de actividad física moderada se consideraron factores de protección para el cáncer de mama. Sin embargo, la menopausia y la historia familiar de cáncer de mama se consideran un factor de riesgo para el cáncer de mama.

Palabras clave:

Cáncer de mama. Vitamina D. Menopausia. Actividad física. Estudio de casos y controles.

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INTRODUCTION

Increasing life expectancy, adoption of western lifestyle, and growing urbanization has raised the global incidence of breast cancer (1). Breast cancer has known risk factors such as the cellular aging, family history, alcohol consumption (2), overweight (3), sedentary lifestyle, and high breast tissue density (4). Other risk factors are related to women's reproductive life, which leads to increased endogenous estrogen levels, as the later age at first childbirth, early menarche, and late menopause (1,3). Some studies indicated that sun exposure and vitamin D levels are inversely proportional to the risk of developing breast cancer (5,6). Moreover, it is suggested a greater benefit if sun exposure occurs during the breast tissue development in young stages of women's life (7). Furthermore, vitamin D may act directly in tumorigenesis, and extrarenal tissues such as breast tissue, expressing the CYP27B1 enzyme, that provide instructions for the synthesis of 1-α-hydroxylase. This enzyme is responsible for converting the inactive precursor of vitamin D to the active form, namely, 1, 25-dihydroxycholecalciferol [$1,25(OH)_2D_3$]. This vitamin can have autocrine or paracrine activity, protecting the breast tissue cells from malignant transformation (8). The production of $1,25(OH)_2D_3$ is controlled by its own levels and is controlled by the parathyroid hormone, fetal growth factor 23, and calcium and phosphorus levels in serum (9).

Parathyroid hormone (PTH) along with vitamin D is involved in calcium homeostasis acting directly or indirectly in organs related to its storage, excretion, and absorption. Moreover, PTH can promote both formation and reabsorption of the bone tissue. Also, drops in calcium levels of 10% are sufficient to increase PTH serum levels (10,11).

In this study, we aimed to evaluate the associations between lifestyle, vitamin D status, PTH, calcium and phosphorus levels in serum, with breast cancer in women in the Hospital Foundation of Belo Horizonte, Minas Gerais, Brazil.

MATERIAL AND METHODS

STUDY POPULATION

This study is a part of hospital-based case-control study, whose source of patients were health services, ambulatory care; hospital; compulsory notification or registration diseases. The study was conducted with women treated in the Mastology Service of Odete Valadares Maternity, of Hospital Foundation of Minas Gerais State (FHEMIG), in Belo Horizonte, Minas Gerais, Brazil. Women who were referred to the ambulatory care were invited to participate in the study as volunteers. Those women who accepted signed the free and clarified consent term. The study followed the Declaration of Helsinki and was approved by the National Committee of Ethics in research (protocol number: 1889/2005) (12,13).

The case group (GCa) was composed by women who were referred for evaluation at the Maternity Odete Valadares, older than 18 years, living in rural or metropolitan region of Belo Horizonte, Minas Gerais, Brazil, with mammography result BI-RADS (14) (Breast Imaging

Reporting and Data System) 0, 3, 4 or 5 (assessment incomplete, probably benign, suspicious abnormality and highly suspicious of malignancy, respectively) and breast cancer confirmed by biopsy. The control group (GCo) was composed by women who were older than 18 years old, lived in same area in Brazil, and mammography's result BI-RADS 1 or 2 (negative and benign findings) (14).

The exclusion criteria were: the age below 18 years old, previous history of any other cancer type, benign, suspicious or indeterminate biopsy result or not having performed mammography.

A total of 378 women were selected for the study, 181 healthy women (GCo group) and 197 women with breast cancer (GCa group). Only those women with ductal or invasive lobular carcinoma were included in GCa group. Women with *in situ* disease, or *Phyllodes* malignant or borderline breast tumor or benign disease biopsy were not included in the study.

STUDY DESIGN

Data collection was carried out in the ambulatory in two steps: In the first step, prior to diagnosis, nutritional interview, lifestyle characteristics, medical history (such as diabetes), family history data and anthropometric measurements were collected. In the second stage, after diagnosis, peripheral blood sample was collected for laboratory analysis.

The anthropometric assessment was performed considering the weight and percentage of fat using the Tanita Body Fat Monitor Scale (model TBF 531©) and height was noted using the vertical stadiometer (Altura Exata©). The waist and hip circumferences were also measured and the body mass index (BMI) was calculated (12,15).

A questionnaire previously validated by Pena et al (12) was applied to the studied population to characterize the sample with regards to socioeconomic issues, medical history (such as diabetes), lifestyle, and food consumption.

Regarding the variables related to women's gynecological history, menarche and menopause age were requested. If they had children, the number of living children, abortion, breastfeeding, duration of breastfeeding for each child, age at first pregnancy, oral contraceptive use, and hormone replacement therapy were also asked.

The patients provided information about lifestyle, as alcohol use, smoking behavior and physical activity Those women who consumed at least one dose (10 g of alcohol) of any alcoholic beverage daily or more than 3 days a week were considered alcoholics (12,16). Those who reported smoking at least one cigarette per day, regardless the time of use, were considered smokers (17). The practice of physical activity was assessed by the International Physical Activity Questionnaire (IPAQ) (18), short version, and the women were classified as sedentary, low active, or active (12) (Fig. 1).

BIOCHEMICAL ANALYSES

Blood samples (4 mL) were collected in opaque bottles containing EDTA at the Odette Valadares Maternity's ambulatory care

on interview day. The blood was centrifuged to obtain plasma and stored at −80 °C for further analysis.

Serum levels of vitamin D, calcium, phosphorus, and PTH were measured in 256 women. For the vitamin D dosage, the ABBOTT© chemiluminescence kit was used and the metabolite 25-hydroxy-vitamin D [25(OH)D] was dosed (imprecision ≤ 10%,accuracy, 8,0 ng/mL detection limit, 8,0-160 ng/mL reference range). PTH was also dosed by chemiluminescence using the Beckman Couter© Kit (imprecision ≤ 8%, accuracy, 1 pg/mL detection limit, 12-88 pg/mL reference range). Calcium and phosphorus were measured by colorimetric method according to the manufacturer's protocol (Beckman Couter©) (calcium; precision 0,95% coefficient of variation, detection limit 0,01 mmol/L, reference range 8,8-10,6 mg/dL, phosphorus: precision < 3% CV, detection limit 1 mg/dL, reference range 3.7-7.2 mg/dL). The analyses were performed in the clinical laboratory of the Health Division at the Federal University of Viçosa, Minas Gerais, Brazil.

STATISTICAL ANALYSIS

The statistical analyses were carried out using Stata program, version 9.1. Kolmogorov Smirnov normality test was performed for each continous variable. The mean values for the variables net income, age, age at first pregnancy, age of menopause, patients' age when they performed the first mammography, weight, BMI, parathyroid hormone, calcium, phosphorus and vitamin D presented asymmetric distribution. Furthermore, Student's *t* test was used to evaluate the differences between the averages of the GCa and GCo groups. The Mann-Whitney test was used for determination of asymmetric distribution. In addition, the association between the breast cancer and each categorical variable was evaluated using the Chi-square test. Univariate analysis (chi-square and raw odds ratio) and multivariate analysis were performed through multivariate logistic regression (cancer was considered dependent variable, and menopause, diabetes, age at first pregnancy, patients age when they made the first mammography, physical activity, income, contraceptive, body fat,

vitamin D, alcoholism, breast cancer history family, nulliparity, age and breast-feeding were considered independent variables). A 5% level of significance was considered for the analysis.

RESULTS

The characteristics of the women in each group are shown in table I. The women with breast cancer were older than healthy controls (p < 0.001). There was no difference between the groups regarding the level of education and rural or urban living. A total of 378 women were evaluated, with a median age of 51 years (44-59 years). Among them, 43.09% of women were from the rural zone and 29.63% had attended full middle school (full middle school, 7th grade, or more) (data not shown).

The average net income of the family and the minimum wage was assessed. Women from control group had higher income than women from case group (p = 0.018) (Table I). In relation to reproductive factors, more women from GCo reported having at least one born child alive than the women from GCa (p = 0.014) (Table I). There was no difference between the groups regarding breastfeeding, (p = 0.120) and use of hormone replacement therapy (p = 0.45). The contraceptive use was higher in control women (p = 0.025). The first term pregnancy equal to or more than 30 years old was higher in GCa group (p = 0.024) (Table I). The average of age of women when they first underwent mammography was higher in the GCa group (43 years × 40 years, p = 0.002). Approximately 80.51% of cases and 41.24% of controls were in menopause (p < 0.001) (Table I). However, no difference was observed for the age of menopause between the groups (p = 0.61). The mean age of menarche was the same in both groups approximately 13 years (p = 0.611) (data not shown).

Differences between groups were not found for anthropometric characteristics, like BMI, weight and waist-hip ratio (WHR) (p = 0.842, p = 0.402 and p = 0.464). To assess the percentage of body fat, patients were classified as normal/eutrophic body fat or high body fat. More women of control group presented high percentage of body fat than in GCa (p = 0.049) (Table I).

The lifestyle, current or previous smoking, alcoholism, and physical activity was assessed. In GCo group, 17.68% of women reported current smoking, while in GCa group, only 12.69% reported smoking (p = 0.014). With regard to alcoholism, more women in GCo group did not consume alcohol in sufficient levels to configure alcoholism than in GCa (p < 0.001) In relation to the practice of physical activity, most of women from control group practiced mild physical activity. However, in case group, most of the women did not practice any physical activity. In GCo 13.41% had moderate physical activity, while in the GCa group 3.57% reported the same (p = 0.001) (Table I).

The median value of vitamin D in GCo group was higher than GCa group (p = 0.008). In GCo group, 34.59% had vitamin D levels above 30 ng/mL, while in the GCa group, only 21.95% had sufficient levels. Approximately 51.13 and 51.22% of controls and cases, respectively, had vitamin D levels considered as insufficient (between 21 and 29.9 ng/mL); while 14.29% in GCo group
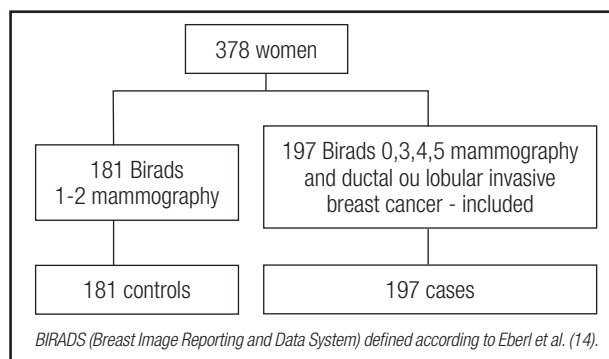
Figure 1.

Study design. Number of women selected in the case and control groups, according to inclusion and exclusion criteria.

Table I. Characteristics of women from case and control groups

Characteristics	n	Case	Control	p
		Groups		
Age (years)[a, **]	377	54 (48-62)	47.5 (42-53)	< 0.001*
Average net income (R$)[a, **]	372	600 (350-1000)	700 (400-1200)	0.018*
Income in minimum wage [a,**]	362	1.714 (1-2.85)	2 (1.14-3.42)	0.018*
Income < R$700,00 (%)[b]	362	54.31	43.09	0.044*
Rural region (%)[b]	369	45.31	40.68	0.369
Schooling (full middle school/7th grade or more) (%)[b]	378	28.43	30.94	0.864
Previous history of benign breast disease (%)[b]	374	17.77	0	< 0.001*
Family history of breast cancer (%)[b]	374	31.12	1.12	< 0.001*
Age of first pregnancy (years) [a,**]	316	23 (19.5-26)	20 (18-24)	< 0.001*
Age first pregnancy > = 30 years (%)[b]	316	14.74	6.88	0.024*
Children (yes, %)[b]		79.70	88.95	0.014*
Children (no, %)		20.3	11.05	
Use of contraceptive (%)[b]	373	51.78	62.43	0.025*
Breast-feeding (%)[b]	371	74.62	82.32	0.120
Menopause (%)[b]	372	80.51	41.24	< 0.001*
Age of first mammography (years)[a,**]	362	43 (39-52)	40 (36-46)	0.002*
High % body fat (%)[b]	363	48.11	58.43	0.049*
Weight (kg)[a, **]	372	63.8 (56.8-75.2)	66.2 (57.2-75.6)	0.402
Body mass index (BMI)[a, **]	372	26.89 (23.57-30.73)	26.95 (24.08-30.49)	0.842
Diabetes mellitus (%)[b]		13.71	7.26	0.043*
Alcoholism (n,%)[b,***]				< 0.001*
Yes	56	31 (15.74)	25 (13.81)	
No	292	139 (70.56)	153 (84.53)	
No answer	30	27 (13.71)	3 (1.66)	
Physical activity (n, %)[b,***]	375			0.001*
Sedentary lifestyle	167	102 (52.04)	65 (36.31)	
Mild	147	87 (44.39)	90 (50.28)	
Moderate	31	7 (3.57)	24 (13.41)	

[a]Mann Whitney, [b]χ^2 Test, **Median (interquartile interval), ***estimated population size and percentage (n,%) are presented.
*Significant difference at p < 0,05.

and 26.83% in GCa group had deficient levels, less than 20 ng/mL (p = 0.014). The analysis of calcium, phosphorous, and PTH did not show any differences (p > 0.05) between the groups (Table II).

The results of the univariate analysis indicate a direct association between the disease and the age of women, nulliparity, age of first successful pregnancy, age the first mammography, *diabetes mellitus*, and family history of breast cancer. Inverse association was verified with moderate physical activity, percentage of body fat, use of hormonal contraceptive, and vitamin D levels (p < 0.05) (Table III).

Finally, a direct association was found between menopause (OR = 5.17, 95% CI = 2.72-9.80) and family history of breast

cancer (OR = 36.37, 95% CI = 4.75-278.50), considered risk factors. Moreover, an inverse association was also observed between vitamin D levels (OR = 0.95, 95%CI = 0.91-0.99) and moderate physical activity (OR = 0.31, 95%CI = 0.10-0.93), with protective effect for breast cancer (Table IV).

DISCUSSION

The results suggest that menopause (12 months or more of amenorrhea) is associated with breast cancer. GCa had more menopausal women than GCo. This result was maintained in the

Table II. Levels of vitamin D, calcium, phosphorus and PTH in case and control groups

Characteristics	n	Groups		p
		Case	Control	
Vitamin D (ng/mL)a,**	256	24.8 (20.5-29.3)	26.9 (22.3-32)	0.008*
Status of vitamin Db (n,%)#	256			0.014*
Deficient		33 (27.05)	19 (14.399)	
Insufficient		62 (50.82)	67 (50.76)	
Sufficient		27 (22.13)	46 (34.85)	
Calcium level (mg/dL)a,**	256	9.9 (9.6-10.3)	9.7 (9.4-10.1)	0.127
Phosphorus level (mg/dL)a,**	256	3.7 (3.3-4)	3.7 (3.4-4.2)	0.236
PTH level (pg/mL)b,##	256	8.5	9.0	0.660

aMann Whitney, bχ^2 Test, **Median (interquartile interval), #mean, estimated population size (n) and percentage (%). ##Mean. *Significant difference at $p < 0.05$.

Table III. Association of patients' characteristics and incidence of breast cancer among the study groups

Characteristics	OR	95% CI	p
Age	1.06	1.04-1.09	< 0.001*
Menopause	5.89	3.70-9.36	< 0.001*
Nulliparity	2.05	1.15-3.66	0.015*
Age at first successful pregnancy	1.08	1.03-1.13	0.001*
Age at first pregnancy > 30 years	2.34	1.10-4.99	0.027*
Age at first mammography	1.04	1.02-1.06	0.001*
Income	1.62	1.08-2.45	0.021*
First mammography performed after 40 years	1.93	1.27-2.94	0.002*
Diabetes mellitus	2.03	1.01-4.07	0.046*
Family history of breast cancer	39.76	9.55-165.52	< 0.001*
Physical activity - sedentary	1	-	-
Mild	0.62	0.40-0.95	0.027*
Moderate	0.19	0.08-0.46	< 0.001*
High % body fat	0.66	0.44-0.99	0.049*
Use of hormonal contraceptive	0.62	0.41-0.93	0.022*
Alcoholism	1.37	0.77-2.43	0.289
Vitamin D	0.94	0.91-0.98	0.001*
Vitamin D levels- Deficient (< 20)	1		
Insufficient (20-29,9)	0.53	0.28-1.03	0.062
Sufficient (≥ 30)	0.34	0.16-0.71	0.004*

OR: Odds Ratio; *95% CI, 95% Confidence Interval. *Significant difference at $p < 0.05$.*

Table IV. Final model of multiple logistic regression analysis of breast cancer-related characteristics

Characteristics	OR	95% CI	p
Menopause	5.17	2.72 - 9.80	< 0.001*
Vitamin D	0.95	0.91 - 0.99	0.012*
Mild physical activity	0.61	0.32 - 1.14	0.122
Moderate physical activity	0.31	0.10 - 0.93	0.037*
Family history of breast cancer	36.37	4.75 - 278.50	0.001*

OR: Odds Ratio; *95% CI, 95% Confidence Interval. *Significant difference at $p < 0.05$.*

it did not influence the association between breast cancer and menopausal status.

Although menopause is not considered a risk factor for breast cancer, the late menopause (after 55 years) is directed associated with the disease (19), due to longer exposure of endogenous hormones. The estrogen exposure has also been associated with conditions like nulliparity, late menopause age, early menarche and giving first birth at later age (20). There is an increased risk each year of late menopause, particularly in positive estrogen receptors tumors (2121). Despite of it, this study did not show difference for the age of menopause (p = 0.600) and late menopause (p = 0.083) between GCa e GCo. This result is due the close menopause age between groups.

Age is considered a risk factor for breast cancer (22). In approximately 80% of cases, the disease occurs after age 50 (4). According to Howlader et al, the risk of developing breast cancer among American women increases 0.44% at 30 years, 2.38% at age 50 and 3.82% at 70 years (23). In this study, the patients of CaG had median age higher than controls, with a direct association with breast cancer. However, this association did not persist after the adjustment in the final model. In a systematic review, Cutler et al. (24) observed an average of cumulative breast cancer inci-

final statistical model, even after the adjusted model for patient age. Despite the breast cancer patients were older than controls,

dence of 0.22% each year of age in women less of 50 years and 0.23% per year among women either 50 years old or with surgically menopausal.

Family history of breast cancer is directly associated with the disease. Having a first degree relative with breast cancer is one of the most consistent risk factors for the disease (19,25). The breast cancer risk in lifetime is 7.8% in women without history family, and rises to 13.3 % with one case in family and 21.1% with two affected relatives (25). The increased risk of breast cancer is associated with different subtypes of the disease, both in cancers with hormone receptor positive and negative (26). Heredity accounts for about 27% of cases of breast cancer, for the sporadic cancer, most cases are related to the environment (27). Family history of breast cancer is also found in cases of hereditary breast cancer associated with gene mutations *BRCA*1 and *BRCA*2, and other mutations, accounting for 5-10% of cases (28).

In our study more women in GCa were reported with family history of breast cancer than in GCo (p < 0.001) and there was a direct association with the disease (OR = 36.37). Moreover, only 1.12% of the patients in GCo presented cases of family history of breast cancer in first-degree relatives. This value is lower than the ones reported in the national literature. For instance, Paiva et al. (29), in a case-control study observed a family history of breast cancer in 12.5% of the patients with breast cancer and in 13.1% of the controls. Matos et al. (30) also showed the prevalence of the disease in 2.3% mother, 3.9% sister and 0.2% daughter affected by the disease.

Some studies have shown that the practice of physical exercise reduces the risk of breast cancer (31-33). In United Kingdon, 3.4% of post-menopausal breast cases in 2010 were associated with lack of exercise (34). Some possible mechanisms involved in breast cancer risk reduction are the reduction of hormone levels induced by exercise (35), body fat reduction (36,37), stimulation of antitumor immune activity and the reduction of insulin-like growth factors, which may increase cell division and inhibit cell death (35,37).

In our study, an inverse association was observed between the practice of moderate physical activity and breast cancer (p < 0.001). A greater number of GCo patients reported moderate physical activity practice (p = 0.001), while sedentary lifestyle was higher among the GCa. The moderate physical activity may have a protective effect against breast cancer according to the results.

The sedentary lifestyle is associated with 8% of breast cancer cases (38). Cohen et al. (39) in 2013 showed in white women that increased physical activity was associated with less chance of developing breast cancer, while sedentary lifestyle was associated with increased risk. The sedentarism is related with weigh gain, and cancer risk, including breast cancer, increases with increasing BMI (40). In a study with Japanese women, an inverse association with breast cancer was found among those who had the habit to walk more than an hour a day than those who walked less than an hour a day (33).

The benefits of practicing physical activity go beyond the disease prevention, also influencing the prognosis of breast cancer patients (41). Friedenreich et al. (42) observed that the practice of moderate to vigorous physical activity in post-menopausal women was enough to reduce the total body fat and adiposity. Therefore, this benefit may be related to the reduced risk of breast cancer in post-menopausal women.

Although there were a greater number of women practicing mild or moderate physical activity in the control group (p = 0.001), higher percentage of body fat (overweight or obese) was found in this group when compared to the GCa (p = 0.049).

The GCo patients had higher levels of 25(OH)D than the GCa patients (p = 0.008) (Table II). After stratification in deficient, insufficient, and sufficient vitamin D levels, the GCo group showed lower number of women with deficient levels and greater number of women with sufficient levels than GCa (p = 0.014) (Table II). Besides, an inverse association with breast cancer in the final model (OR = 0.95, 95% CI 0.91-0.99, p = 0.012) was observed (Table IV). Therefore, sufficient levels of vitamin D have given protective effect against breast cancer. Similar to our study, Park et al. (43) also dosed the vitamin D levels and stratified the women in groups by the levels of vitamin D, as deficient, insufficient, and sufficient (20 ng/dL, 20-29.9 ng/dL, and 30 ng/dL, respectively). It was assessed the levels of 25(OH)D in Korean women with breast cancer and in the general population, and found a direct association with breast cancer in women with deficient vitamin D serum levels when compared to those with sufficient levels, with no variation of menopausal status.

In Brazil, studies have shown insufficient levels of vitamin D in the elderly. Camargo et al. (2014) found concentration of 25(OH)D of 24.8 ng/mL in patients aged 67.9 ± 8.6 years (44). Arantes et al. (2013) demonstrated deficient levels (< 20 ng /mL) at 17% in women with age between 60 and 85 years old (45). Saraiva et al. (46) also found insufficient levels of 25(OH)D in 43.8% of outpatients and 71.2% of institutionalized elderly patients. Saraiva et al. (2005) found Vitamin D deficiency in 15.4% and insufficiency in 41.9% of patients aged 79.1 ± 5.9 years (47).

According to the *Nurses' Health Study* results, comparing the highest quintile with the lowest quintile of vitamin D in patients, there was a 30% breast cancer reduction risk (48), with even more impact in postmenopausal women aged over 60 years. The vitamin D action in the risk reduction of breast cancer could be explained by the inhibition and control of mammary cell growth demonstrated in animal model (49,50), by the antiproliferative action of $1,25 (OH)_2D$ in tumor-derived endothelial cells, by modulating the cell cycle, and by affecting tumor cell signaling (51). Vitamin D also has action on the expression of genes involved in growth, differentiation and apoptosis (52). The overexpression of p73 is associated with induction of apoptosis in animals and in humans and can be influenced by calcitriol (52,53). The presence of 25-hydroxyvitamin D3 1-α-hydroxylase (CYP27B1) in mammalian cells indicate that local production of vitamin D would lead to a paracrine or autocrine action in mammary cells protection (54). In the present study, breast cancer patients with low vitamin D levels, would probably have less protection against malignant transformation and tumor growth.

The menopausal *status* seems to influence the association between vitamin D and breast cancer. However, there is no con-

sensus in the literature. In a recent meta-analysis evaluating nine prospective studies, no association of risk reduction was found for breast cancer in pre-menopausal women, however, for post-menopausal women a decrease of risk, especially for 25(OH)D levels between 27 and 35 ng/mL was verified (55). The protective effect of adequate levels of this vitamin in preventing breast cancer among post-menopausal women was demonstrated by Crew et al. (56). Other studies have found benefit in reducing breast cancer risk in pre-menopausal women with normal weight, mainly with an intake of 5 µg/day of vitamin D (57,58). Abbas et al.(59) found an inverse association between breast cancer in pre-menopausal women and vitamin D levels, without interaction with BMI and family history of breast cancer, also demonstrated among Japanese women.

Fedirko et al. (60) also demonstrated an inverse association between circulating levels of vitamin D and breast cancer among pre-and post-menopausal Mexican women in a case-control study. This study had a greater number of menopausal women among the cases. The $25(OH)D_3$ average was 18.6 ng/mL in the cases and 21.9 ng/mL in controls. Furthermore, among the controls, 36% of women had deficient vitamin D levels ($25(OH)D_3 <$ 20 ng/mL) and 9% sufficient levels (> 30 ng/mL). Similarly, in this study, in GCa group, a greater number of menopausal women was observed. Among GCo patients, 14.29% had deficient levels and 34.59% sufficient levels of vitamin D (Table II).

The serum levels of calcium, phosphorus and PTH did not present difference between case and control groups, independent of vitamin D status and despite the vitamin D levels. The calcium and the vitamin D supplement intake may have a role in reduction of breast cancer risk (61-63), but more studies are needed to confirm this association.

The direct association was found in this study between *diabetes mellitus* and breast cancer in univariate analysis, but it did not remain after the final model analysis.

CONCLUSION

This case-control study demonstrated that menopause and family history of breast cancer were considered risk factors for breast cancer in women independent of the women age.

The serum level of vitamin D was considered protective factor for breast cancer. Despite the vitamin D levels found in women with breast cancer being lower than those of the control group, both averages were stratified as sufficient level. The vitamin D status is not yet used for breast cancer screening in women. However, this result suggests that vitamin D may have a positive impact as strategy of breast cancer prevention or reduction. Thus, an interesting strategy would be to screening women with insufficient and deficiency vitamin D status to provide supplementation of this vitamin. Another strategy could be the encouragement of physical activity practice for health improvement, since the practice of moderate to intense physical activity had a protective effect for breast cancer. Thus, our findings will contribute to fill an important gap in the scientific literature.

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