Subclinical Hypovitaminosis D and Osteoporosis in Breast Cancer Patients

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ABSTRACT

Objective: This study was designed to detect 25-hydroxy vitamin D serum levels and bone mineral density (BMD) status in breast cancer patients, and to determine their relation to treatment and disease stages.

Patients and methods: The study included 74 female patients with breast cancer and 52 healthy volunteers as the control group. Serum levels of 25-hydroxy vitamin D, calcium, phosphorus, and alkaline phosphatase were measured using ELISA kits, while dual energy x-ray absorptiometry (DXA) was performed to assess the BMD. Twelve patients received chemotherapy only; 12 received chemotherapy and hormonal therapy, 22 received chemotherapy and radiotherapy while 28 received chemotherapy, hormonal therapy and radiotherapy.

Results: Serum levels of phosphorous and 25-hydroxy vitamin D were significantly lower (p =0.0001), and alkaline phosphatase was significantly increased (p =0.0001) in patients compared to the control. Hip, spine, and forearm DXA were significantly lower in patients than in controls (p =0.0001). The worst bone status was in those receiving both chemotherapy and hormonal therapy. The grade of tumor significantly correlated with the serum phosphorus level (p =0.048) and negatively with the serum 25- hydroxyl vitamin D level (p =0.03) as well as with the DXA of hip (p =0.01) and spine (p =0.0001).

Conclusion: Our study supports findings of increased incidence of hypovitaminosis D, osteoporosis and osteopenia in breast cancer patients. Hence, we throw light on the importance of offering calcium and vitamin D supplements to breast cancer patients. It is recommended that breast cancer patients have a DXA scan on a yearly basis.

Key words: Breast Cancer, DXA, 25-hydroxy vitamin D, bone mineral density.

Introduction

Among the long term problems associated with breast cancer is an increased incidence of bone loss and osteoporosis. This may be attributed to the disease itself or to the effect of chemotherapy, radiotherapy, and/or hormonal therapy [1,2]. Osteoporosis is a disease that affects bone structure and strength, leading to increased fracture risk [3]. Menopausal women experience a gradual decrease in bone density due to the effects of estrogen decline [4]. Many breast cancer patients experience a premature menopause that may be related to the effects of chemotherapy, direct radiotherapy, or surgical removal of the ovaries. There are specific chemotherapeutic agents (doxorubicin, cyclophosphamide, methotrexate, and 5-fluorouracil) that may play a major part in this process. In addition, hormonal treatment by aromatase inhibitors (AIs) such as anastrazole, letrozole, and exemestane play a pivotal role. Inhibition of the aromatase enzyme blocks the conversion of adrenal androgen into estrogen [5]. Using letrozole for 2 years had an impact on the bone mineral density (BMD), as the patients experienced a noticeable decline at the hip and lumbar spine, with more women becoming osteoporotic [6]. Corticosteroids that are commonly used in breast cancer metastases are known to cause bone loss. Moreover, breast cancer itself plays a role in this loss through activation of osteoclasts [7]. Vitamin D may help in prevention of breast cancer. While the association between vitamin D and breast cancer risk/prognosis is still controversial, a high proportion of women at-risk or affected by the disease have deficient vitamin D levels (<20 ng/ml) [8]. The best way to prevent bone loss associated with AIs is unclear, but it is advisable to practice exercises, receive calcium, vitamin D and bisphosphonate especially in post-menopausal women with a T-score less than -2.0 regardless of the fracture risk factors [9]. A guideline for the monitoring and treatment of bone loss associated with breast cancer has been published by the American society for clinical oncology (ASCO) [10]. Experimental studies have shown that 25(OH) vitamin D [11] calcium [12] and parathyroid hormone (PTH) [13] might affect tumor development. High levels of 1,25(OH) vitamin D in the breast might have an antitumor effect through the induction of cell differentiation, inhibition of cell growth and regulation of apoptosis in normal and malignant cells [14]. Vitamin D exerts its anti tumor effect via its receptor to form a nuclear receptor-ligand complex which regulates the expression of target genes [15]. Not only does the active form of vitamin D inhibit breast cancer cells from growing, but it makes them grow and die more like natural cells. Moreover vitamin D has anti-angiogenesis effect [16]

The two naturally occurring vitamin D forms Ergocalciferol (vitamin D2) and colecalceferol (vitamin D3) can be obtained from natural foods, fortified products or supplements and D3 can also be synthesized from 7-dehydrocholesterol in skin exposed to ultraviolet radiation [17]. Following its synthesis in the skin or oral intake, vitamin D is converted to 25-hydroxy vitamin D in the liver. The 25(OH) D3 is the predominant circulating metabolite and correlates with vitamin D status [18]. Thereafter, 25(OH) D undergoes renal hydroxylation, tightly regulated by PTH and calcium concentrations [19]. Due to the widespread use of screening mammography and early detection programs leading to breast cancer diagnosis at a much earlier stage and the recent introduction of more effective anticancer therapy, more women are surviving their breast cancer, which highlights the need for survivorship programs that address issues like bone health [20].

The present cross-sectional study aims to evaluate the circulating concentration of 25-hydroxy vitamin D and the bone mineral density status of breast cancer patients and to study their relation to the treatment received and the stage of breast cancer.

Patients and Methods

Seventy-four female patients with breast cancer were randomly recruited from the oncology department of Saudi German Hospital during the period of April 2013 to April 2014. Complete history was obtained and rheumatological examination performed. Fifty-two age and sex matched healthy adult females were recruited as controls.

Exclusion criteria from the study involved active hyper- or hypoparathyroidism, uncontrolled thyroid disease, clinically relevant vitamin D deficiency, malabsorption syndromes, Paget's disease, Cushing's disease, pituitary diseases, bone diseases, renal dysfunction, other malignancies, and diseases known to influence bone metabolism. Patients on long-term treatment with anticonvulsants, anti-coagulants, sodium fluoride, calcium supplements, and bisphosphonates were excluded from this study. The study was performed in accordance with the Declaration of Helsinki, and all women patients gave written consent for enrollment in the study.

Biochemical analysis: All patients and controls were required to provide a full history and undergo a clinical examination. Non-fasting venous samples were separated and stored at -80 °C. Assays were performed for the serum alkaline phosphatase, serum phosphate and serum calcium levels. Serum 25-hydroxy vitamin D level: was measured using ELISA kit (Eagle Biosciences, Inc., 20A Northwest Blvd., Suite 112, Nashua, NH 03063 north of Boston, MA, USA); sensitivity of the kit was 0.02 pico mole /l; Intra-assay and inter-assay coefficient of variation (CV) were 3.2% and 8.6%.

Dual energy X-ray absorptiometry (DXA): was performed to assess bone mineral density (BMD) status for the hips, forearms, and spines of all participants. Patients were considered to have osteopenia if their adjusted T scores were -1.0 to -2.5 and osteoporosis if their adjusted T scores were ≤ -2.5 at any measurement site [21].

Statistical analysis of data was performed with a statistical package for the social sciences (SPSS) version 21. Data were presented as mean ± standard deviation or number and percentage as appropriate. Chi-square test was used for analysis of non-parametric data and unpaired Student's t-test, ANOVA, and linear correlation were used for parametric data. A p-value of less than 0.05 was considered significant.

Results

Thirty breast cancer patients were included with a mean age of 46.3±6.3 years. Thirty age and sex matched controls had a mean age of 48.1±9.66 years. None of the patients or control were smoking. Twenty-one patients were menstruating (5 with irregular menses) and 9 postmenopausal. The age, laboratory and DXA results of the cancer patients and controls are shown in Table 1. Breast cancer was unilateral in all the patients (10 on the right side and 64 on the left). In cancer breast cases, there was osteopenia at the hip region in 14 (18.9%) patients, at the forearm in 20 (27%) and at the spine in 14 (18.9%) while osteoporosis was present in 8 (10.8%) patients at the hip, 2 (2.7%) at the forearm and in 9 (12.2%) at the spine.

Twelve (16.22%) patients received chemotherapy only, another 12 (16.22%) received chemotherapy and hormonal therapy, 22 (29.70%) received chemotherapy and radiotherapy, and 28 (37.80%) received chemotherapy, hormonal therapy, and radiotherapy. The chemotherapy regimens used were (5-fluorouracil, doxorubicin and cyclophosphamide) for 6 cycles, or (docetaxel, doxorubicin and cyclophosphamide) for 6 cycles, or sequential (doxorubicin and cyclophosphamide) for 4 cycles then paclitaxel for 4 cycles. The dose of 5-fluorouracil was 600 mg/m2, doxorubicin 60mg/m2, cyclophosphamide 600 mg/m2, docetaxel 75 mg/m2, and paclitaxel 175mg/m2. Hormonal treatment included aromatase inhibitors (AIs) such as letrozole 2.5mg daily, anastrozole 1mg daily, examestene 25mg daily and the anti-estrogen tamoxifen 10mg bid daily or a luteinizing hormone-releasing hormone (LHRH) agonist goserelin 3.6mg SC monthly. All patients underwent tumor resection, completed chemotherapy and/or radiation therapy within one year of study entry, and had no evidence of residual disease.

Regarding stages of the disease, 2 cases (2.7%) were in stage I, 18 cases (24.3%) were in stage II, 40 (54.1%) were in stage III, and 14 (18.9%) were in stage IV.

On considering the treatment regimen received by the patients; in those receiving chemotherapy only (n=12) there was hip osteoporosis in 4 cases (33.3%), forearm osteopenia in 33.3% and spine osteoporosis and osteopenia detected in 4 patients each; in those receiving chemo and hormonal therapy (n=12), hip osteopenia was present in 6 (50%), forearm osteopenia found in 8 (66.7%), spine osteopenia and osteoporosis in 33.3% and 25% respectively; in those receiving chemo and radiotherapy (n=22) osteopenia and osteoporosis of the hip and spine were present in 9.1% of cases, forearm osteopenia was found in 18.2% while osteoporosis in 9.1%; and in those receiving chemo, hormonal and radiotherapy (n=28), hip osteopenia was present in 21.4% while forearm and spine osteopenia were present in 14.3%. Comparison of biochemical data and DXA score of breast cancer patients according to treatment regimen are presented in Table 2.

Table 1: Comparison between age, biochemical data and DXA score of control and breast cancer patients

Mean±SD (range)	Cancer breast (n = 74)	Control (n = 52)	t	p-value
Age (years)	46.3±6.3 (27-65)	48.1±9.66 (37-60)	- 0.34	0.74
Biochemical data				
Ca (mg/dl)	9.1±0.7 (7.9-10.2)	9.1±0.7 (8.05-10.3)	0.08	0.93
P (mg/dl)	1.6±0.4 (0.9-2.5)	2.2±0.4 (1.6-2.8)	7.58	**0.0001**
ALP (U/L)	221.9±50 (139-3054)	188.2±48.9 (105-277)	- 3.75	**0.0001**
25-OH D (IU/L)	18.4±6.3 (7-33)	23.7±5.2 (17.9-34.6)	5.02	**0.0001**
DXA t score				
Hip	-0.2±1.04 (-0.3 – 1)	0.3±0.47 (-0.7 – 0.9)	4.33	**0.0001**
Forearm	-0.1±1.05 (-0.2.7 – 1.7)	-0.1±0.63 (-0.9 – 0.98)	2.07	**0.0001**
Spine	-0.2±1.07 (-2.8 – 1.5)	0.06±0.5 (-0.8 – 0.9)	3.21	**0.0001**

Ca: Calcium, P: Phosphorus, ALP: Alkaline phosphatase, 25-OH D: 25-hydroxy vitamin D, DXA: Dual energy x-ray absorptiometry. Bold values are significantly different at $p<0.05$

Table 2: Comparison of biochemical data and DXA score of breast cancer patients according to treatment regimen

mean±SD	Treatment regimen in cancer breast patients (n=74)				
	CT only (n = 12)	CT & HT (n = 12)	CT & RT (n = 22)	CT, HT & RT (n = 28)	ANOVA F
Biochemical data					
Ca (mg/dl)	9.4 ± 0.5	9.3 ± 0.3	9.1 ± 0.9	8.8 ± 0.7	3.2
P (mg/dl)	1.2 ± 0.2	1.5 ± 0.1	1.7 ± 0.6	1.8 ± 0.4	6.1
ALP (U/L)	203.3 ± 4.1	255.8 ± 37.6	208.3 ± 49.6	226 ± 56.6	3.3
25-OH D (IU/L)	15.3 ± 6.1	18.8 ± 1.3	17.67 ± 6.6	20.1 ± 6.95	1.9
DXA t score					
Hip	-0.8 ± 1.5	-1.1 ± 1.4	-0.3 ± 1.1	-0.2 ± 0.9	2.4
Forearm	-0.8 ± 0.9	-0.8 ± 1.5	-0.3 ± 1.2	-0.2 ± 0.7	1.4
Spine	-0.9 ± 1.6	-0.96 ± 1.3	-0.3 ± 1.04	-0.3 ± 0.9	1.9

CT: Chemotherapy, HT: Hormonal therapy, RT: Radiotherapy, Ca: Calcium, P: Phosphorus, ALP: Alkaline phosphatase, 25-OH D: 25-hydroxy vitamin D, DXA: Dual energy x-ray absorptiometry. Bold values are significantly different at $p<0.05$

A significant correlation was found between the grade of tumor and serum phosphorus (r=0.231, p=0.048) while negative correlations were found between tumor grading with the serum 25- hydroxyl vitamin D level (r=-0.26, p=0.03) as well as with the DXA of hip (r=-0.3, p=0.01) and spine (r=-0.41, p=0.0001).

Discussion

Vitamin D has also been reported to have anticancer activities against many cancer types, including breast cancer. The breakthrough that breast epithelial cells can locally manufacture active vitamin D from circulating precursors, makes the effect of vitamin D in breast cancer biologically conceivable [22]. In the present study, there was a significant decrease in serum phosphorus and 25-hydroxy vitamin D in breast cancer patients compared to healthy controls. These results were in accordance with the results of Crew et al., who stated that there is an "inverse association identified between 25-hydroxy vitamin D levels and breast cancer development" [23]. This is in harmony with the results of Lin et al who investigated and followed up 276 premenopausal and 743 post menopausal women for 10 years and stated that higher intakes of calcium and vitamin D were moderately associated with a lower risk of premenopausal breast cancer [24]. Similarly, in another study including 636 females, with incident breast cancer, a decreased risk was found with the increase in serum 25 (OH) vitamin D3 concentrations [25]. On the other hand, there were three comparable studies where no association between 25-hydroxy vitamin D levels and breast cancer risk was seen [26-28], and one study showed only a borderline association [29]. Combined vitamin D and calcium supplementation can reduce fracture risk. However, evidence is not sufficiently robust to draw conclusions regarding the benefits or harms of vitamin D supplementation for the prevention of cancer [30]. Future research is needed to better understand potential differences in breast cancer risk by vitamin D source and hormone receptor status [31]. The results of the present study have proven that the bone status of breast cancer patients is severely compromised, as indicated by DXA scores compared to the control group. There was a marked significant decrease in the DXA scores for the hips, spine, and forearms in patients compared to controls (p=0.0001). These results are consistent with those of Hadji et al. study assessing the bone status of 53 pre-menopausal breast cancer patients who received chemotherapy for one year. A significant decline in the DXA scores for the hips and lumbar spine was present in the patients compared to the controls (p=0.001) [32]. Our results are also in agreement with those of Marques Conde et al., who studied 51 postmenopausal patients with breast cancer and found a significant decrease in BMD [33]. In premenopausal women, both chemotherapy and gonadotropin-releasing hormone (GnRH) agonists exerted their effects on the bone, possibly through induction of premature ovarian failure causing a marked decline in estrogen levels; moreover, third generation AIs and tamoxifen produces a negative effect on bone due to estrogen exhaustion [20]. On assessing the bone status of patients with breast cancer in the present study according to the type of treatment it was found that the worst was in those receiving both chemotherapy and hormonal therapy, followed by those who received radio, chemo, and hormonal therapy. Our results agree with those of Vehmanen et al who compared between two groups of premenopausal women with breast cancer. The first group, which had hormone receptor-negative tumors, was considered a control group, and the second group had hormone receptor-positive tumors. Both groups received standard chemotherapy, and the second group started hormonal therapy with tamoxifen after six months from the beginning of chemotherapy. A significant increase in bone loss (decrease in BMD) was found among patients who had received both hormonal therapy and chemotherapy, compared to the group that had received chemotherapy only [34]. Aromatase inhibitors (AIs) are highly effective medicines in cancer care that may contribute to the occurrence of hip fractures. These drugs block estrogen production in peripheral tissues and the third generation (AIs) (anastrozole, letrozole and exemestane) reduces circulating estrogen levels, leading to accelerated bone loss and increased risk of fractures. The bone effects of AIs are comparable to other serious adverse reactions of other drugs and considered a frequently unrecognized cause of morbidity and mortality among cancer patients [35]. This may explain the

significantly reduced BMD DXA t score of the spine and forearm in patients with malignancy [36]. Moreover, in a previous study, patients receiving AIs were found to be at a higher risk of developing osteoporosis compared to normal subjects [37].

Conclusion

In conclusion, the vitamin D levels and BMD of the hip, forearms and spine are obviously reduced in breast cancer patients. The sub-clinically detected hypovitaminosis D, osteoporosis and osteopenia all throw light on the importance of offering calcium and vitamin D supplements to breast cancer patients. It is further recommended that breast cancer patients have a DXA scan performed at baseline and repeatedly on a yearly basis. Conducting the study longitudinally on a larger scale of patients is required to confirm our results.

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