Calcium Homeostasis and Bone Turnover in Tamoxifen and Chemotherapy Treated Postmenopausal Women with Breast Cancer

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Abstract: Preserving healthy bone among Postmenopausal women with breast cancer receives priority. The goal of study was to find out changes in calcium homeostasis and bone turnover in those patients. 82 patients were recruited and were classified into: newly diagnosed (ND, n= 15), tamoxifen (TT, n= 10) and chemotherapy treated (Chem, n= 57) groups. Serum was used for determination of calcium, phosphorus, vitamin D, Parathyroid hormone (PTH) and bone markers (osteocalcin, carboxy terminal propeptide of type I collagen and cathepsin K). Both hypocalcaemia and hypercalcaemia were detected in all groups with hypocalcaemia predominant among Chem group. No significant change in phosphate was noted between Chem and ND groups, while higher values were observed in TT compared to ND. PTH showed no significant variation between all studied groups. Vitamin D was almost below sufficient levels in ND and Chem groups, while improvement was noted by tamoxifen treatment. Both tamoxifen and chemotherapy treatment at the tested doses and duration had no effect on bone turnover markers. It is recommended to follow up total and ionized calcium and vitamin D before starting breast cancer therapy and during treatment and provide supplement when their levels falls.
[Amani F.H. Noureldeen, Hanaa M. Gashla and Wafaa T. Arab. **Calcium Homeostasis and Bone Turnover in Tamoxifen and Chemotherapy Treated Postmenopausal Women with Breast Cancer.** *Life Sci J* 2014;11(5):288-294]. (ISSN:1097-8135). http://www.lifesciencesite.com. 40

Keywords: Postmenopausal; Breast cancer; Tamoxifen; Chemotherapy; Bone turnover.

1. Introduction

Breast cancer is the most common cancer among women worldwide and the main cause of death in women ages 40 to 59 [1] which accounts for about 26 % of all female cancers [2]. In fact, breast cancer is the single leading cause of cancer death for women 20 to 59 years of age [3], thus posing a major public health concern. Regular and early screening and therapeutic developments have played an important role in increasing the survival rate, and that more patients are now receiving long-term adjuvant treatments.

Breast cancer is the most common cancer among Saudi females for the past 12 consecutive years [4]. Data on female patients with invasive breast carcinoma reported from different regions in Saudi Arabia showed that most patients are in the age group of 40 to 50 years. More than 50% were stage II and III, while ductal carcinoma in situ represented <5% of this population [5].

Preserving healthy bone among postmenopausal survivors with breast cancer gains priority because such women are at increased risk for osteoporosis and increased incidence for bone fractures, which may adversely affect their quality of life, even if they achieve a complete cure of their malignancy. Many therapies significantly reduce the recurrence are known to treat breast cancer, but unfortunately might impose negative effect on bone. Previous studies have demonstrated bone loss and fractures in women with chemotherapy-induced early menopause for breast cancer [6,7], although bone loss was not found in patients who retained ovarian function after chemotherapy treatment [7]. On the other hand, women diagnosed before age 55 had an increased relative risk of 1.78 for vertebral compression fractures by chemotherapy treatment [8]. Tamoxifen, a synthetic estrogen inhibitor which is widely used, significantly reduces the recurrence rate and increases overall survival. In premenopausal women, tamoxifen inhibits estrogen effects on bone tissue, thereby inducing bone loss [9]. Moreover, a study in patients with premature menopause, induced by adjuvant chemotherapy treatment for breast cancer, showed that tamoxifen reduced the rate of bone loss at the lumbar spine, by about 50%, but not at the hip [10].

The goal of the present study was to evaluate calcium homeostasis and bone turnover in tamoxifen and chemotherapy treated postmenopausal women with breast cancer, so that osteoporosis and hence fracture could be avoided.

2. Subjects and Methods

I- Subjects

This study was approved by Unit of Biomedical Ethics committee- King Abdulaziz University

Hospital, Jeddah, Saudi Arabia. After obtaining informed consent from each participant, patients underwent clinical examination, chest x-ray and bone scintigraphy to rule out hematogenic metastases. Subjects were interviewed using structured questionnaire to collect data including: age, weight, height, life style (smoking, exercise, dietary intake, etc.), clinical history including concomitant disease and use of hormonal replacement therapy, age and duration at menopause, if they had an oplicet to remove their uterus and /or one or both of ovaries and DXA scan results to assess bone status. All patients were postmenopausal (more than 6 month) with invasive breast cancer. Patients who did not had regular follow-up or patients who had local recurrence or developed metastatic disease during follow-up or history of any bone disease or any disease or medications that might affect bone turnover rate were excluded.

II- Study Design

82 patients who were treated at King Abdulaziz University Hospital, Jeddah, Oncology Department, were recruited in the study. Patients were divided into three main groups: **Newly diagnosed breast cancer (ND) group:** included 15 postmenopausal breast cancer patients. Patients in this group were not under breast cancer treatment. Blood samples were obtained from those patients just after surgery (mastectomy or lumpectomy). **Tamoxifen treated (TT) group:** included 10 postmenopausal breast cancer patients. Patients in this group were receiving both chemotherapy and hormonal therapy (10mg/day tamoxifen). **Chemotherapy treated (Chem) group:** this group included 57 postmenopausal breast cancer patients who were under chemotherapy treatment (doxorubicin/cyclophosphamide). This group was further subdivided into four subgroups, according to chemotherapy dose: **Chem 1:** included postmenopausal 10 breast cancer patients. Blood samples were taken from those patients after 21 days from 1st chemotherapy dose. **Chem 2:** included 13postmenopausal breast cancer patients. Blood samples were taken from those patients after 21 days from 2nd chemotherapy dose (after 6 weeks from 1st dose). **Chem 3:** included 12 postmenopausal breast cancer patients. Blood samples were taken from those patients after 21 days from 3rd chemotherapy dose (after 9 weeks from 1st dose). **Chem 4:** included 22 postmenopausal breast cancer patients. Blood samples were taken from those patients after 21 days from 4th chemotherapy dose (after 12 weeks from 1st dose).

III- Methods

Whole blood was collected from each patient in a plain tube, serum was separated and was subjected to determination of:

A) Markers of calcium homeostasis:

1- Calcium (Ca): using colorimetric method with a kit supplied by Randox Company (Cat. No. CA590)
2- Phosphorus (P): using Photometric UV test, with a kit supplied by Human Company, Germany (Cat. No. 10027)
3- Parathyroid hormone (PTH) using Enzyme-linked immune-sorbent assay (ELISA) Kit purchased from ALPCO Company, USA (Cat. No. 21-IPTHU-E01)
4- 25-Hydroxy Vitamin D_3 using electrochemiluminescence immunoassay (ECLIA) Kit provided by Elecsys and Cobas analyzers, (Cat. No. 06268668001V3).

B) Markers of bone turnover:

1- Osteocalcin (OC) using ELISA Kit supplied from ALPCO Company (Cat. No. 38-OSTHU-E01)
2- Carboxy terminal propeptide of type I Collagen (PICP) levels using ELISA Kit purchased from Quidel Company, Germany (Cat. No. 8003)
3- Cathepsin K using ELISA Kit provided from Biomedica Company (Cat. No. BI-20432).

IV) Statistical analysis

Statistical analysis was performed using SPSS 20.0 for windows (SPSS Inc, USA). Descriptive statistics are shown as arithmetic mean ± standard error of the mean, to describe the continuous data & using percentages to describe ordinal variables. Kruskal-Wallis test was performed for comparing more than two groups. Independent samples, Mann-Whitney (U) test was used as post hoc test to compare the means of two groups to determine if they are statistically different. *P*-value less than 0.05 was considered statistically significant.

3. Results

I- Patients' characteristics and Ca homeostasis:

No significant differences in all patients' characteristics between all studied groups were noted (Table 1). No significant change was observed in serum calcium by tamoxifen treatment compared to ND patients, meanwhile, chemotherapy treatment resulted in decreased serum Ca compared to ND group, being only significant after first (chem1) and third (chem3) doses. In relation to tamoxifen treatment, significant reduction in serum Ca was noticed after first chemotherapy dose (Table 2). Abnormally decreased serum calcium was more common in all chemotherapy treated than untreated and tamoxifen treated patients. On the other hand, elevated calcium levels were detected in 7 %, 30 %, 20 % and 9 % of patients in ND, TT, Chem1 and Chem4 (Table 3).

Table 1: Characteristics (X ± SE) of postmenopausal newly diagnosed (ND), tamoxifen treated (TT) and chemotherapy treated (Chem) breast cancer groups

Groups / Variables	ND	TT	Chemotherapy Treated			
			Chem 1	Chem 2	Chem 3	Chem 4
Age (years)	57.1 ± 1.19	56.7 ± 1.51	56.2 ± 1.55	55.2 ± 0.82	52.5 ± 1.05	56.2 ± 0.96
P vs ND	----	NS	NS	NS	NS	NS
P vs TT	NS	----	NS	NS	NS	NS
Weight (Kg)	80.0 ± 5.21	71.2 ± 3.81	77.1 ± 4.77	74.2 ± 4.75	76.5 ± 5.98	75.6 ± 2.62
P vs ND	----	NS	NS	NS	NS	NS
P vs TT	NS	----	NS	NS	NS	NS
Height (Cm)	154.5 ± 1.15	153.6 ± 1.52	153.2 ± 1.27	153.7 ± 1.41	155.5 ± 2.63	152.1 ± 1.51
P vs ND	**—**	NS	NS	NS	NS	NS
P vs TT	**NS**	----	NS	NS	NS	NS
BMI (Kg/cm^2)	33.4 ± 1.96	30.1 ± 1.29	32.8 ± 1.84	31.5 ± 2.03	32.4 ± 2.78	32.5 ± 0.80
P vs ND	----	NS	NS	NS	NS	NS
P vs TT	NS	----	NS	NS	NS	NS
Age at menopause (years)	49.1 ± 1.27	49.11 ± 1.43	48.7 ± 1.35	48.1 ± 1.27	45.6 ± 1.29	50.35 ± 0.63
P vs ND	----	NS	NS	NS	NS	NS
P vs TT	NS	----	NS	NS	NS	NS
Duration of menopause(months)	99.2 ± 14.45	82.7 ± 10.48	90.0 ± 16.98	90.5 ± 10.57	88.4 ± 16.82	81.2 ± 10.38
P vs ND	----	NS	NS	NS	NS	NS
P vs TT	NS	----	NS	NS	NS	NS

ND: newly diagnosed- TT: tamoxifen treated- chem 1, chem 2, chem 3 and chem 4: chemotherapy treated after Ist, 2nd, 3rd and 4th doses.
P > 0.05 is considered none significant (NS).

Table 2: Calcium homeostasis parameters (X ± SE) of postmenopausal newly diagnosed (ND), tamoxifen treated (TT) and chemotherapy treated (Chem) breast cancer groups.

Groups / Variables	ND	TT	Chemotherapy Treated			
			Cem 1	Chem 2	Chem 3	Chem 4
Calcium (8.4-10.2 mg/dl)	9.0 ± 0.29	9.9 ± 0.88	7.3 ± 0.31	8.5 ± 0.37	8.2 ± 0.74	8.8 ± 0.34
P vs ND	---	NS	0.001	NS	0.05	NS
P vs TT	NS	---	0.01	NS	NS	NS
Phosphate (3-4.5 mg/dl)	4.3 ± 0.45	5.9 ± 0.45	3.6 ± 0.27	4.2 ± 0.27	3.6 ± 0.38	3.8 ± 0.27
P vs ND	----	0.02	NS	NS	NS	NS
P vs TT	0.02	---	0.001	0.01	0.001	0.001
PTH (15.1-65.6 pg/ml)	50.6 ± 5.86	38.8 ± 5.18	61.9 ± 13.20	67.2 ± 11.43	86.8 ± 14.42	60.1 ± 6.27
P vs ND	----	NS	NS	NS	NS	NS
P vs TT	NS	---	NS	NS	NS	NS
25(OH) D (≥ 75 nmol/l)	26.7 ± 3.48	45.4 ± 6.19	25.4 ± 5.22	25.4 ± 4.47	19.1 ± 2.88	24.2 ± 2.32
P vs ND	---	0.01	NS	NS	NS	NS
P vs TT	0.01	---	0.03	0.01	0.001	0.001

ND: newly diagnosed- TT: tamoxifen treated- chem 1, chem 2, chem 3 and chem 4: chemotherapy treated after Ist, 2nd, 3rd and 4th doses.
Numbers between brackets indicate normal range for each parameter.
P < 0.05 is considered significant, while *P* > 0.05 is considered none significant (NS).

Table 3: The percent of patients with normal and abnormal Calcium (Ca), phosphate (Ph), parathyroid hormone (PTH) and 25(OH) vitamin D_3 (Vit D_3) values in postmenopausal newly diagnosed (ND), tamoxifen treated (TT) and chemotherapy treated (Chem) breast cancer groups

Groups / Variables	ND	TT	Chemotherapy Treated			
			Chem 1	Chem 2	Chem 3	Chem 4
% with decreased Ca	20	30	60	54	67	41
% with normal Ca	73	40	20	46	33	50
% with elevated Ca	7	30	20	0	0	9
% with decreased Ph	13	0	20	8	42	27
% with normal Ph	60	10	70	61	41	41
% with elevated Ph	27	90	10	31	17	32
% with normal PTH	87	90	80	69	50	90
% with elevated PTH	13	10	20	31	50	10
% with Sufficient Vit D_3 (75-250 nmol/L)	0	10	0	0	0	0
% with Insufficient Vit D_3(25-75 nmol/L)	40	80	50	46	25	50
% with Deficient Vit D_3(< 25 nmol/L)	60	10	50	54	75	50

ND: newly diagnosed- TT: tamoxifen treated- chem 1, chem 2, chem 3 and chem 4: chemotherapy treated after I^{st}, 2^{nd}, 3^{rd} and 4^{th} doses.

Significantly higher serum phosphate in TT group relative to ND and chemotherapy treatment was detected. No significant variations in the mean value of serum phosphate between ND breast cancer and all chemotherapy treated subgroups were obtained (Table 2). The percent of patients in each group with abnormal serum phosphate level is indicated in Table 3.

Mean serum PTH was not changed by treatment, either by tamoxifen or chemotherapy, compared to untreated patient group (Table 2). The percent of patients with normal and elevated serum PTH levels in each group is shown in Table 3. None of the untreated or treated breast cancer patients was presented with decreased serum PTH. Normal serum PTH was predominant than elevated values.

Significantly elevated vitamin D_3 mean values in TT compared to ND and Chem groups (Table 2) was obtained. According to Vitamin D_3 levels, we classified patients into 3 categories as: sufficient [25(OH) D_3: 75-250 nmol/L], insufficient [25(OH) D_3: 25-74 nmol/L] and deficient [25(OH) D_3 < 25 nmol/L]. Results indicated that all patients in ND group and Chem subgroups were away from sufficient level, while improvement was noted with tamoxifen treatment, (Table 3).

II- Bone turnover:

Although bone formation markers (OC & PICP) showed slight trend towards elevation by chemotherapy and almost comparable values to ND group by TT, however this variations did not reach statistical significance and all individual data remained within normal range. Most patients in all studied groups had non detectable cathepsin K values below assay sensitivity (1.1 pmol/L), Table 4.

Table 4: Markers (X ± SE) of bone formation including osteocalcin (OC) and carboxy terminal peptide of type I collagen (PICP) and bone resorpition marker (cathepsin K) in postmenopausal newly diagnosed (ND), tamoxifen treated (TT) and chemotherapy treated (Chem) breast cancer groups

Groups / Variables	ND	TT	Chemotherapy Treated			
			Cem 1	Chem 2	Chem 3	Chem 4
OC (3-8 ng/ml)	3.86 ± 0.59	3.84 ± 0.78	3.45 ± 1.01	6.66 ± 277	6.90 ±2.55	6.27 ± 1.68
P vs ND	---	NS	NS	NS	NS	NS
P vs TT	NS	---	NS	NS	NS	NS
PICP (50-170 ng/ml)	68.59 ± 4.45	80.67 ± 10.12	91.91 ± 17.20	71.83 ± 6.01	99.97 ± 21.17	104.58 ± 12.75
P vs ND	---	NS	NS	NS	NS	NS
P vs TT	NS	---	NS	NS	NS	NS
cathepsin K (pmol/L) % with detectable values	7	10	0	0	14	30
% with non detectable values	93	90	100	100	86	70

ND: newly diagnosed- TT: tamoxifen treated- chem 1, chem 2, chem. 3 and chem. 4: chemotherapy treated after I^{st}, 2^{nd}, 3^{rd} and 4^{th} doses.
Numbers between brackets indicate normal range for each parameter. $P > 0.05$ is considered none significant (NS).
Detectable and non detectable cathepsin K: values above and below assay sensitivity (1.1 pmol/L).

4. Discussion

Both hypo-and hypercalcaemia were previously observed in different types of cancer. Many authors [11,12] reported that hypocalcaemia was an uncommon but not unexpected during the course of various types of malignant disease. Different possible causes for hypocalcaemia were reported by different authors [13-15], among them are vitamin D deficiency, hyperphosphataemia (due to libration of phosphate from lysed malignant cells into the circulation after cytotoxic therapy) and hyperparathyroidism which were detected in some, but not all, cases in the present study. In this study we analyzed total serum calcium that includes: 40% of calcium bound to albumin, 10% of calcium fraction bound to other ions (e.g. phosphate), in addition to 50% as Ca^{+2} [16]. Therefore, factors that might affect ionized or bound calcium might affect total calcium. Unfortunately we did not measure serum Ca^{+2} concentrations, however, in a previous report [17] decreased Ca^{+2} was observed in TT patients compared to control group. In normal individual, blood Ca^{+2} concentrations are remarkably stable due to a complex regulatory system involving the action PTH, active vitamin D and calcitonin on target organs. PTH is highly dependent on Ca^{+2} concentrations in extracellular fluid. Serum PTH increases as serum Ca^{+2} decreases and vice versa [18]. Calcitriol also increases serum ionized calcium and phosphate by different mechanisms [19]. Therefore, increased prevalence of hypocalcaemia among our patients could be due to decreased serum [Ca^{+2}] which in turn might leads to increased PTH, or as a result of decreased serum vitamin D levels or due to increased serum phosphate (all were detected in our patients). On the other hand, the observed hypercalceamia among some patients in our study might be due to decreased serum phosphate which was detected in ND, and Chem groups. Hypercalcaemia in newly diagnosed breast cancer relative to healthy controls were reported by other investigators [16]. Our findings of elevated serum calcium in some cases in all studied groups could be also due to pathophysiology of breast cancer either through local or systemic effects on the skeleton which are referred to as local osteolytic hypercalcemia (LOH) and humoral hypercalcemia of malignancy (HHM), where the latter has emerged as the dominant subtype [20]. Others [21] indicated that approximately 30% of women with breast cancer develop hypercalcemia at some point in their disease that results from local osteolysis of bone. However, even early stage breast cancers may be associated with aberrations in calcium homeostasis that are caused by parathyroid hormone related protein (PTHrP), the agent of HHM. Consequently, others [22] reported that serum levels of PTHrP.

Obesity in postmenopausal women was reported to be one of the different causes for elevated serum calcium observed in some cases [23]. In our study, most patients with hypercalcemia were obese with BMI range from 32-37 kg/cm^2.

In the present study, the different pattern of serum calcium could not be attributed to differences in dietary calcium intake, since others [24] suggested that serum calcium levels is not only affected by dietary calcium intake but also by other dietary factors, while others [25] found no significant association between dietary calcium intake and serum calcium levels. It is worth mentioning to indicate that none of the patients were receiving either calcium and/or vitamin D supplement.

In this study, none of the ND or treated postmenopausal breast cancer patients was presented with primary hyperparathyroidism. Although others [16] reported an incidence of 1.58 % among women with incident breast cancer, however higher values, 4.7 % [26] and 7 % [27], among treated breast cancer patients, were also detected. In the present study, differences in serum PTH did not reach statistical significance between ND group and all other treated groups, which was supported by some studies [28,29].

The present study demonstrated that serum 25(OH) D_3 was highly deficient in ND and in Chem groups, while improvement was achieved by hormonal treatment. Ecological studies have associated high level of sunlight exposure with low breast cancer incidence and mortality [30]. Increased prevalence of 25 (OH) D_3 deficiencies in breast cancer patients before therapy was previously reported [31-33]. In different studies [32,34], 74 %, and 69% of breast cancer patients were 25 (OH) D deficient, and despite daily supplementation of vitamin D, still many remained deficient. Recently, it was reported that vitamin D status was worse during chemotherapy but recovered by combined treatment (both chemo & hormonal therapy), which is consistent and support our study [35].

The significant reduction in serum vitamin D_3 levels in all patients groups could be related to the increased BMI in our patients. An increased storage of 25(OH) D_3 in adipose tissue results in lower circulating levels of it [36]. Alternatively, increased total body fat may also be a consequence of low vitamin D levels as it has been hypothesized that low vitamin D status, by causing PTH excess and calcium influx into adipocytes, may promote weight gain [37].

When evaluating data of bone turnover markers from women, generally, the effect of age or menopausal status on such markers is very important. It is important to indicate that the mean values of age, BMI and menopausal status were matched in all studied groups and none of the patients had bone

metastasis. In this study, postmenopausal women treated with chemotherapy had elevated bone formation markers (OC and PICP) relative to ND; although differences were not significant and all individual values were within normal range. OC mean values were increased by 72.53 %, 78.76 % and 62.44 % in Chem 2, Chem 3 and Chem 4 , while PICP mean values were increased by 33.99 %, 4.72 %, 45.75 % and 52.47 % in Chem 1, Chem 2, Chem 3 and Chem 4 compared to ND.

Serum cathepsin K is of interest because it is the primary proteolytic enzyme used by osteoclast to degrade bone type I collagen during resorption. Cathepsin k was suggested to be a valuable marker of bone resorption [38]. Results indicated that most cases in ND and treated breast cancer groups showed none detectable cathepsin K values, which could be due to minor changes in bone resorption that could not be detected by the employed kit. In previous study [39], changes in some bone turnover markers and bone mineral density among breast cancer patients treated with tamoxifen were not significant by time compared to untreated patients. In postmenopausal women with breast cancer, tamoxifen lead to a normalization of bone turnover, demonstrated by decreased bone resorption and formation [40].

Recently, Jacot *et al.* [31] reported that vitamin D insufficiency was associated with changes in the calcium/RANKL/OPG axis, with significantly decreased serum calcium levels and the RANKL/OPG ratio. Elevation of RANKL/OPG ratio was reported [41,42] to be associated with de novo bone metastasis and the progression of skeletal tumor. The authors did not find any variation in the metabolism of bone markers. They suggested that in early stage breast cancer, the bone is not or only weakly metabolized by the low level of vitamin D. Results obtained from the present study (including decreased serum calcium, vitamin D deficiency, non significant change in bone formation markers, with their individual values within reference range, in addition to the minor change in bone resorption) support the previous study.

Conclusion

It is important to asses serum total and ionized calcium and vitamin D before starting therapy and during treatment and to use supplement when their levels falls to deficiency levels. It is essential to assess bone formation and resorption, so in case of elevated values, metastases might be considered.

Acknowledgement

The authors are grateful to King Abdulaziz City for Science and Technology for the financial support; project research number: S-F-0243-11.

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3/15/2014