

Effect of Adjuvant Chemotherapy is Responsible for Decreasing Segmental and total BMD in BC Postmenopausal Women

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Received date: January 08,2018; Accepted date : February 22,2018; Published date: March 28,2018. Citation for this Article: Srinivasa Rao Konijeti and Srileela Movva, Effect of Adjuvant Chemotherapy is Responsible for Decreasing Segmental and total BMD in BC Postmenopausal Women, J Cancer Research and Cellular Therapeutics, Doi: 10.31579/2640-1053/021

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Abstract

Background

Women with breast cancer are at increased risk for the development of osteoporosis and skeletal fractures, as consequences of aromatase inhibition or chemotherapy-induced ovarian failure. We investigated the effect of adjuvant chemotherapy on biochemical markers of bone formation and resorption as well as on bone mineral density (BMD) of non-metastatic breast cancer (NMBC) postmenopausal Egyptian women.

Methods

We followed 100 newly diagnosed women with T1-3 N0-2 M0 breast cancer, who had a mean age (\pm SD) of 55.06 \pm 8.78 year, before and after receiving 6-cycles of CAF chemotherapy treatment protocol. All participant women were subjected to blood biochemical analysis for determining serum levels of: erythrocyte sedimentation rate, calcium, alkaline phosphatase (ALP), bone specific alkaline phosphatase (S.ALP), Osteocalcin, carboxyterminal peptide of collagen type I (CTX-I), 25-Hydroxyvitamin D, Parathyroid Hormone (PTH) and tumor marker CA15-3. Segmental and total BMD were also investigated using Dual X-ray Absorptiometry technique.

Results

We found ALP, S.ALP, and CTX-I levels were significantly lower ($p < 0.001$), while PTH levels to be significantly higher for all women after chemotherapy as compared to their initial state before chemotherapy. Both segmental and total BMD, and consequently T- and Z-Scores after chemotherapy were significantly ($p < 0.01$) lower than their levels before chemotherapy. We developed prediction mathematical formulae for spine, pelvis and total BMD for all women before and after chemotherapy.

Conclusions

Adjuvant chemotherapy is responsible for decreasing both biochemical markers of bone formation and resorption as well as for decreasing segmental and total BMD in NMBC postmenopausal Egyptian women. We believe the mathematical formulae developed on basis of the two individual variables Age and BMI can be useful for assisting the clinician to frequently monitor bone health status of breast cancer patients in similar conditions.

Keywords: chemotherapy; bone turnover; bone mineral density; osteoporosis; dual-energy X-ray absorptiometry; multivariate regression analysis

Introduction

Breast cancer (BC), which forms in ducts and lobules of breast tissues, occurs in both men and women, although male breast cancer is rare [1]. It is the most common cause of cancer death among women worldwide; its incidence rates being high in developed countries whereas rates in developing countries and in Japan are low but increasing. BC accounts for 37.6% of all reported tumors in Egyptian females, with an age-adjusted incidence rate of 49.6 per 100,000 females [2]. BC, prostate cancer, and multiple myeloma have particularly shown strong association with skeletal metastases and related bone loss, resulting in fracture, hypercalcemia, pain, and declines in mobility and performance status [3,4].

Although adjuvant chemotherapy represents a significant advance in the management of patients with BC, which has prolonged their survival by decreasing the systemic relapse, it causes a significant reduction in their bone mineral density (BMD) [1,2]. Thus, women with BC are at increased risk for the development of osteoporosis and skeletal fractures, giving rise to significant morbidity and some mortality [3], as a consequence of aromatase inhibition or chemotherapy-induced ovarian failure [4,5]. Exemestane and anastrozole, two chemotherapeutic

aromatase inhibitors, have been shown to directly inhibit osteoclast differentiation and bone resorption markers leading to osteoporosis in postmenopausal women with non-metastatic breast cancer (NMBC) [6]. The bone resorption cross-linked carboxyterminal peptide of collagen type I (CTX-I) combined with BMD measurements, can be used for assessing bone health status in postmenopausal women [7, 8].

The objectives of this study were to investigate biochemical markers of bone formation and resorption as well as segmental and total BMD in NMBC postmenopausal Egyptian women before and after receiving a 6- cycles adjuvant chemotherapy treatment protocol.

Methods

Patients and material

The study population was comprised of 100 postmenopausal women [mean age (\pm SD) 55.06 \pm 4.78 year and body mass index (BMI) 38.28 \pm 4.13 kg/m²] with newly diagnosed T1-3 N0-2 M0 BC, who were studied longitudinally before and after receiving 6-cycles of a three-drug combination protocol containing Cyclophosphamide (600 mg/m²),



Adriamycin (40 mg/m²), and 5-Fluorouracil (600 mg/m²) (CAF), as detailed elsewhere [9,10]. Participants were recruited from the Department of Cancer Management and Research, Medical Research Institute, Alexandria University, Alexandria, Egypt; where they were hospitalized for diagnosis and/or treatment; and were referred to the Medical Biophysics and Chemical Pathology Departments, Medical Research Institute, Alexandria University, Alexandria, Egypt for subsequent bone densitometric measurements and blood biochemical analysis, respectively. The study was conducted in accordance with ethical guidelines of the 1975 declaration of Helsinki and the Ethics Committee of the Medical Research Institute, Alexandria University approved the study protocol. All participant women were asked to freely volunteer to the study protocol and provided a signed informed consent prior to their enrollment.

Clinical staging of BC was carried out according to the recent guidelines of the AJCC on basis of: a detailed physical examination, imaging studies, operative findings and pathologic examination of the breast and other tissues [11]. Exclusion criteria from the study protocol were the following: (1) serum creatinine greater than 150 mmol/L; (2) peptic ulcer; (3) hysterectomy or bilateral oophorectomy; (4) osteoporosis; (5) untreated hypothyroidism; (6) bisphosphonate, calcitonin or peroral steroid therapy; (7) pregnancy or lactation; and (8) other malignancies.

All participant women were interviewed regarding general health, bone aches, history of fractures, medications, and menopausal status and were subjected to a complete physical examination stressing on bone, joints, and neurological examination. The following analyses were carried out, using standard methods.

Blood biochemical analyses

Fasting blood samples were collected from all participant women to determine serum levels of: erythrocyte sedimentation rate (ESR, mm/hr) [12] and total calcium (Ca, mg/dl) [13] using a semi-automatic chemical analyzer (Olympus AU 400, Olympus Life and Material Science, Europe GmbH, Hamburg, Germany); osteocalcin (OC, ng/ml) [14], 25-hydroxyvitamin D (25-VitD, pg/ml) [15], parathyroid hormone (PTH, pg/ml) [16] and tumor marker CA15-3 (U/ml) [17] by chemiluminescence technique (Immulate 1000, Siemens Healthcare Diagnostics Inc., Flanders, NJ, USA); and total alkaline phosphatase (ALP, U/l), bone specific alkaline phosphatase (S.ALP, µg/L) [16] and carboxyterminal peptide of collagen type I (CTx-I, ng/ml) [18] by ELISA technique (ELISA ELx 800, Bio-Tek Instruments Inc., Winooski, UT, USA).

Imaging, body-composition and bone densitometric measurements

Imaging studies were carried out for all participant women using chest X-ray, abdominal and pelvic ultrasound and mammography. Demographic and body-composition variables were also measured for all participant women. Specifically, body weight (kg) (participants clothed in underwear, bare feet) was measured using a digital scale sensitive to the nearest 0.01 kg (Electronic Body Scale, TCS-200-RT, China). Height (m) was measured using a stadiometer. BMI was calculated as Weight/Height² (kg/m²). Segmental (i.e., head, arms, trunk, ribs, spine, pelvis and legs) and total bone mineral content (BMC) and BMD, as well as fat mass (FM), lean bone-free mass (LBFM) and tissue bone-free mass (TBFM) were assessed using a Dual-energy X-ray Absorptiometry (DXA) total body scanner (Lunar DXP Pro, GE Health Care, USA), as detailed earlier by our group [19- 22].

Statistical analysis

Data analysis was carried out using the StatView[®] statistical software package (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were calculated for the mean±SD of all relevant variables and their frequency distributions were examined. Analysis of the continuous variables showed them to be normally distributed. Paired Student's t-test of significance was used to compare differences before and after adjuvant chemotherapy for various variables.

Differences were considered to be significant only if p values were less than 0.05. Furthermore, multivariate linear regression analysis was performed to examine the interrelations among demographic variables and segmental and total BMD for NMBC women using simple and partial correlation coefficients. Prediction equations based on two independent variables (i.e., Age and BMI) were developed and their cumulative correlation coefficients (R) and standard error of estimation (SEE) were calculated, as detailed elsewhere [20,22].

Results

Demographic and body-composition data for NMBC women before and after chemotherapy are shown in Table 1. Although numerically low, neither weight nor its tissue components (i.e., FM, LBFM, and TBFM) were statistically different after chemotherapy. However, segmental and total BMC and BMD, consequently, total T- and Z-Scores, were all significantly (p<0.05) lower after chemotherapy as compared to their levels before chemotherapy (data only for BMD are shown in Figure 1). Biochemical analysis (Table 2) showed comparable levels of serum ESR, Ca, OC, 25-VitD and CA15-3; yet ALP, S.ALP and CTx-I levels were significantly lower after chemotherapy as compared to their levels before chemotherapy. Only PTH serum levels were significantly higher after chemotherapy in comparison with its levels before chemotherapy.

	Before chemotherapy		After chemotherapy	
Age (year)	55.06±4.78	(48.80–72.84)	55.63±4.60	(49.07–73.10)
Weight (kg)	94.25±13.77	(66.80–118.10)	88.01±18.82	(44.30–112.90)
Height (m)	1.57±0.07	(1.32–1.67)	1.53±0.05	(1.32–1.64)
Body mass index (BMI, kg/m ²)	38.28±4.13	(28.65–46.84)	37.51±6.24	(24.37–42.96)
Fat mass (FM, kg)	45.27±9.06	(25.61–62.16)	42.88±12.60	(15.79–61.40)
Lean bone-free Mass (LBFM, kg)	43.40±6.70	(31.43–60.23)	39.36±7.65	(21.09–58.66)
Tissue bone-free Mass (TBFM, kg)	88.67±12.86	(64.81–107.98)	82.25±17.60	(40.97–104.78)
T-score	1.25±1.36	(-1.60–2.70)	0.55±1.20*	(-1.90–2.20)
Z-score	0.92±0.92	(-0.80–3.10)	0.40±1.01*	(-1.60–2.70)

Values are expressed as Mean±SD and numbers in parenthesis are ranges (minimum–maximum). Statistical analysis was carried out using unpaired student's t-test of significance. *p<0.001 versus women before chemotherapy.

Table 1 : Demographic and body-composition parameters measured for women (n=100) before and after receiving 6-cycles of a three-drug cyclophosphamide, adriamycin, and 5-fluorouracil (CAF) chemotherapy treatment protocol.

	Before chemotherapy		After chemotherapy	
Erythrocyte sedimentation rate (ESR, mm/hr)	73.57±25.91	(23–120)	59.68±35.68	(17–130)
Calcium (Ca, mg/dl)	9.42±0.3	(8.6–9.8)	9.43±0.37	(8.8–9.9)
Alkaline phosphatase (ALP, U/l)	78.82±24.05	(41–127)	58.90±20.42*	(30–101)
Bone specific alkaline phosphatase (B.ALP, mg/l)	43.33±6.43	(36–48)	29.33±9.30*	(23–40)
Osteocalcin (OC, ng/ml)	11.91±2.2	(7.5–16.3)	9.92±1.02	(7.8–11.9)
Carboxyterminal peptide of collagen Type I (CTx-I, ng/ml)	1.26±0.53	(0.33–2.11)	0.61±0.07*	(0.07–1.28)
25-Hydroxyvitamin D (25-VitD, ng/ml)	28.49±3.25	(21.99–34.98)	29.01±3.14	(22.72–35.26)
Parathyroid hormone (PTH, pg/ml)	59.50±27.01	(19.1–113)	86.34±35.02*	(24.8–143)
Tumor marker CA15-3 (CA15-3, U/ml)	24.99±8.74	(14.1–41.7)	32.51±17.84	(15.6–88.5)

Values are expressed as Mean±SD and numbers in parenthesis are ranges (minimum–maximum). Statistical analysis was carried out using unpaired student's t-test of significance. *p<0.001 versus women before chemotherapy.

Table 2 : Biochemical parameters measured in serum for women (n=100) before and after receiving 6-cycles of a three-drug cyclophosphamide, adriamycin, and 5-fluorouracil (CAF) chemotherapy treatment protocol.

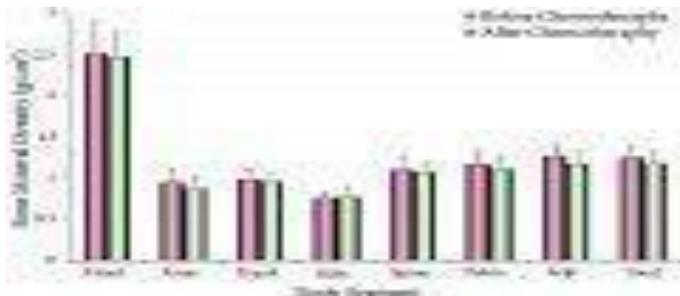


Figure 1 : Bar plot of segmental and total bone mineral density (BMD, g/cm²) measured using the dual-energy X-ray absorptiometry (DXA) technique for women (n=100) before and after receiving 6-cycles of a three-drug cyclophosphamide, adriamycin, and 5-fluorouracil (CAF) chemotherapy treatment protocol.

	Before chemotherapy			After chemotherapy		
	b	SEE	p	b	SEE	p
Lumbar spine BMD						
Age (year)	0.002	0.004	0.001	0.008	0.006	0.015
Body mass Index (BMI, kg/m ²)	0.027	0.003	0.006	0.016	0.003	0.033
Weight (kg)	0.018	0.032	0.592	-0.023	0.021	0.297
Height (m)	0.006	0.021	0.583	-0.003	0.016	0.451
Fat mass (FM, kg)	-0.046	0.186	0.808	0.074	0.329	0.823
Lean bone-free mass (LBFM, kg)	0.012	0.003	0.356	0.014	0.003	0.491
Tissue bone-free mass (TBFM, kg)	0.008	0.004	0.266	0.007	0.005	0.311
Pelvis BMD						
Age (year)	-0.002	0.005	0.001	0.009	0.005	0.013
Body mass index (BMI, kg/m ²)	0.033	0.026	0.005	0.016	0.014	0.047
Weight (kg)	0.013	0.003	0.486	0.007	0.002	0.812
Height (m)	0.002	0.019	0.611	0.010	0.005	0.412
Fat mass (FM, kg)	-0.005	0.227	0.984	0.089	0.288	0.761
Lean bone-free mass (LBFM, kg)	0.012	0.213	0.198	0.014	0.215	0.122
Tissue bone-free mass (TBFM, kg)	0.008	0.114	0.119	0.007	0.115	0.112
Total BMD						
Age (year)	-0.001	0.002	0.001	0.006	0.003	0.001
Body mass index (BMI, kg/m ²)	0.034	0.002	0.001	0.022	0.002	0.001
Weight (kg)	-0.443	0.101	0.233	0.283	0.190	0.151
Height (m)	0.006	0.002	0.451	-0.011	0.012	0.386
Fat mass (FM, kg)	-0.048	0.018	0.712	-0.004	0.002	0.978
Lean bone-free mass (LBFM, kg)	-0.035	0.011	0.306	0.004	0.009	0.639
Tissue bone-free mass (TBFM, kg)	-0.032	0.012	0.505	0.003	0.008	0.522

β: regression coefficient; SEE: standard error of estimation; p: significance level at <0.05 for individual parameter.

Table 3: Coefficients of initial multiple linear regression of predictors for lumbar spine, pelvis, and total bone mineral density (BMD) for women (n=100) before and after receiving 6-cycles of a three-drug cyclophosphamide, adriamycin, and 5-fluorouracil (CAF) chemotherapy treatment protocol.

	Prediction Equation ^a	R ²	SEE	WSSE			Entry
Before chemotherapy	$BMD_{Spine} = -0.002 \times Age + 0.027 \times BMI$	0.98	0.98	0.16	18.20	1	
After chemotherapy	$= -0.008 \times Age + 0.016 \times BMI$	0.98	0.98	0.11	51.30	2	
Before chemotherapy	$BMD_{Pelvis} = -0.002 \times Age + 0.033 \times BMI$	0.98	0.98	0.15	18.08	3	
After chemotherapy	$= -0.009 \times Age + 0.016 \times BMI$	0.97	0.97	0.17	29.73	4	
Before chemotherapy	$BMD_{Total} = -0.001 \times Age + 0.034 \times BMI$	0.99	0.99	0.11	19.43	5	
After chemotherapy	$= -0.006 \times Age + 0.022 \times BMI$	0.99	0.99	0.11	35.63	6	

^aAll prediction equations are significant (p<0.0001) using unpaired student's t-test versus women before chemotherapy. Age is in years and body mass index (BMI) is in kg/m². R²: coefficient of determination; SEE: standard error of estimation; WSSE: weighed sum of squared errors.

Table 4 : Final prediction equations based on two independent variables for lumbar spine, pelvis, and total bone mineral density (BMD) for women (n=100) before and after receiving 6-cycles of a three-drug cyclophosphamide, adriamycin, and 5-fluorouracil (CAF) chemotherapy treatment protocol.

Discussion

Osteoporosis is a global public health concern currently affecting more than 200 million people worldwide, about 80% of them are women [23]. Not only patients with cancer may be at risk for primary osteoporosis, but also for secondary osteoporosis due to cancer therapies; which may alter the gonadal function and negatively affect bone turnover [24-27]. BMD testing is considered highly effective for establishing a diagnosis of osteoporosis and monitoring its progression, since an inverse relationship exists between BMD and future fracture risk. BMD is expressed as a T- or Z-Score, which are the standard deviation of BMD from the expected BMD for a young adult or an age-matched normal population of the same sex, respectively.

Although, data of body-composition (i.e., FM, LBFM, and TBFM) were comparable before and after chemotherapy (Table 1), segmental and total BMC and BMD distribution were significantly (p<0.05) lower after chemotherapy in comparison with their levels before chemotherapy. Consequently, total T- and Z-Scores after chemotherapy were also significantly (p<0.001) lower than those before chemotherapy, confirming that bone loss was directly related to treatment with adjuvant chemotherapy. In line with this, many studies reported that adjuvant chemotherapy may cause a rapid bone loss, increasing the risk of osteoporosis for pre- and postmenopausal women with BC later in life [1- 7,24-27]. It has been shown that bone loss with aging occurs because hypogonadism may progress to primary osteoporosis. However, secondary osteoporosis due to cancer therapies-induced bone loss results from other factors (e.g., chronic diseases, nutritional deficiencies, drugs, etc.) that negatively alter bone remodeling. Both cases cause elevation of PTH levels, greater bone resorption than synthesis, impaired neuromuscular functioning, and increased risk for falls and fractures [25-27].

Biochemical analysis showed that, albeit comparable levels of serum Ca and 25-VitD, both ALP and S.ALP were significantly lower after chemotherapy as compared to their initial levels before chemotherapy. CTx-I levels were also significantly lower after chemotherapy, denoting a condition of decreased bone resorption in post-chemotherapy NMBC women (Table 2). Although both markers of bone formation and resorption were significantly lower after chemotherapy as compared to their levels before chemotherapy, consequently denoting a lower bone turnover activity, which was evidenced by the significantly lower segmental and total BMD for postchemotherapy NMBC women (Figure 1). In line with this, Greep et al., [25] previously reported that postmenopausal women with early BC who received adjuvant chemotherapy had lower BMD in comparison with their counterparts who did not receive any chemotherapy.

Moreover, Rodríguez-Rodríguez et al. [1], had also previously detected significant decreases in BMD at lumbar, trochanter, intertrochanter and total hip after adjuvant treatment for NMBC women. The significantly higher PTH levels after chemotherapy (i.e., 86.34 ± 35.02 vs. 59.50 ± 27.01 pg/ml for that before chemotherapy, $p < 0.001$) may justify the observed decrease of bone formation in post-chemotherapy NMBC patients as compared to their status before chemotherapy.

The use of markers of bone turnover for monitoring bone metastases in BC and in response to therapy had been shown earlier [28-30]. Chemotherapeutic aromatase inhibitors (e.g., exemestane and anastrozole) have been shown to directly inhibit osteoclast differentiation and bone resorption markers leading to osteoporosis in postmenopausal women with early BC [6], yet with apparently increased bone resorption biochemical markers, as also had been shown earlier [31,32]. It has been shown that osteoporotic bone loss and bone metastasis ultimately share a pathophysiologic pathway that stimulates bone resorption by increasing the formation and activity of osteoclasts [4]. Osteolytic lesions commonly seen in BC can cause severe pain, pathologic fracture, and compression syndromes of the nerve root or spinal cord, as well as metabolic disturbances (e.g., hypercalcemia, phosphate imbalances, disruptions in acid/base and neurological homeostasis, and nephrolithiasis) [33]. Combined osteolytic and osteoblastic lesions, which cause increased bone resorption through osteoclasts within osteoblastic lesions and compensatory, secondary bone formation through osteoblasts within osteolytic lesions, have been observed in BC patients [33,34]. Therefore, it is advised that women with BC who are undergoing hormonal therapy, chemotherapy, radiation, and bisphosphonate therapy should be closely monitored for BMD loss and skeletal health maintenance prescriptions [5]. It is noteworthy, current recommendations for avoiding the skeletal complications of BC therapy include adequate intake of Ca and vitamin D, regular weight-bearing exercise, cessation of smoking, reduction in alcohol intake, and bisphosphonate treatment for those found to be osteoporotic [5,34].

As a fast and reasonably accurate method for frequently monitoring bone health, we developed prediction mathematical formulae for spine and pelvis BMD, which are the sites most susceptible to fracture risks, together with the total BMD of NMBC women. Multiple linear regression analysis showed that the covariates age and BMI were significantly associated with BMDspine, BMDpelvis, and BMDtotal independently ($R = 0.99$, $p < 0.0001$ for all associations). Table 3 shows the continuous prediction equations developed for BMDspine, BMDpelvis and BMDtotal based on two independent variables for NMBC women before and after chemotherapy. These equations gave estimations, which were on average $< -0.70\%$ for BMD spine, $< 0.30\%$ for BMDpelvis, and $< 2.00\%$ for BMDtotal of all participants, which did not result in false negative or positive diagnosis of BMD status. Similar studies carried out on postmenopausal healthy and cirrhotic Italian women [20] as well as on Egyptian patients with β -Thalassemia Major [22] using anthropometric variables have proven useful for the continuous monitoring of their bone health with comparable levels of SEE. We believe these formulae will permit the physician to identify patients at risk of fracture, so that preventive strategies or treatment can be targeted towards those at greatest fracture risk.

One of the limitations of the present study is the small sample size, being focused only on NMBC women who don't have: serum creatinine greater than $150 \mu\text{mol/L}$; peptic ulcer; hysterectomy or bilateral oophorectomy; osteoporosis; untreated hypothyreosis; bisphosphonate, calcitonin or peroral steroid therapy; pregnancy or lactation; and any other malignancies. Thus, to validate the developed formulae, there is a need to study a bigger population of BC women, possibly extending and taking into consideration other factors like bone metastasis and the accompanying physiologic mechanisms responsible for lowering segmental and total BMD.

Conclusions

Adjuvant chemotherapy is responsible for decreasing segmental and total BMD in BC postmenopausal women, which can be clinically evaluated by the significant changes in both T- and Z-Scores as well as biochemical markers of bone turnover. The decrease in segmental and total BMD was mainly due to significant decrease in the levels of ALP and S.ALP rather than an increase in CTx-I markers. Thus, measurements of BMD and biochemical markers of bone formation and resorption for BC women before starting any adjuvant chemotherapy is important to assess initial status of bone health. We believe, the simple mathematical formulae developed on basis of the two individual variables Age and BMI can be useful for assisting the clinician to frequently monitor bone health status of BC patients in similar conditions, being able to manage possible bone losses rapidly and efficiently.

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