



STUDY OF SERUM BIOCHEMICAL BONE MARKERS IN PATIENTS OF CARCINOMA BREAST UNDERGOING CHEMOTHERAPY AT CIMS, BILASPUR

Dr. Chandrahas Dhruv

Assistant Professor, Department of Radiotherapy CIMS, Bilaspur

Dr. Vijay Kumar Manwani*

Associate Professor, Department of Community Medicine CIMS, Bilaspur.

*Corresponding Author

ABSTRACT

Background: Breast cancer is the most common cancer and the leading cause of cancer-related deaths among women worldwide. The annual global incidence of breast cancer is estimated to be >1.3 million cases and approximately 465,000 women die of this disease every year. Osteoporosis is a common chronic problem in postmenopausal women that increases the risk for spinal compression fractures and fractures of the femoral neck, causing life-threatening complications. Cancer-induced osteoporosis is a long-term complication associated with cancer therapy that can directly or indirectly affect bone metabolism.

Objective: To analyze and compare the Serum Biochemical Bone Markers in Patients of Carcinoma Breast before and after Chemotherapy.

Material & Method: This prospective clinical study involves 27 histopathologically proven cases of carcinoma breast patients which were conducted during October 2017 to August 2018 to estimate the Serum Biochemical Bone Markers in Patients of Carcinoma Breast before and after chemotherapy. The categorical clinical characteristics between the two treatments are compared using chi-square test.

Result: in this study the Majority of the patients had stage IIIB and IIB disease 12 out of 27(44.4%) patients presented with IIIB disease, 9 out of 27(33.3%) belongs to stage IIB, 4 out of 27 (14.8) were in IIIA, 3 patients out of 27(11.1%) had IIIC disease. Majority of the patients, i.e. 88.8% patients (24/27) had Serum Calcium in normal range. None of the patients had hypocalcaemia (Serum Calcium <8.5mg/dl), 3 patients out of 27(11.1%) had hypercalcaemia, i.e. >11.0 mg/dl. In patients completing CT and follow-up, 95.2% (20/21) had Serum Calcium in normal range with mean of $10.10 \pm .72$. Only one patient had hypercalcaemia (Serum Calcium >11.0mg/dl). The mean pre-treatment Serum Phosphorus 3.59mg/dl in all 27 patients and 3.44 ± 0.68 mg/dl in patients completing treatment. The maximum and minimum values were 4.93 mg/dl and 0.8 mg/dl respectively. Approximately 30% patients (8/27), had serum Alkaline Phosphatase level in the range of 200-300 U/L. The mean serum Alkaline Phosphatase level was 341.41 ± 167.35 in all 27 patients; whereas in patients completing treatment it was 184 ± 57.31 U/L.

Conclusion: In our study we found that most of the patients with newly diagnosed carcinoma breast had pre-existing osteopenia and osteoporosis. In patients completing chemo radiotherapy, significant difference was seen between pre-treatment and post-treatment serum Calcium levels among patients in postmenopausal age group. Significant difference was also seen between pre-treatment and post-treatment Serum Phosphorus levels among patients in both premenopausal and postmenopausal groups. No statistically significant change was seen in Serum Alkaline Phosphatase levels with regard to premenopausal and postmenopausal patient groups, before and after treatment.

KEYWORDS : Osteoporosis, Chemotherapy, Bone Resorption.

INTRODUCTION

Breast cancer is the most common cancer and the leading cause of cancer-related deaths among women worldwide.^[1] The annual global incidence of breast cancer is estimated to be >1.3 million cases and approximately 465,000 women die of this disease every year. Advances in technologies for early diagnosis and therapies for breast cancer have substantially improved survival and clinical outcomes in recent years, especially in US and other developed countries.^[2] Breast cancer significantly influences the women's health and is assuming greater importance in the developing countries due to the rising incidence, delay in presentation and dismal outcome.^[3] Osteoporosis is a systemic disease with low bone mass and micro architectural changes that compromises bone strength leading to skeletal fragility and fracture. With increase in life expectancy, osteoporosis is one of the major and serious public health problems and common cause of morbidity and mortality in postmenopausal women and men above 60 years.^[4] Osteoporosis is a common chronic problem in postmenopausal women that increases the risk for spinal compression fractures and fractures of the femoral neck, causing life-threatening complications.^[5] Women treated for cancer may be at risk for osteoporosis and fracture. Cancer-induced osteoporosis is a long-term complication associated with cancer therapy that can directly or indirectly affect bone metabolism. Osteoporosis in women treated for cancer occurs more rapidly and tends to be more severe compared with the normal age-related bone loss.

Cancer treatment induces bone loss, which causes bone fragility and an increased susceptibility to fractures. Osteoporosis is one of the problems that have been identified with increasing frequency in patients with cancer.^[6] Bone loss in women treated for cancer occurs more rapidly and tends to be more severe compared with the normal age-related bone loss.^[7] Bone turnover markers, in combination with other risk factors for osteoporotic fracture, may be used to define fracture risk and intervention thresholds. In women with low bone mass, bone turnover markers are independent predictors of fracture risk; vertebral fracture is directly correlated with bone turnover marker

concentration and negatively with vertebral bone mineral density.^[8] Thus, there is a scope for evaluating the biochemical serum bone markers in carcinoma breast patients undergoing chemo radiotherapy by measuring serum Calcium, serum Phosphorus & serum Alkaline Phosphatase.

OBJECTIVE

To analyze and compare the Serum Biochemical Bone Markers in Patients of Carcinoma Breast before and after Chemotherapy.

Material and Methods

The present prospective clinical study involving 27 patients of carcinoma breast was conducted during October 2017 to August 2018 in the Department of Radiotherapy, in collaboration with the Department of Community Medicine, Chhattisgarh Institute of Medical Sciences, CIMS Bilaspur, Chhattisgarh. The Departmental Research Committee has approved the study and the informed written consent of the subjects has been recorded individually.

Patient Inclusion criteria:

The patients taken up for the study were required to meet the following criteria.

- Cytologically and histopathologically proven cases of carcinoma breast.
 - Premenopausal/postmenopausal women.
 - Age more than 25 years and less than 50 years.
 - ECOG performance score of 0 or 1.
 - Patient with normal liver function test, renal function test and haematological parameters.
- Patient with normal electrocardiogram

CLINICAL EVALUATION

A detailed history was taken and clinical evaluation was done in all the patients. A thorough general physical examination was carried out with measurement of parameters such as height, weight, body surface area, body mass index, temperature, pulse, blood pressure and respiratory

rate. Examination for detection of abnormality in central nervous system, cardio-vascular system, respiratory system, gastro-intestinal system and genitourinary system was done for all the patients.

INVESTIGATIONS

The following investigations were carried out in all the patients.

Blood investigations: Haemoglobin, Total and Differential Leucocyte Count, Platelet Count, Random Blood Sugar, Renal Function Test and Liver Function Test.

Radiological investigation: Chest X-ray PA view and Ultrasound abdomen and pelvis.

Electrocardiogram,

Tissue biopsy from breast lump.

FNAC from palpable axillary lymph node.

Specific investigations were done to measure biochemical markers in all the patients before start of treatment:

(1) Serum Calcium

(2) Serum Phosphate

(3) Serum Alkaline Phosphatase

The above specific investigations were repeated in all the patients, during 3rd monthly follow up after completion of therapy,

Specimen Collection:

Blood samples were collected by venipuncture taking care to avoid haemolysis. For monitoring the individual patient, follow up samples were collected under same conditions as that of baseline samples. Heparinised samples were used for analyzing plasma. Paired serum sample of Serum Calcium, Serum Phosphorus and Serum Alkaline Phosphatase were collected both before starting therapy and during 3 months follow up.

STATISTICAL ANALYSIS:

In this study, frequency tables with counts and percentages are used to describe pre-treatment and treatment characteristics for each group. The categorical clinical characteristics between the two treatments are compared using chi-square test. For continuous variables, mean and median values were compared between the groups using the t-test. The comparison between treatment arms were done using log-rank test. A p-value of <0.05 was taken as significant. Data are analysed using the statistical software SPSS for windows (version 19.0).

FOLLOW UP

After completion of treatment, patients were followed up on a monthly basis in the department of Radiotherapy. A minimum of 3 monthly follow up per patient was recorded. They were assessed for loco-regional recurrence and /or distant metastasis by clinical examination and/or by necessary investigations.

Results

The present prospective study was carried out on 27 histopathologically proven cases of Carcinoma Breast during the period October 2017 to August 2018. All patients were evaluated with a detailed history, clinical examination, haematological and radiological investigations. In addition, Serum Calcium, Serum Phosphorus, Serum Alkaline Phosphatase measurements were done in all patients. 3 patients defaulted either before or during treatment and 3 patients expired during treatment. Hence, only 21 patients completed chemotherapy, and all investigations were repeated during the follow up period.

The observations were recorded as per the proforma.

Age

21 out of 27 patients (77.7%) among all patients completed chemotherapy and follow-up on time were in range of 34-50 yrs. And 3 out of 27 patients (11%) who expired after first measurement were in the range of 40-48 yrs. And 3 patients out of 27 (11%) who defaulted in follow-up were in range of 34-40 yrs. mean age were 44 ± 4.9 yrs in all patients completing treatment. From among the 27 patients, the youngest patient was 34 years old and oldest was 50 years of age. (Table-1)

Table 1 Age wise Distribution of Patients

Age Groups (years)	All Patients Nos. (%) (n=27)	Patients Completing treatment Nos. (%) (n=21)
31-40	8 (29.6)	6 (28.5)

41-50	19 (70.3)	15 (71.4)
Mean \pm S.D.	43.8 \pm 4.9	44 \pm 4.9
Minimum	34yrs	35yrs
Maximum	50yrs	50yrs

Menopausal Status

21 patients (77.7%) among all 27 patients were postmenopausal and rest 6 patients (22.2%) were premenopausal. In patients completing treatment and follow-up, 18 out of 21 patients (85.7%) were postmenopausal and the rest 3 patients (14.2%) were premenopausal. (Table-2)

Table 2 Menopausal Status of the patients

Menopausal Status	All Patients Nos. (%) (n=27)	Patients completing treatment Nos. (%) (n=21)
Premenopausal	6 (22.2)	3 (14.2)
Postmenopausal	21 (77.7)	18 (85.7)

TNM

Majority of the patients had stage IIIB and IIB disease. 12 patients out of 27(44.4%) presented with IIIB disease, 9 out of 27(33.3%) belongs to stage IIB, 4 out of 27 (14.8) were in IIIA, 3 patients out of 27(11.1%) had IIIC disease. (Table-3)

Table 3 Distribution of Patients according to TNM Stage

TNM Stage	All Patients Nos. (%) (n=27)	Patients completing treatment Nos. (%) (n=21)
IIIB	12 (44.4)	13 (61.9)
IIB	9 (33.3)	5 (23.8)
IIIA	4 (14.8)	3 (14.2)
IIIC	3(11.1)	2 (9.5)

Body Mass Index (BMI)

Majority of the patients, i.e. 19/27 (70.3%) had a BMI in the normal range of 18.5 to 24.9kg/m². BMI <18.5 kg/m² was seen in 1 out of 27 patients (3.7%). None of the patients were obese (i.e. BMI >30 kg/m²). In patients completing treatment, 14 patients out of 21 (66.6%) had BMI in normal range (18.5-24.9 kg/m²) and in 1 patient (4.7%) it was less than 18.5 kg/m² (i.e. underweight). (Table-4)

Table 4 Distribution of patients according to Body Mass Index (BMI)

Body Mass Index (kg/m ²)	All Patients Nos. (%) (n=27)	Patients completing treatment Nos. (%) (n=21)
<18.5	1 (3.7)	1 (4.7)
18.5-24.9	18 (66.6)	14 (66.6)
25.0-29.9	8 (29.6)	6 (28.5)
>30.0	0 (0)	0 (0)
Mean \pm SD	23.48 \pm 3.12	23.91 \pm 3.2
Minimum	17.8kg/m ²	17.8kg/m ²
Maximum	29.5kg/m ²	29.5kg/m ²

Pre-treatment Serum Calcium

The normal range of Serum Calcium is 8.5 to 11.0 mg/dl. Majority of the patients, i.e. 88.8% patients (24/27) had Serum Calcium in normal range. None of the patients had hypocalcaemia (Serum Calcium <8.5 mg/dl), 3 patients out of 27(11.1%) had hypercalcaemia, i.e. >11.0 mg/dl. In patients completing CT and follow-up, 95.2% (20/21) had Serum Calcium in normal range with mean of $10.10 \pm .72$. Only one patient had hypercalcaemia (Serum Calcium >11.0 mg/dl). (Table-5)

Table 5 Pre-treatment Serum Calcium level

Serum Calcium (mg/dl)	All Patients Nos. (%) (n=27)	Patients completing treatment Nos. (%) (n=21)
<8.5	0 (0)	0 (0)
8.5-11.0	24 (88.8)	20 (95.2)
>11.0	3 (11.1)	1 (4.7)
Mean \pm SD	10.10 \pm .72	9.47 \pm .71
Minimum	8.7mg/dl	8.7mg/dl
Maximum	11.4mg/dl	11.4mg/dl

Pre-treatment Serum Phosphorus

22 of 27 patients (81.4%), had serum Phosphorus in the range of 3 to 4.5 mg/dl, followed by 18.5% patients (5/27) with serum Phosphorus ranging from 1.5 to 3 mg/dl. Similarly, in patients completing CT and follow-up, 95.2% (20/21) had Serum Phosphorus in the range of 3-4.5 mg/dl range. The mean pre-treatment Serum Phosphorus 3.59mg/dl

in all 27 patients and 3.44±0.68mg/dl in patients completing treatment. The maximum and minimum values were 4.93 mg/dl and 0.8 mg/dl respectively. (Table-6)

Table 6 Pre-treatment Serum Phosphorus level

Serum Phosphorus (mg/dl)	All Patients Nos. (%) (n=27)	Patients completing treatment Nos. (%) (n=21)
1.50-3.00	5(18.5)	2(9.5)
3.01-4.50	22(81.4)	19(90.4)
4.51-6.00	0	0
Mean ± SD	3.59 ± .82	3.29±.60
Minimum	0.8mg/dl	0.8mg/dl
Maximum	4.93mg/dl	4.93mg/dl

Pre-treatment Serum Alkaline Phosphatase

Approximately 30% patients (8/27), had serum Alkaline Phosphatase level in the range of 200-300 U/L. The mean serum Alkaline Phosphatase level was 341.41 ± 167.35 in all 27 patients, whereas in patients completing treatment it was 184±57.31 U/L. (Table-7)

Table 7 Pre-treatment Serum Alkaline Phosphatase level

Serum Alkaline Phosphatase (U/L)	All Patients Nos. (%) (n=27)	Patients completing treatment Nos. (%) (n=21)
90-200.0	5(18.5)	5(23.8)
200.1-300.0	8(29.6)	6(28.5)
300.1-400.0	4(14.8)	2(9.5)
400.1-500.0	5(18.5)	4(19)
>500	5(18.5)	5(23.8)
Mean ± SD	341.41 ± 167.3	184±57.31
Minimum	95U/L	95U/L
Maximum	723U/L	602U/L

Change in Serum Calcium level and Menopausal status

In 18 post-menopausal patients, the mean pre and post-treatment Serum Calcium level were 10.19 mg/dl and 9.5 mg/dl, respectively. The mean pre-treatment Serum Calcium levels showed no significant difference between pre-menopausal and post-menopausal patients (p=0.816). Similarly, the mean post-treatment serum Calcium level also had no statistically significant difference between premenopausal and postmenopausal patients (p=0.099). When comparing the mean pretreatment with the post-treatment values, the difference was statistically highly significant in postmenopausal patients (p=0.019), whereas it was insignificant in premenopausal patients (p=0.095). (Table-8)

Table 8 Change in Serum Calcium level after treatment according to Menopausal Status

Menopausal status (Nos.)	Pre-treatment (mg/dl)	Post-treatment (mg/dl)	P value (Between paired group)
Premenopausal (3)	9.5±0.1	9.2±0.2	0.816
Postmenopausal (18)	10.19±0.66	9.52±0.76	0.099
Total (21)	10.09±0.66	9.47±0.71	0.003
P value (Between above groups)	.019	.095	

Change in Serum Phosphorus level and Menopausal status

In 18 post-menopausal patients, the mean pre and post-treatment serum phosphorus level were 3.58 mg/dl and 3.32 mg/dl, respectively. The mean post-treatment serum Phosphorus level had significant difference between pre-menopausal and post-menopausal patients (p=0.014). Whereas the difference in mean pre-treatment serum Phosphorus level was not statistically significant between premenopausal and postmenopausal patients (p=0.424). Highly significant difference was found between the mean pre-treatment and post-treatment serum Phosphorus level among both pre and postmenopausal groups (p<0.05). (Table-9)

Table 9 Change in Serum Phosphorus level after treatment according to Menopausal Status

Menopausal status (Nos.)	Pre-treatment (mg/dl)	Post-treatment (mg/dl)	P value (Between paired group)
Premenopausal (3)	4.00 ±0.20	3.15 ±0.08	0.033
Postmenopausal (18)	3.58 ±0.90	3.32 ±0.65	0.038

Total (21)	3.64 ± 0.84	3.29 ± 0.60	0.005
P value (Between above groups)	0.424	0.014	0.033

Table 10 Change in Serum Alkaline Phosphatase level after treatment according to Menopausal Status

Menopausal status (Nos.)	Pre-treatment (U/L)	Post-treatment (U/L)	P value (Between paired group)
Premenopausal (3)	254.33±126.5	240.0±107.6	0.775
Postmenopausal (18)	310.44±135.2	175.33±43.21	0.424
Mean ± SD	302.43±132.48	184.57±57.31	0.390
P value (Between above groups)	0.998	0.927	

Discussion

Breast cancer is the most common cancer and the leading cause of cancer-related deaths among women worldwide.^[1] The annual global incidence of breast cancer is estimated to be >1.3 million cases and approximately 465,000 women die of this disease every year. Advances in technologies for early diagnosis and therapies for breast cancer have substantially improved survival and clinical outcomes in recent years, especially in US and other developed countries.^[2] Breast cancer significantly influences the women's health and is assuming greater importance in the developing countries due to the rising incidence, delay in presentation and dismal outcome.^[3]

In the present study 21 out of 27 patients (77.7%) who completed treatment were in range of age 34-50 yrs. 3 out of 27 (11%) who expired after first measurement were in the age range of 40-48 yrs. And 3 patients out of 27 (11%) who defaulted in follow-up were in the range of 34-40 yrs mean age was 44±4.9 yrs in patients completing treatment. From among the 27 patients, the youngest patient was 34 years old and oldest was 50 years of age. 21 patients (77.7%) among all 27 patients were postmenopausal and rest 6 patients (22.2%) were premenopausal. In patients completing treatment, 19 out of 21 patients (90.4%) were postmenopausal and the rest 2 patients (9.5%) were premenopausal. Majority of the patients had stage IIIB and IIB. 12 patients out of 27(44.4%) presented with IIIB disease, 9 out of 27(33.3%) had stage IIB, 4 out of 27 (14.8) were in IIIA, 3 patients out of 27(11.1%) were at the time of presentation had IIIC. Majority of the patients, i.e. 19/27 (70.3%) had a BMI in the normal range of 18.5 to 24.9kg/m². BMI <18.5 kg/m² was seen in 1 out of 27 patients (3.7%). None of the patients were obese (i.e. BMI>30 kg/m²). In patients completing treatment, 14 patients out of 21 (66.6%) had BMI in normal range (18.5-24.9 kg/m²) and in 1 patient (4.7%) it was less than 18.5 kg/m² (i.e. underweight).

Cancer-induced osteoporosis is common in women and men with breast cancer or men with prostate cancer who receive chemotherapy, hormone therapy, or surgical castration; these treatments are associated with hypogonadism and induce bone loss. In a study by Shapiro PL et al. (2001)^[9] of 49 premenopausal women with stage I or II breast cancer receiving adjuvant chemotherapy, 35 experienced ovarian failures, defined as more than 3 months of amenorrhea. In another study (Kanis et al. 1999),^[1] the annual incidence of vertebral fracture was compared in women on follow-up after primary breast cancer treatment to general population and was found to be nearly five times greater (P<0.0001). In women with recurrent disease it was more than 20 times greater (P<0.0001).

In the present study, patients completing treatment, the paired serum Calcium levels were measured both before and after treatment with the mean pre and post-treatment serum Calcium levels as 10.0 mg/dl and 9.4 mg/dl, respectively. The post-menopausal patients had mean pre and post-treatment serum calcium level as 10.0 mg/dl and 9.4 mg/dl, respectively. The mean post-treatment serum Calcium level also had no statistically significant difference between premenopausal and postmenopausal patients (p=0.099). Whereas, highly significant difference was found between the mean pre-treatment and post-treatment serum Calcium level in postmenopausal group (p=0.016), it was statistically insignificant in pre-menopausal patients (p=0.095).

In this study, the mean values of pre-treatment and post-treatment serum Phosphorus level were 3.64mg/dl and 3.29 mg/dl respectively. The difference between pre and post-treatment serum Phosphorus was statistically significant (p=0.005). Significant difference was found

between the mean pre-treatment and post-treatment serum Phosphorus level among both pre and postmenopausal groups ($p < 0.05$). In this study, mean values of pre-treatment and post-treatment serum Alkaline Phosphatase level were 302.43 U/L and 184.57 U/L, respectively. The difference between mean pre and post-treatment serum Alkaline Phosphatase level was statistically insignificant ($p = 0.390$). No significant difference was seen in pre-treatment and post-treatment serum Alkaline Phosphatase samples, according to age groups, menopausal status, ($p > 0.05$).

Conclusion

In our study we found that majority of the premenopausal patients were osteopenic, whereas majority of the postmenopausal patients were osteoporotic. Rapidly progressing bone resorption results in worsening of osteoporosis which causes bone fragility and an increased susceptibility to fractures at Lumbar spine and Hip region. In patients completing chemo radiotherapy, significant difference was seen between pre-treatment and post-treatment serum Calcium levels among patients in postmenopausal age group. Significant difference was also seen between pre-treatment and post-treatment Serum Phosphorus levels among patients in both premenopausal and postmenopausal groups. No statistically significant change was seen in Serum Alkaline Phosphatase levels with regard to premenopausal and postmenopausal patient groups, before and after treatment. Clinicians should be aware of the risk of bone loss, so that such predisposed patients can be screened and treated appropriately to prevent the long-term morbidity and mortality of osteoporosis in these patients.

REFERENCES

1. Kanis JA McClosky, EV, Powels T et al. A high incidence of vertebral fracture in women with breast cancer. *Br J Cancer* 1999; 79: 1179-1181.
2. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of randomized trials. *Lancet* 2005; 365: 1687-717.
3. Kumar A, Singh S, Pradhan S, et al. Doppler ultrasound scoring to predict chemotherapeutic response in advanced breast cancer. *World J Surg Oncol* 2007; 5:99-104.
4. Cummings SR, Phillips SL, Wheat ME, et al. Recovery of function after hip fracture. *J Am Geriatr Soc*. 1988; 36:801-806.
5. H.G. Shenoy a, M.B. Peter a, Y.A. Masannat a, B.J.G. Dall b,D. Dodwell c, K. Horgan. Practical advice on clinical decision making during neoadjuvant chemotherapy for primary breast cancer. *Surgical Oncology* 2009; 18:65-71.
6. Adler RA. Cancer treatment-induced bone loss. *Curr Opin Endocrinol Diabetes Obes* 2007; 14:442-445.
7. Michaud LB, Goodin S. Cancer-treatment-induced bone loss, part 2. *Am J Health Syst Pharm* 2006; 63:534-546.
8. Riggs BL, Wahner HW, Seeman E, et al. Changes in bone mineral density of the proximal femur and spine with aging: Differences between the postmenopausal and senile osteoporosis syndromes. *J Clin Invest*. 1982; 70:716-723.
9. Sowers M, Corton G, Shapiro B. Changes in bone density with lactation. *JAMA* 1993; 269: 3120-2.