A Cross-sectional Study: Bone Markers in Different Body Mass Index Groups of Newly Diagnosed Breast Cancer Females in Karachi, Pakistan

Shamim A. Qureshi1, Shamsa K. Udani1, Mishghan Zehra1, Tooba Batool1, Tooba Lateef1, Farooq Ghani2, Muhammad Bilal Azmi3

ABSTRACT

Background: Pakistan is a big victim of breast cancer and vitamin D deficiency. Interestingly, bones are the common site of breast cancer metastasis and vitamin D deficiency makes this condition more worst. The present study designed to estimate bone markers and minerals in different BMI groups of newly diagnosed breast cancer patients. Materials & Methods: diagnosed breast cancer females were approached and their characteristics including age, marital status, menstrual & family history, receptor status, tumor grade & type and presence of metastasis were noted from their medical reports. Whereas bone markers and minerals viz., alkaline phosphatase (ALP), bone specific ALP (BALP), vitamin D, carboxyl terminal collagen crosslinks (CTX), human epidermal growth factor 2 receptor (Her2) protein, albumin, calcium (Ca), phosphorus (P) and magnesium (Mg) were estimated plus body mass index (BMI) measured. Results: Most of the recruited females found aged less than 50 yrs, wedded, belonged to plump to obese BMI groups, had invasive ductal carcinoma, expressed triple positive receptor status and tumor grade II. Very few had metastasis and family history of breast cancer. Patients in all BMI groups showed insufficient level of vitamin D but normal levels of ALP, Ca, Mg, P, albumin, Her2 protein and CTX. Whereas Ca and BALP found slightly low in underfed BMI group patients. Conclusion: The results concluded and recommended that vitamin D levels must be monitored in breast cancer patients before and after treatment otherwise it will decrease more and may affect other bone markers.

Key words: Breast cancer, vitamin D deficiency, bone markers.

INTRODUCTION

Breast cancer is unfortunately affecting female gender in the world including females in Pakistan are not safe and showed high mortality rate because of this health problem. Many factors contributing the high prevalence of breast cancer like family history, age, age giving child birth, daily habits (alcohol intake, nutritional status), lifestyle, native country, BRAC 1 & BRAC 2 genes mutation, breast feed ignorance, age of starting periods and menopause, taking hormonal replacement therapy (HRT), and exposure of chest to radiation. Recent studies claimed that body mass index (BMI) and vitamin D deficiency are also accelerating the risk of this life-threatening problem in population which ultimately leads to bone deterioration. The breast cancer cells have high affinity to metastasis into bones that alter bone homeostasis. In addition, different treatments of breast cancer like aromatase inhibitors also induce loss of bone density and accelerate the risk of developing fractures. Vitamin D deficiency makes this condition more pathetic. Studies have shown that daily intake of vitamin D not only save bones but also to notably extend diminishing the risk of breast cancer in females in pre- and post-menopause plus breast cancer females with 50 ng/ml vitamin D levels are more protected from developing breast cancer than women having 30 ng/ml of same vitamin. For that reason, this study was planned to investigate the levels...
of vitamin D and other bone markers in breast cancer patients of different body mass index (BMI).

**METHODS**

**Study Design, Subject and Protocol**

It was a cross-sectional study based on biochemical assessment of bone markers and minerals in newly diagnosed breast cancer females and permitted by Ethical Review Committee of Aga Khan University and Hospital (AKUH). The female patients were recruited from the breast cancer clinics in the same hospital. The study population was advised to sign and fill consent form, followed by providing blood samples.

All females histologically newly diagnosed with breast cancer patients prior to any treatment, aged ≥ 18 yrs and resident of Pakistan were included in the study whereas diagnosed breast cancer females less than the decided age (< 18 yr) or pregnant or breast cancer treated or suffering from any chronic disease which affects the metabolism of vitamin D were excluded. By keeping these criteria in mind, three hundred and one (301) patients were approached, of which 201 agreed to participate in the study by filling consent form, providing required medical information and giving their blood samples.

Data regarding the demographic detail viz., height, weight, marital status, and family history were collected from patients whereas clinical record including tumor grade (blooms richard grading system), histological type, presence of metastasis and receptor status including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor (HER-2) receptor 2 expression were obtained from pathological reports of patients. For estimating biochemical parameters, appropriate volume (ml) of blood was collected from each patient in a gel tube for separating serum and kept at -80°C until analyzed.

**Bone Markers and Minerals Plus Physical Parameter**

Phosphorous (P), magnesium (Mg), calcium (Ca), albumin, and alkaline phosphatase (ALP) were measured by Advia 2400, Siemens.[13-16] 25-hydroxy vitamin D (25OHD) was assayed by electro chemiluminescence on Liaison, Siemens.[17] Her2 protein levels were assayed by electro chemiluminescence on Centaur, Siemens.[18] Bone specific alkaline phosphatase (BALP) and carboxy terminal collagen crosslinks (CTX) were determined by electro chemiluminescence on E-170 and colorimetric method respectively.[19,20] Body Mass Index (BMI) of each patient of study population was calculated by using the following formula.

\[
\text{BMI (kg/m}^2) = \frac{\text{Weight (kg)}}{\text{Height (m}^2)}
\]

Patients were grouped on the basis of age and BMI to study the bone markers especially 25-hydroxy vitamin D (25OHD).

**Statistical Analysis**

Statistical package for social sciences (SPSS version 22.0) was used to determine percentage and frequencies of each variable of demographical detail through descriptive statistics and mean ± standard deviation (S.D) were calculated for quantitative variables by using independent student’s t-test.

**RESULTS**

**Demographic Characteristics of Study Population**

The characteristics of enrolled patients including age, BMI, marital status, menstrual & family history, receptor status, tumor grade & type and presence of metastasis, all are summarized in Table 1-3.

**Bone Markers and Minerals**

There was no significant difference in age of patients found in different BMI groups from underfed to obese. Similarly, no prominent variation was observed in serum levels of bone markers and minerals including ALP, BALP, vitamin D, CTX, Her2 protein, albumin, Ca, P and Mg. However, Ca slightly rose in underfed group (Table 4-5). On the basis of vitamin D level, overall study population categorized into deficient, insufficient and sufficient (Figure 1).

**DISCUSSION**

In Asia, Pakistan has high prevalence of breast cancer in females and it is reported that every 1 in ninth women is prone to this health hazard.[21] Different studies have shown that the incidence rate of breast cancer in Pakistan is getting higher.
horrible and it is also affecting the females of younger age group most probably due to the lack of awareness, lack of confidence and showing hesitation in going for breast examination.[22] The similar situation was found in present study that 13.9 % of total study population belong to 21-36 yrs of age class interval, 29 % within 37-51 yrs, whereas 44 and 11.94 % were found in age above 52 and 67 yrs of females (Table 1). However, the overall recruited diagnosed breast cancer females (201) showed mean age of 51.69 ± 12.47 yr.

Table 3: Histological Types of Breast Cancer Found in Study Population

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<th>S. No.</th>
<th>Histological Type</th>
<th>Percentage (%)</th>
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<td>1</td>
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<td>4.98</td>
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<tr>
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Body fats are also increasing the risk of breast cancer in women.[23] In this regard, age, gender, genetic factor, imbalance diet, irregular menstrual cycle, post married life, parity and sedentary life style are all contributing the most.[21, 24] Studies state that ingestion of foods rich in saturated fats raise the chances of developing breast cancer by inducing obesity which in turns alter the BMI.[25,26] Beside this, it has been estimated that fat enriched diets containing 35-40% of fat can induce the formation of breast tumor since fatty foods increase the levels of cholesterol that also accelerate the biosynthesis of estrogen and other steroid hormone required for the proliferation of cancer cells.[27] In the present study, most of the enrolled breast cancer patients were found plump (44.27 %; BMI 23.1-30 kg/m²) and obese (24.37 %; BMI >30 kg/m²) followed by 8.4 and 22.38 % belonged to underfed and standard groups of BMI and this was also confirmed by observing BMI 25.87 ± 5.29 (kg/m²) of overall study population with mean weight of 65.89 ± 13.08 (kg) and height 5.12 ± 0.4 (ft). However, study claimed high death rate in breast cancer patients belonged to underfed BMI group most probably due to the lack of awareness, lack of confidence and showing hesitation in going for breast examination.[22] The similar situation was found in present study that 13.9 % of total study population belong to 21-36 yrs of age class interval, 29 % within 37-51 yrs, whereas 44 and 11.94 % were found in age above 52 and 67 yrs of females (Table 1). However, the overall recruited diagnosed breast cancer females (201) showed mean age of 51.69 ± 12.47 yr.

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Another marker is tumor staging that indicate the metastasis of tumor.[32] In which cancerous cells initially arise in breast and spread (metastasis) to its nearby area including lymph nodes, sternum, chest wall, lungs, liver, brain or bones.[33] Present study showed only 7% metastasis cases. One more prognostic marker is hormone (estrogen/progesterone) and epidermal human growth factor 2 (Her2) receptors status, which helps in making the decision for treatment.[29,34,35] Out of 201 patients, mostly (51.7 %) found triple positive (ER/PR+ Her2+), followed by triple negative ER/PR- Her2- (25.8 %), 14.4 % ER/PR- Her2+ and 7.9 % ER/PR+ Her2-. Studies claimed that triple positive is more aggressive and showed higher grade, triple negative is unresponsive to targeted therapy because of the absence of cell receptors and these patients commonly have BRCA 1 gene mutation, ER/PR-Her2+ have rapid growth and spread quickly and ER/PR+ Her2- have slow growth and responsive to hormonal therapy.[35]

Her2 protein encoded by Her2 gene and its increase concentration reflects the over expression of Her2 receptors on breast cancer cells which involved in their abnormal proliferation.[34,36] In present study, the levels of same protein were observed normal with the mean level ranging from 13 – 15.65 ng/ml respectively in all four BMI groups of breast cancer females. Bone is the frequent site for breast cancer metastasis.[37] Beta C-terminal Telopeptide (β-CTX) act as a re-sorption marker for the breakdown of bone type I collagen by osteoclasts. The significant type I collagen fragments are the C-terminal Telopeptides (CTX) in which alpha aspartate isomerizes into beta-aspartate. This type I collagen is synthesized in the bone and about 90% of the organic bone matrix comprises of this type I.[38,39] Bone is continuously going through the process of bone remodeling, unfortunately bone re-sorption becomes accelerated than formation when breast cancer metastasized into bones results an increase circulation of type I collagen fragments in serum.[34,36] In present study, normal CTX levels were estimated with mean of 321 – 402 pg/ml in all BMI groups of breast cancer women. It may be due to fewer number of breast cancer patients with metastasis found in this study.

On the basis of vitamin D, 16 % of total study population was categorized as vitamin D deficient, 48% showed insufficient levels of same vitamin whereas 36% displayed sufficient amount of vitamin D (Figure 1). However, when same vitamin D was estimated in different groups of BMI then unfortunately breast cancer women present in all four BMI groups showed insufficient levels of vitamin D (Table 4). No doubt, high incidence of vitamin D deficiency has been reported in Pakistani population. Plus diagnosed breast cancer females having low levels of vitamin D in comparison to normal ones in Pakistan.[10,40,41] Another study conducted in Pakistan also claimed protective role of same vitamin D in decreasing the risk of breast cancer.[42]

ALP involved in hydrolytic cleavage of phosphate from many substrates nucleotides, proteins, carbohydrates and alkaloids. One of its isoenzymes is bone-specific alkaline phosphatase (BALP) chiefly resides in osteoblasts and is thought to be involved in the calcification of bone matrix or bone formation.[43] It is also considered as a surrogate indicator of bone metastasis.[44] When breast cancer metastasized to bone the levels of BALP rises and becomes an important prognostic tool of cancer progression and bone metastasis.[45,46] Studies report that deficiency of vitamin D also affects the levels of BALP as it found increased in vitamin D deficient patients of breast cancer.[47]
In present study, normal levels of ALP were found from 75.91 U/l in all the groups of BMI. On contrary, BSALP was found slightly higher (18.61 U/l) in underfed breast cancer females, which clearly reflects the low health status of these females.

Calcium is an important mineral required by the cells to perform a number of vital functions like involved in transmission of nerve impulse, metabolism, contraction of muscles and also play a key role as a secondary messenger and participate in cell signaling system, cell proliferation and apoptosis.[49] The level of calcium is regulated by vitamin D which enhances its absorption in intestinal mucosa so they both are interrelated metabolically. Therefore an inverse relationship exists in between vitamin D and calcium levels in blood.[50] The high levels of calcium in the blood can leads to calcium, phosphorus, magnesium, Her2 protein, C-terminal telopeptide and albumin.

Phosphate is another important mineral which is required in body to synthesize phospholipids, nucleic acids and high energy compounds such as adenosine triphosphate (ATP), creatine phosphate as these are required by the actively dividing cells and muscles. Again, the metabolism of phosphorus is controlled by vitamin D and increased levels of phosphorus observed in vitamin D deficient individuals. Interestingly, its increased levels in turn enhance the progression of cancer.[51,52] Similarly, magnesium is the second most bountiful positively charged mineral found within the cells. It modulates a number of essential functions including the activation, metabolism and absorption of vitamin D.[53] Beside these, Mg inhibits the progression of breast cancer by inducing genomic stability, controlling cell differentiation, proliferation or apoptosis and preventing angiogenesis therefore deficiency of magnesium not only induce vitamin D deficiency but also leads to cancer.[53-55] In the present study, there was no noticeable variation found in values of Mg and P in different groups of BMI of breast cancer patients. Whereas mean Ca level was observed significantly (p<0.05) decreased in patients belonged to underfed group of BMI.

Serum albumin levels are considered as an indicator of nutritional status, disease progression, severity and its prognosis in breast cancer patients as nutrient deficiency is very common in these patients.[56,57] However, in this study, albumin levels were found almost normal in female patients belong to all BMI groups.

![Figure 1: Categories of Breast Cancer Patients on the Basis of Vitamin D Level](image-url)

**CONCLUSION**

Most of the newly diagnosed breast cancers females of present study were belonged to plump and obese BMI group with high percentage of triple positive receptor status. In all four BMI groups, all patients have insufficient level of serum vitamin D and normal levels of other markers including calcium, phosphorus, magnesium, Her2 protein, C-terminal telopeptide and albumin.

**REFERENCES**


