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Evaluation of The Serum Level of Osteocalcin in Breast Cancer Patients, and Its Association with Estrogen and Progesterone Receptors

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ABSTRACT

Background: Breast cancer is the second leading reason of cancer-related deaths in women. Osteocalcin (OC) is increased when bone metabolism is raised. Cancer cells with estrogen and progesterone receptors be determined by estrogen and related hormones, such as progesterone, to grow.

Aim: To find the level of serum osteocalcin, and its association with estrogen receptors and progesterone receptors.

Methods: Study design: A cross-sectional study was designed; taking 45 patients with breast cancer and 22 controls women whose ages were between 25-70 years old, from beginning of January 2021 to ending of June 2021. These patients came to Kirkuk Teaching Hospital. Age, weight, length, and body mass index (BMI) were recorded. Biochemical analysis: The serum osteocalcin, progesterone receptors (PRs), and estrogen receptors (ERs) levels were measured by an automated immunoassay system.

Results: Mean of BMI of control group was (25.63 ± 1.93) kg/m^2 and of the patients' group was (26.97±4.08) kg/m^2. There was a significant difference in osteocalcin between the control and patients' group. There was a significant difference in ER between the control and patients' group. There was a significant difference in PR between the control and patients' group.

Conclusion: Biochemical marker of bone metabolism may identify patients with bone disease who are at high risk for skeletal-related events.

Keywords: Breast Cancer; Osteocalcin; ER; PR; BMI.

INTRODUCTION

Breast cancer is one of the top causes of death due to cancer, and it is about one in every eight females in the USA country. Breast cancer cells are commonly metastasized to different parts of body such as bone, where survival rate of 5-year is about less than 10% **(1)**.

The bone is frequently remodeling in adults. Beneath perfect conditions, the osteoclast bone-resorbing cell excavates cavities and osteoblast cell bone-depositing yield matrix to procedure a new bone, so the bone will not lose or gain. Exclusions to these situations include the following: (1) bone destruction as a consequence of mature and osteoporosis , (2) the bone damage as physical activity or exercise and (3) Normal bone makeover disconcertion by metastatic cancer bone **(2)**. The osteolytic feature is the common form of metastatic breast cancer to the bone, where the bone deposition was lesser than bone resorption **(3)**.

The osteolytic types of the bone lesions are frequently associated with increased calcium level, severe bone pain, and skeletal-related events such as spinal cord compression and bone fractures. On other fact, metastases may cause a rise in bone deposition were reflected by osteoblastic activity [4]. Remarkably, precise tools that provoke development of osteoblastic injuries in metastasis of breast cancer to the bone are not completely identified [5].

Bone destruction was associated with bone metastasis caused by the relations among osteoblasts, osteoclasts, and metastatic cancer cells [6].

Although osteoclasts have long been reflected the chief effector cells and consequent the treatment goal of tumor stimulates the osteolysis, increasing recommendation that supports of mesenchymal stem cell line express the essential parts in the microenvironment regulating metastatic scattering of metastatic bone, potential and growing, osteolysis, and fudging of anti-cancer immunity [7].

In strictly, the spreading of tumor cells primary keeps of an osteoblastic position and then will be capable to procedure a micro-metastatic tumor clusters closely to the endo-steal external as it will appear by several preclinical simulation's types and patient trial samples **(8)**. Successively, in the early stage of the tumor metastatic to the bone, the osteoblasts will be activated, multiply, and growth reasons needed to osteoclastogenesis, tumor progression, osteolytic effects, and also anti-tumoral immunity[9].

Osteocalcin (OC), which is a bone-specific protein that is produced via the osteoblasts cell and is the main non-collagen type protein in the bone matrix. The osteocalcin molecular weight is about 5,800 Da and includes 49 amino acids, it contains three gamma carboxyl residues that enable the connection of hydroxyapatite to OC in bone. The level of serum OC, a subtle indication of bone production, and it is related to all bone revenue percentage and reduces the bone mineral density (BMD), and also it associates with histomorphometric indexes of the bone formation [10].

The patients with breast cancer that have positive estrogen receptor (ER) and/or positive progesterone receptor (PR) involve lesser threats of mortality rate compared with females that had negative ER- and/or PR[11].

The aim of this study was to calculate the serum level of osteocalcin in patients with breast cancer patients, and to find the correlation with estrogen and progesterone receptors.

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PATIENTS AND METHODS

The study design was a cross-section designed taking 45 women with breast cancer as group one and 22 controls women as a group 2, and the ages of all were between (25-70) years old. The sample collection was from beginning of January to the end of June 2021. These female patients came to Kirkuk Teaching Hospital. The interview was done with them by using planned questionnaire, which was including characteristics such as weight, height, age, … etc.

Venous blood was collected from each of patients' group and control group. We centrifuged the blood samples directly at 3000 r/min for about 10 minutes, and then we collected the samples in specific tube. Then we stored the samples at -8°C.

Regarding the biochemical tests: serum osteocalcin measured by (Osteocalcin Elisa Kit, Elabscience , USA, ERs, and PRs levels were tested by (Progesterone receptor Elisa Kit , and Estradiol receptor Elisa Kit , Elabscience, US) which was an automated by DS2™ - Automated ELISA System Dynex USA.

Statistical analysis

The data were analyzed using the SPSS software, version 20.0. All of the data were presented as mean ± standard deviation (SD) to estimate the significance of differences between the patients' group and control group. ***P value is accepted at p≤0,05.***

Ethical approval:

This study was ethically approved by Ibnsina Univers ity's Research Ethics Committee (REC). All procedures performed in studies involving human participants were under the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from each participant after explaining all steps of this study.

RESULTS

Serum osteocalcin determination

The level of serum OC in patient group was elevated significantly as compared with the control group (Table 1). Also, correlation of OC level with BMI in patients' group was significant and in control group was non-significant (Figs. 1,2)

Table (1): Level of BMI and Serum Osteocalcin (ng/ml) in patient cases and control.

Measurements	Control group (22 participant)	Patient group (45 patients)	P-value
	Mean± SD	**Mean± SD**	
BMI (kg/m^2)	25.63 ±1.93	26.97 ±4.08	NS
Osteocalcin (ng/ml)	24.79 ±9.53	51.80 ±3.15	< 0.01

BMI= Body Mass Index, P=probability, NS= non-significant.

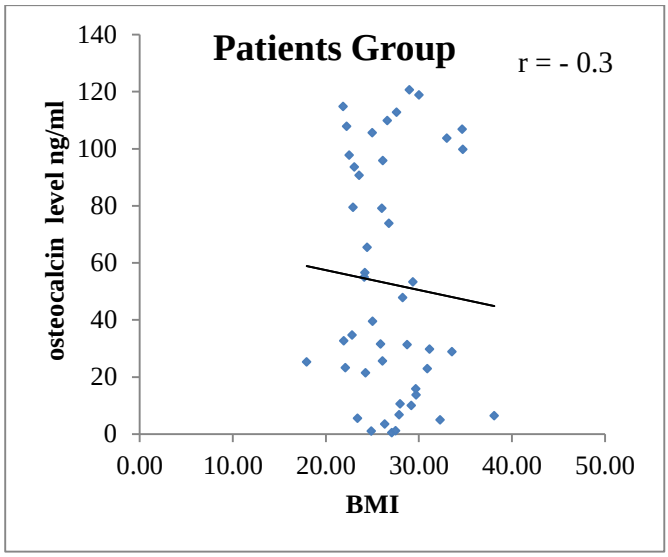

Figure 1: **Correlations between osteocalcin and BMI in breast cancer patients.**

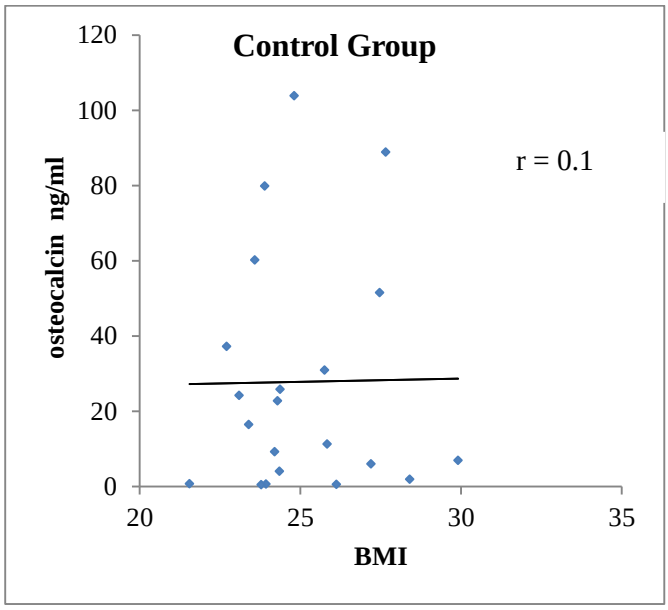

Figure 2: Correlations between osteocalcin and BMI in control cases

Determination the estrogen, and progesterone receptors,

The mean of estrogen receptor level demonstrated a significant raise in patients' group as compared with the control group (Table 2). Also, the correlation of ERs with OC levels in the control group was significant (P<0.05), while in the patients' group was not significant (Figs. 3,4).

The difference was significant between the level of serum PRs in patients' group and the control group (Table 2). Also, the correlation of PRs with OC level was negative but not significant in control group, but in the patients' group was positive and also not significant (Figs. 5,6)

Table (2): Comparison of estrogen and progesterone receptors levels in the control and patients' group

Parameters	Control group (22 participant)	Patient group (45 patients)	P value
	Mean± SD	**Mean± SD**	
ERs pg/ml	417.99±60.34	593.18±89.99	< 0.01
PRs ng/ml	5.50±1.49	2.19±0.2	< 0.01

ER= Estrogen receptor , PR= Progesterone receptor .

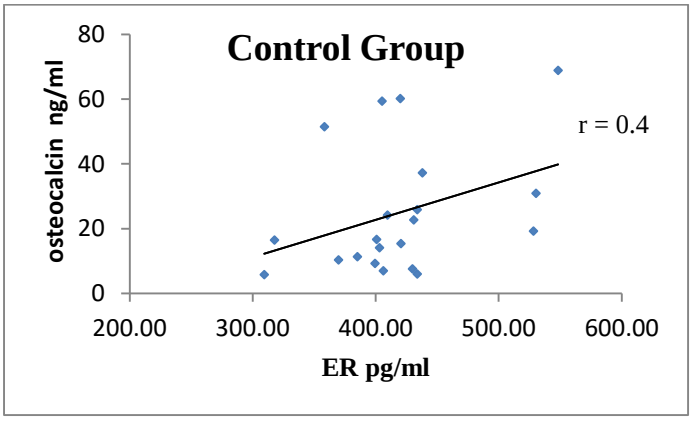

Figure 3: Correlations between osteocalcin and ER in control cases.

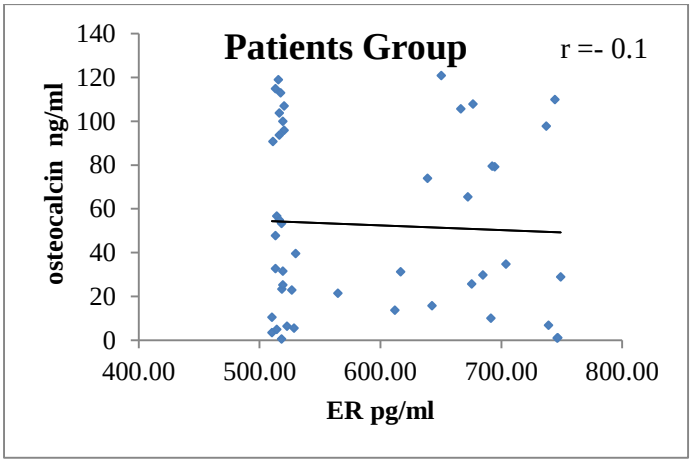

Figure 4: Correlations between osteocalcin and ER in breast cancer patients.

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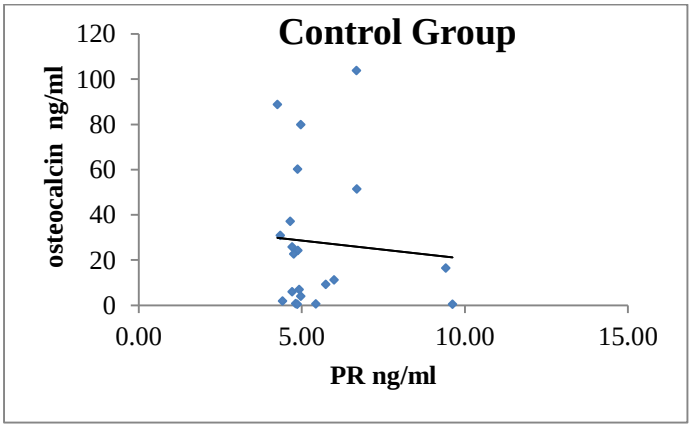

Figure 5: Correlations between osteocalcin and PR in control cases.

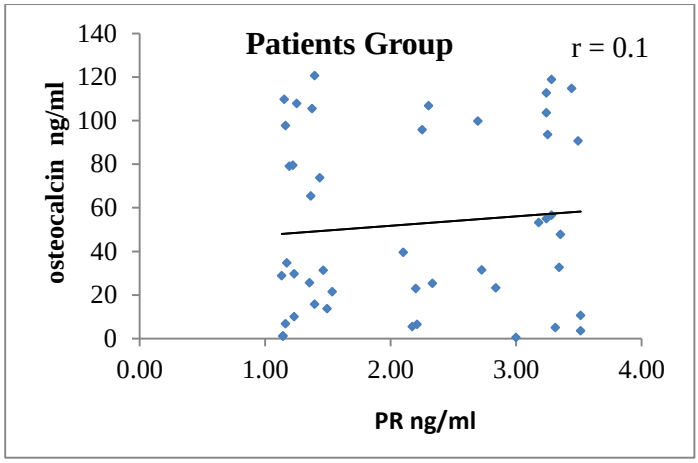

Figure 6: Correlations between osteocalcin and PR in breast cancer patients

DISCUSSION

In bone matrix the osteocalcin is regarded as the main non-collagenous protein, it is calcium-binding protein for bone-specific, as it is vitamin K dependent. The OC is formed by the osteoblasts during bone synthesis process. After the osteoblasts release it, the OC is not adapted in the bone matrix solitary, but also it is secreted in the blood. Therefore, OC level in the serum was associated with the bone turnover ratio in numerous bone metabolism disorders. Hence the OC was labeled as marker of bone turnover and can be used for this determination. Consequently, the level of OC was probable to follow up the management of bone tumor disease. An elevated level of OC after beginning the management for about 1 month may be used as prognostic factors of the management efficacy[12].

In this current study, numerous observations were significant, like the mean of the age of patients was (48.1 years) old, which represents the early appearance of the breast cancer and which is before the menopause.

This finding was also found by numerous studies, which were directed in Middle East [13]. Nevertheless, these outcomes were unlike the developed countries results[14], as the breast cancer frequently happens at the postmenopausal time, or may be in advanced oldness. In the females; the initial incidence of breast malignancy in Ivory Coast is in premenopausal age because of the reasonable short life probability, the premature age of first delivery, and increased gravidity. Furthermore, in United States, the early time of the first pregnancy[15], and the multiple pregnancy [(16)] could be the major risk factors for developing of breast malignancy. So, these explanations can clarify the increased occurrence of breast tumor in premenopausal ages.

Also, the present study found that there was a negative significant correlation between the serum OC level and BMI ratio in breast cancer group ($P<0.05$, $r = -0.3$). This result agrees with many studies like **Kord-Varkaneh**[17] , which found an overall significant inverse relation between the serum level of OC with body mass index of patients.

In healthy Chinese females the level of serum OC was negatively correlated with BMI, and fasting blood sugar

(FBS), so, the OC influence the development of diabetes mellitus (DM) and increased obesity [18].

Another study found that increased plasma OC levels were significantly related to reduced BMI, which suggests that OC may be associated with energy metabolism in humans [19]. Multiple regression investigations revealed that OC level is considered as important negative signal of BMI, along with triglyceride (which is considered as progressive predictor). Body fat ratios were found to be reduced as plasma OC levels augmented in female participants. Overweight and obese patients have been revealed to have lower plasma OC levels, and two forms of OC can be released from both omental adipose tissue and sub-surface skin in vitro . [20]. However, there are other studies signifying that plasma OC levels are not associated with BMI. Recently, weight and body fat loss were not established to be linked with OC types under vitamin D and vitamin K supplementation [21].

Jie Wang *et al*. established that the movement, and bone attack of MDA-MB-231 cells may be disallowed by quieting the gene of bone sialoprotein[22].

In present study the difference was significant (P value was <0.05) in the level of OC between the breast cancer patients' group and the control group (51.80 ±33.15, 24.79 ±19.53 (ng/ml)) respectively.

Engblom *et al*.[23] found that OC level was associated to the incidence and development of the tumor. Similarly, it can assist the progress and metastasis of lung cancers via the neutrophils load.

The Many studies advised the role of OC in glucose increasing metabolic rate, and its correlation with creation of cancer [24]. Also, **Ye *et al***.[25] and **Kayed *et al***.[26] established role of OC in development of prostatic cancer through the PC-3 lines and the same for pancreatic tumor. Another study by **Lee *et al***.[27], explained the use of OC as analytic factor for early detection of metastasis the breast malignancy. And **Salem *et al*** .[28] recommended that the OC level in patients complaining of breast malignancy that had metastasis to the bone was more than control group.

In osteosarcoma and pancreatic malignancy, many research verified the OC role in development of the tumor. Also, another research documented the association of rise of the level of OC in bone metastasis of breast malignancy[29].

In this study, there difference between the control and patients' group was significant in the levels of ERs, PRs between the control and patients' group (417.99±60.34 and 593.18±89.99 respectively) pg/ml and (5.50±1.49 and 2.19±0.92 respectively) ng/ml.
Also, the correlation of ERs with OC level was significant in control group ($P < 0.05$, $r = 0.4$), while in patients' group was non-significant ($r = -0.1$).

Estrogen receptor expression is a predictable marker of breast cancer in females. In normal epithelium of women mammary gland ER is identified in 7–17% of cells. It is estimated that around 70–80% of breast tumors in women express ER. These tumors are described by slower growth, differentiation, and well prognosis with a suitable treatment procedure, which associates with the length of existence after surgical removal[30].

For illustration, a recent study recommended that the vertebral fracture is more incident, about 5 times in newly patients with breast tumor in 3 years period, than well people[31]. Furthermore, in a study by **Hammod *et al***.[32] the patients that complained from the osteoporosis were about quarter of the total patients during a period of five years duration. Further studies documented the females had a higher bone density increased the risk of breast malignancy[33].

Many studies revealed that estrogen is one of important reasons for development the breast cancer; its levels in breast malignancy are advanced than in well persons[34]. Exactly, the proliferation impact and death the cells of breast tissue via connecting to the receptors of estrogen or up-regulation. Meanwhile the estrogen implicates in the effects of breast malignancy, the possible role was to avoid the breast cancer development by removal of both ovaries, however the management with drugs such as selective-estrogen-receptor-modulators also adjust the level of estrogen[35].

CONCLUSIONS

The osteocalcin, which is a biomarker of the bone metabolism could recognize the patients at high risk of bone disease and it may need more follow up.

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