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A Comparative Study Of The Levels Osteoproteger In (OPG), Vitamin D, Parathyroid Hormone, And Estrogen Hormone Between Healthy Females And Breast Cancer Patients

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Abstract

Breast cancer (BC) is one of the most widespread cancers around the world and it is the second main reason for death after lung cancer among women Therefore, the current research is looking at comparing the levels of Osteoprotegerin, vitamin D, parathyroid hormone and estrogen between healthy women and breast cancer patients. The study includes three groups: the control group **(G1),** the early diagnosis group for breast cancer **(G2),** and the chemotherapy group **(G3),** all cases selected at the beginning of the disease diagnosis.According to the statistical values, the results of vitamin D showed a decrease in vitamin D levels in groups with breast cancer compared with the control group. It showed a highly significant difference in all studied groups. G1 with G2 **(20.04 ± 2.80), (4.98 ± 1.67),** P-value **(0.001)** and G1 with G3 **(20.04 ± 2.80), (9.38 ± 1.43),** P-value **(0.001)** and also G2 with G3 **(4.98 ± 1.67), (9.38 ± 1.43),** P-value **(0.001),** respectively.It also showed an increase in the levels of parathyroid hormone in groups diagnosed with breast cancer compared with the control group. It showed a highly significant difference between G1 with G2 **(68.52 ± 20.44), (167.79 ± 35.21),** P-value **(0.001)** and G1 with G3 **(68.52 ± 20.44), (136.52 ± 58.56),** P-value **(0.001),** respectively. But it showed a non-significant difference between G2 with G3 **(167.79 ± 35.21), (136.52 ± 58.56),** P value **(0.779).**Moreover, estrogen levels were within the normal limits for groups diagnosed with breast cancer compared with the control group, and there was non-significant difference in all studied groups. G1 with G2 **(87.93 ± 37.01), (75.60 ± 24.99),** P-value **(0.185)** and G1 with G3 **(87.93 ± 37.01), (76.74 ± 21.87),** P-value **(0.247)** and also G2 with G3 **(75.60 ± 24.99), (76.74 ± 21.87),**p-value **(0.985)**, respectively.As for the results of the levels of Osteoprotegerin (OPG) were within the normal limits for groups diagnosed with breast cancer compared with the control group, and there was non-significant difference in all studied groups. G1 with G2 **(324.11 ± 104.73), (315.0 ± 123.98),** P-value **(0.941)** and G1 with G3

(324.11 ± 104.73), (313.38 ± 109.02), P-value **(0.919)** and also G2 with G3 **(315.0 ± 123.98), (313.38 ± 109.02),** p-value **(0.998),** respectively.

Conclusion :- Groups affected with breast cancer had severe deficiency in vitamin D levels and high in parathyroid hormone, which gives evidence for the role of vitamin D as an anticancer agent. The levels of estrogen and Osteoprotegerin (OPG) were within normal limits, and this indicates that the bones were not destroyed for the purpose of obtaining calcium due to the rise of the parathyroid hormone in the early stages of the disease, but with age and disease, the results may change .

Key words: Breast Cancer, Osteoprotegerin (OPG),Estrogen, Parathyroid Hormone, Vitamin D

Introduction

Breast cancer (BC) is one of the most widespread cancers around the world and it is the second main reason for death after lung cancer among women[1]. Many reports demonstrated a reverse correlation between vitamin D concentration and the occurrence of 15 types of cancers, including breast, lung, kidney, colorectal, and pancreatic cancer [2].

Osteoprotegerin (OPG) is a homodimeric glycoprotein known as “TNFRSF11B and is a member of the Tumor Necrosis Factor Receptor (TNFR) superfamily of proteins”. OPG is involved in many biological processes. its role is of particular importance in “bone metabolism, inflammation, tumorigenesis and other processes where cell differentiation, survival, and death are controlled” [3]. Osteoprotegerin (OPG) consists of "401 amino acid residues" and is primarily produced as a "55-62 kDa" glycosylated monomer. Then it undergoes a homogenization process, after which it is excreted as a homodimer bound to disulfide (the mature form 110-120 kDa). The binary OPG has a higher affinity for RANKL than the monomeric [4]. OPG proteins consist of seven functional domains. [5] Domains 1-4 are cysteine-rich N-terminal domains that interact with RANKL during binding [6]. Domains 5-6 are the areas of death that contribute to the dimerization of OPG[7]. Domain 7 is the c-terminal heparin-binding domain of cysteine (Cys-400) which also plays an important role in the dimerization of OPG.[5,6]. Osteoprotegerin (OPG) was first characterized as a negative regulator of bone turnover. Bone homeostasis is maintained by the interplay between the receptor activator of nuclear factor kappa-B (RANK), its soluble activation ligand (RANKL), and OPG. OPG binds RANKL as a decoy receptor, inhibiting the activation of RANK by RANKL and preventing the differentiation of bone marrow precursor (monocyte/macrophage) cells to osteoclasts — cells that are central in the process of bone resorption [8]. Besides bone, OPG is expressed in other tissues, including the stomach, intestines, skin, liver, heart, lung, kidney, and breast. This expression across diverse tissue types indicates that its biologic functions may extend beyond bone metabolism. In relation to breast cancer, OPG was initially investigated as a potential inhibitor of metastasis-related osteolysis [7].

Vitamin D Synthesis in humans by more than one steps the first step starts when ultra-violate B (UVB) radiation approximately (290- 315nm) reaches the skin and stimulates conversion 7-dehydrocholesterol to pre-vitamin D_3 [9]. Approximately 90% of vitamin D is endogenous production under the skin The direct equilibrated thermally to produce vitamin D_3, while vitamin D_2 and D_3 taken from the diet are together with D_3 synthesis from the skin

were bound with proteins and lipoproteins for transported to the liver and produced 25-hydroxyl vitamin D 25 (OH)D, this step induced hydroxylation by vitamin D-25-hydroxylase enzyme encoded by (CYP2R1) Then undergo another hydroxylation in the kidney to yield1,25-dihydroxy vitamin D 1,25(OH)2Dby by the enzyme 25(OH)D-1α-hydroxylase which encoded by (CYP27B1). Vitamin D plays an essential role to absorbed dietary calcium in the human body and this led to giving healthy bones, so as without enough vitamin D for a prolonged period due to poor bone health and other diseases like Diabetes, Osteomalacia, Osteoporosis, Rheumatic arthritis, Parkinson, Alzheimer diseases, and fractures in adults and breast cancers [10].

Parathyroid Hormone (PTH), also called parathormone or parathyrin, is a hormone secreted by the parathyroid glands that regulate the serum calcium concentration through its effects on bone, kidney, and intestine [11]. It is a polypeptide containing 84 amino acids, which is a prohormone. It has a molecular mass of around 9500 Dalton. Its action is opposed by the hormone calcitonin[12]. secreted by parathyroid glands, which play an essential role in bone remodeling by alternately resorbed and rebuilt, PTH was secreted in response to low serum calcium levels in the blood, so it indirectly stimulates osteoclast activity with bone marrow this due to release more ionic calcium (Ca^{+2}) into the blood to raise calcium levels in sera [11]. Many epidemiologic studies suggested that PTH has carcinogenic and tumor-promoting effects which maybe increase breast cancer so that PTH and 1,25$(OH)_2$D levels are against correlated with each other and this led to increasing calcium levels in blood [13].

Estrogen is one of the female sex hormones that are responsible for the development and regulation reproductive system for female and secondary sex characteristics [14]. There are three major endogenous estrogens that have estrogenic hormonal activity: estrone (E_1), estradiol (E_2), and estriol (E_3) Estradiol and estrange are the most potent and prevalent. Another estrogen called estetrol (E_4) is produced only during pregnancy. Estradiol(E_2) is the elevated biological activity [15]. High levels of estrogen especially after menopause are considered to be the causes of breast cancer there are also a number of significant risk factors that have been implicated in the genesis of breast cancer, almost 5–10% of patients are suffering from genetic changes related to breast cancer [16]. High concentrations of circulating estrogens have been found to be strongly associated with increased risk for breast cancer in postmenopausal women [16]. This hormone has a main and secondary role in normal cases according to a previous study More than 75% of breast tumors express the estrogen receptor (ER), suggesting that the majority of breast cancer injuries are hormone-dependent and respond to the hormone estrogen [17]. E_2-activated ER is a key organizer of breast carcinogenesis by leading complex biological pathways that control different functions of cells, such as growth, apoptosis, migration, and angiogenesis [18].

Aim of the study

The current study aims to measure the levels of Osteoprotegerin (OPG) in women with breast cancer and compare it with the control group. As well as to find the relationship between vitamin D, parathyroid hormone, and Estrogen.

Materials and Methods

- **Study Groups**

All blood samples were collected from Medical City, Oncology Teaching Hospital, Department of Early Diagnosis of Breast Cancer.The period for collecting blood samples was from 1/9/2020 to 1/3/2021. These analyzes include the control group, newly diagnoses group, and the chemotherapy group of breast cancer. The second and third groups consisted of 45 blood samples, and the first group consisted of 40 blood samples from Iraqi women. The first group represents the control group **(G1).** The second group includes the newly diagnosed group **(G2).** The third group includes women who were given the first and second doses **(G3).** The women were between 25 and 45 years old before menopause.

- **Blood Sampling**

Blood samples were obtained by taking five milliliters of blood with a syringe for healthy and breast cancer patients. Then the samples are placed in a gel tube and left for a certain period of time to coagulate before being placed in the separator. Samples were then placed in a centrifuge at 3000 rpm for 10 min. After that, the serum is isolated and divided into seven sections and placed in a special storage tube called Abendorf. Then the samples are stored at a temperature of (-20 ° C) for the purpose of measuring parameters such as (Vitamin D - Osteoprotegerin (OPG) - Estradiol (E_2) - Parathyroid (PTH)) that are measured by the ELISA technique.

- **Parameters Measurement**

Evaluations of Osteoprotegerin (OPG), Vitamin D, PTH, Estrogen were achieved by the ELISA technique.

- **Statistical Analysis**

The results were obtained as a mean ± SD (standard deviation). P-values of <0.001 and <0.05 were expressed as highly significant and significant respectively, as well as Pearson's correlation coefficient, was utilized to determine the correlation between two continuous variables.

Results

The results showed vitamin D, parathyroid hormone, estrogen, and Osteoprotegerin (OPG) in the control group **(G1),** early diagnosis group **(G2),** and chemotherapy group **(G3)** in the table (1).

The results of the study showed a decrease in vitamin D levels in two groups of women with breast cancer compared with the control group, which showed a highly significant difference in all groups studied. G1 with G2 **(20.04 ± 2.80), (4.98 ± 1.67),** P-value **(0.001)** and G1 with G3 **(20.04 ± 2.80), (9.38 ± 1.43),** P-value **(0.001)** and also G2 with G3 **(4.98 ± 1.67), (9.38 ± 1.43),** p-value **(0.001),** respectively

As for the parathyroid hormone results, high significant differences appeared between G1 with G2 **(68.52 ± 20.44), (167.79 ± 35.21),** P-value **(0.001)** and G1 with G3 **(68.52 ± 20.44), (136.52 ± 58.56),** P-value **(0.001)** respectively. But it showed a non-

significant difference between G2 with G3 **(167.79 ± 35.21), (136.52 ± 58.56),** P-value **(0.779).**

There was non-significant difference in estrogen levels in all studied groups. G1 with G2 **(87.93 ± 37.01), (75.60 ± 24.99),** P-value **(0.185)** and G1 with G3 **(87.93 ± 37.01), (76.74 ± 21.87),** P-value **(0.247)** and also G2 with G3 **(75.60) ± 24.99), (76.74 ± 21.87),** p-value **(0.985),** respectively.

Also, there was non-significant difference in the levels of Osteoprotegerin (OPG) in all the groups studied. G1 with G2 **(324.11 ± 104.73), (315.0 ± 123.98),** P-value **(0.941)** and G1 with G3 **(324.11 ± 104.73), (313.38 ± 109.02),** P-value **(0.919)** and also G2 with G3 **(315.0 ± 123.98), (313.38 ± 109.02),** p-value **(0.998),** respectively.

Table (1): Comparison Among Osteoprotegerin, Vitamin D, PTH and E2 Levels in Control and Groups Diagnosed with Breast Cancer.

Parameters	Con. (G1) Mean ± SD	Mal. (G2) Mean ± SD	Chem. (G3) Mean ± SD	P-Value G1vs.G2	P-Value G1vs.G3	P-Value G2vs.G3
Vit. D	20.04±2.80	4.98 ±1.67	9.38 ±1.43	0.001	0.001	0.001
PTH	68.52 ± 20.44	167.79±35.21	136.52 ± 58.56	0.001	0.001	0.779
E_2	87.93 ± 37.01	75.60 ± 24.99	76.74±21.87	0.185	0.247	0.985
OPG	324.11±104.73	315.0±123.98	313.38±109.02	0.941	0.919	0.998

In addition to that, There was a weak positive correlation between OPG levels and Vitamin D and E_2 and there was a negative (inverse) correlation between OPG levels and PTH. There was a non-significant between OPG and Vitamin D, PTH and E_2 in patients with early diagnosis. As shown in Table (2)

Table (2):- The Correlation Between OPG and Studied Parameters in Early Diagnosis patients.

Parameter	R	P-value
Vitamin D (ng/mL)	0.026	0.867
PTH (pg/mL)	-0.210	0.165
Estradiol (pg/ml)	0.017	0.911

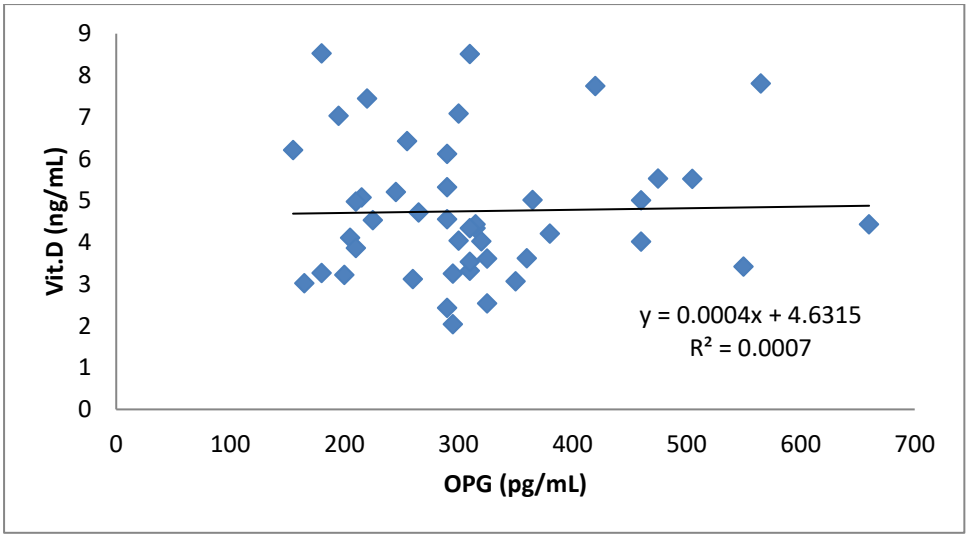

Figure (1): OPG vs. Vit.D in early diagnosis patients.

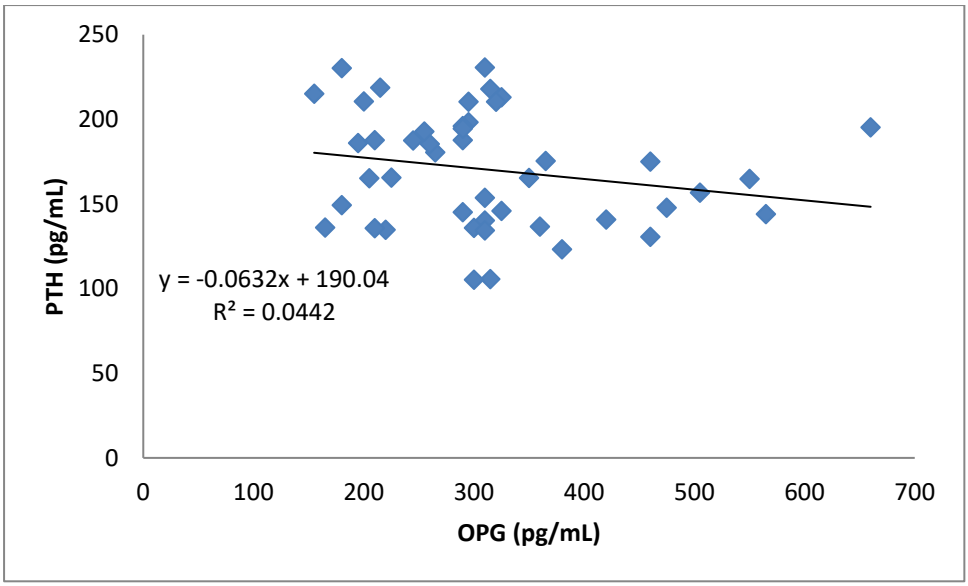

Figure (2): OPG vs. PTH in early diagnosis patients.

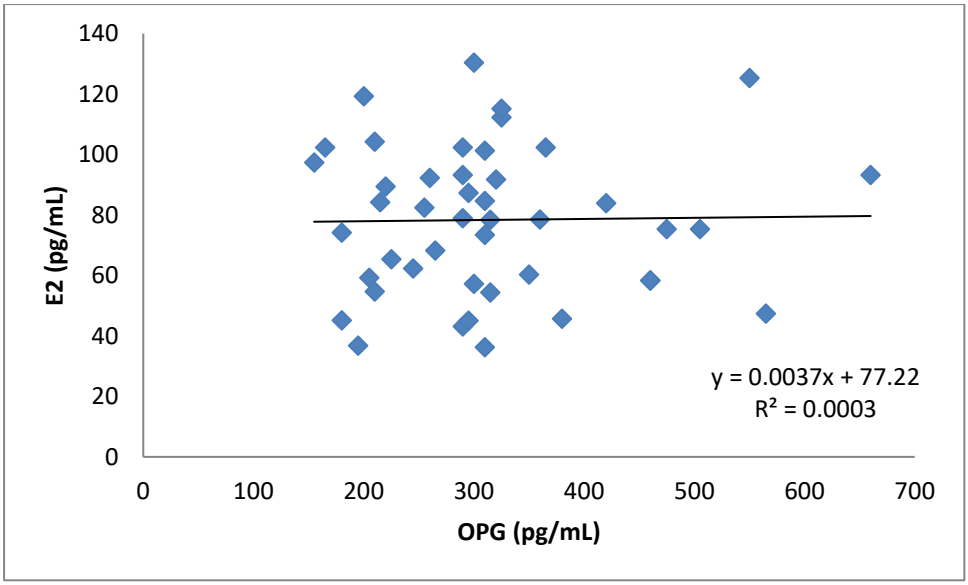

Figure (3): OPG vs. E_2 in early diagnosis patients.

While the results in chemotherapy patients showed a weak positive relationship between OPG and PTH and a weak negative (inverse) relationship between OPG and Vitamin D and E_2. There was a non-significant between OPG and Vitamin D, PTH and E_2 in chemotherapy patients. As shown in Table (3)

Table (3):- The Correlation Between OPG and Studied Parameters in Chemotherapy Patients.

Parameter	r	P-value
Vitamin D (ng/mL)	-0.016	0.918
PTH (pg/mL)	0.081	0.599
Estradiol (pg/mL)	-0.133	0.384

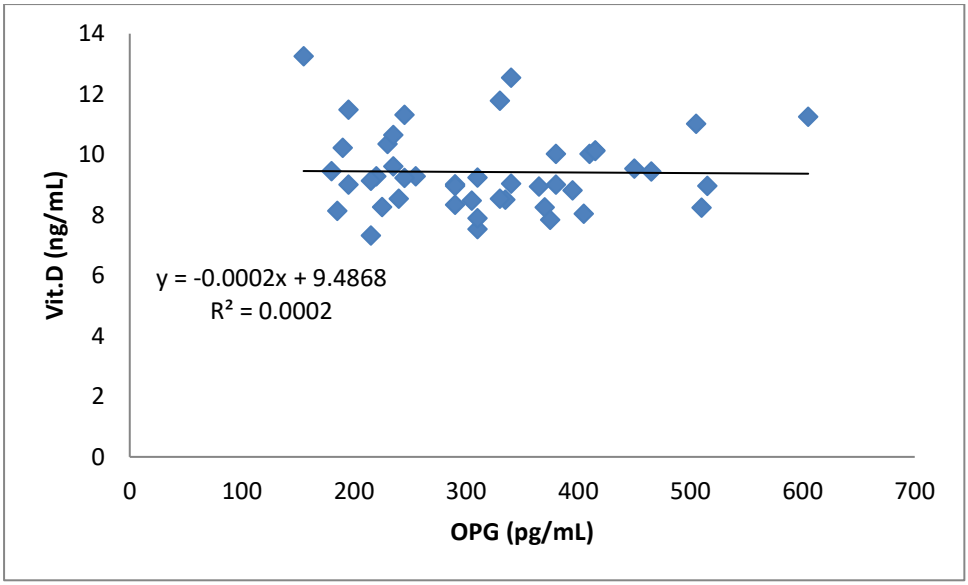

Figure (4): OPG vs. Vit.D in Chemotherapy patients.

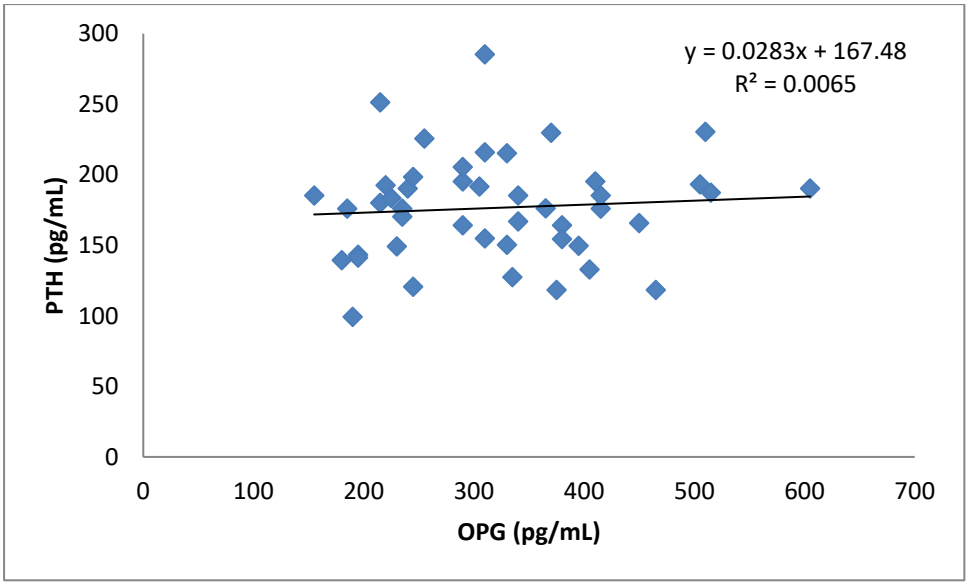

Figure (5): OPG vs. PTH in Chemotherapy patients.

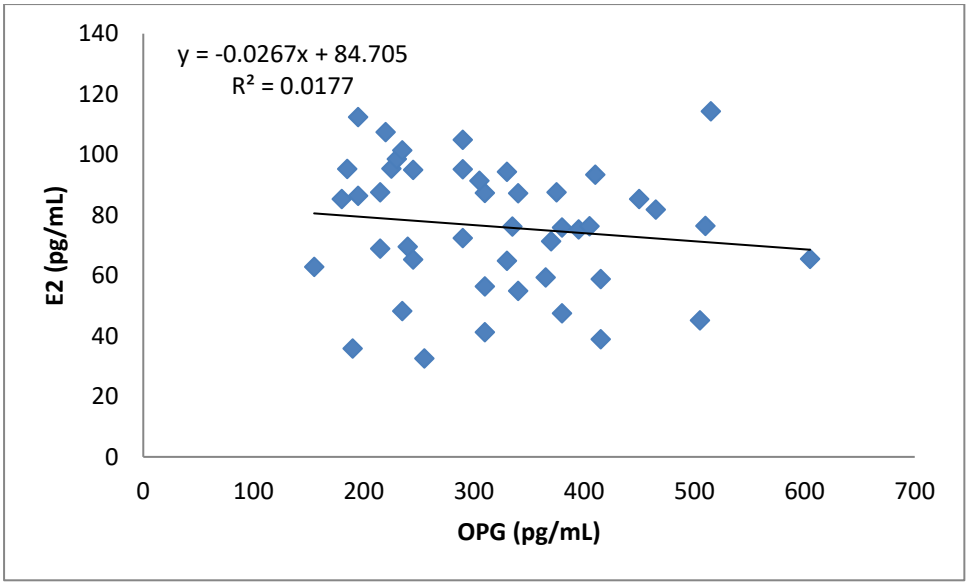

Figure (6): OPG vs. E_2 in Chemotherapy patients.

Discussion

The current study showed a contributing relationship to vitamin D depletion with regard to the incidence of breast cancer, but the results of vitamin D levels for the group of women with breast cancer were very low compared to the control group, and this indicates an increased risk of breast cancer by different mechanisms.

This severe deficiency of vitamin D in blood will affect the incidence of the disease on more than one side. Some epidemiological studies linked vitamin D and breast cancer. A recent research study that agreement with this study, found 90% or more of people with breast cancer have vitamin D deficiency, however has been discovered that a high incidence of breast cancer is the relationship with low vitamin D levels [19]. vitamin D has a role through its effect on the estrogen receptors. Estrogen has been found to play a key role in breast cancer inducing the breast cell to occur uncontrolled division and thus tumor [20]. The effect of this hormone through the hormone receptors where the low level of vitamin D consider positive effect by an increase of estrogen receptor (ER) therefore vitamin D has an estrogenic effect on the spilled of chest cell to form tumor vitamin D can reduce the risk of breast cancer in these women since it can down-regulate the expression of estrogen receptors and attenuate the synthesis and signal of these hormones [21]. The proposed second effect of vitamin D influenced the aromatase [22]. $1,25(OH)_2D3$ decreases the expression of aromatase (CYP19), the enzyme that catalyzes estrogen synthesis selectively in breast cancer cells and the adipose tissue surrounding them, by directly restrictive aromatase transcription and down-regulating ER-α decrease in vitamin D levels increases gene expression of this enzyme which increases the efficacy of the enzyme and increases estrogen concentration which has a positive effect on the disease [21]. Either the third effect of vitamin D on cancer patients by its effect on the shortening of the programmed death of cancer cells or their formation, Calicitrol stimulate apoptosis involves the generation of reactive oxygen species (ROS), mitochondrial disruption and eliminate cytochrome C [23]. Other mechanisms of suppressive effects of calcitriol on invasion and metastasis include

potent antiangiogenic activity that might contribute to its actions to inhibit invasion and metastasis [24]. Other mechanisms of vitamin D against cancer include anti-inflammation is characterized by mediators such as cytokines, chemokine, prostaglandins (PGs), and reactive oxygen and nitrogen species in tumor tissue [25]. Other research proposed mechanism comprising Angiogenesis is the process of generating new blood vessels from existing vasculature and is a crucial step that causes progression, and metastasis of tumors [26].

On the other hand, many clinical studies have suggested an elevated breast cancer risk with increased levels of PTH [27]. where previous studies suggest that high levels of the parathyroid hormone have carcinogenic and tumor-stimulating effects that are associated with the risk of breast cancer [28]. according to these studies indicate the carcinogenic effect of parathyroid hormone increased the risk of breast cancer, also three of the previous cohort studies that agree with these results, that found an association between higher levels of parathyroid hormone and breast cancer risk [27]. This study depends on the measurement levels of vitamin D and the factors affecting stimulation in patients with breast tumors on the one hand and another side the measurement levels of PTH as another criterion on the accuracy of measurements of vitamin D where the levels of this hormone rise when the decreased level of vitamin D and that obtained from measurements and this is due to the occurrence of one form of hyperparathyroidism types, expansion of one or more of the parathyroid glands causes excessive hormone production called primary hyperparathyroidism, which leads to rising levels of calcium concentration in blood (hypercalcemia), and increase the risk breast cancer incidence [29].It is well established that there is an inverse relationship between serum 25-OHD and serum PTH [30]. It also has the responsibility to increase levels of PTH when the blood calcium level is too low, PTH is released to bring the calcium level back up to normal, to compensate for a decrease in calcium levels by gut due to decrease activity of vitamin D effective because low 25(OH)D levels below normal values cause reduced efficiency intestinal calcium absorption, on the other hand, increased secretion of PTH [31].

According to the results of the study, estrogen levels were within normal limits, and this does not mean excluding the role of this hormone is causing the disease because it is one of the main causes of breast cancer. High concentrations of circulating estrogens have been found to be strongly associated with increased risk for breast cancer in postmenopausal women [32]. The increase in levels of estradiol supports the evidence that suggested the role of these hormones in breast cancer [33]. According to a previous study, more than 75% of breast tumors occur through estrogen receptors (ER), suggesting that the overwhelming majority of breast cancers are hormone-dependent and grow in response to the hormone estrogen [34].Estrogen plays a role through estrogen receptors, promoted cell proliferation, and initiates mutations that occur as a function of errors during Deoxyribonucleic acid (DNA) replication [35]. Either second mechanism the role of estrogen in breast cancer can be explained by direct effect by the formation of oxygen free radicals which have a role in the genetic changes in cell chest or through metabolism processes of estrogen that occur inside of breast cells by addition of hydroxyl group at the 2-, 4- and 16α-positions of E_2. The 2 and 4 catechol estrogen metabolites are undergone redox cycling to formation oxygen free radicals, the formation of free radicals which damage DNA-bound guanine to form 8-oxo–guanine [36]. The quinone-adducts and 8-OXO-guanine bases are

unsteady and are eliminated from the affected DNA sections through a process called depurination" [36]. Error DNA repair of spaces results to generate mutations at the depurated positions. a collection of these mutations will lead to developing breast cancer [37]. In premenopausal patients with multiple estrogen metabolizing genes, the incidence of the disease is four times higher than in women without genetic mutations [38].

As for the levels of Osteoprotegerin (OPG), they were within the normal limits, and this indicates that the bones were not subjected to demolition for the purpose of obtaining calcium due to the high parathyroid hormone in the early stages of the disease, but with age and disease the results may changeThe role of OPG in bone turnover via the OPG/ RANK/RANKL system has been well characterized and is the subject of a number of reviews [39].OPG is central in the regulation of bone turnover and a number of studies have assessed the role of OPG in bone-related diseases. In particular, several studies have assessed the role of OPG in osteoporosis and rheumatoid arthritis. Osteoporosis is a disease characterized by low bone mineral density (BMD) due to increased osteoclast activity, and one of the major causes in women is a postmenopausal estrogen deficiency. studies have also demonstrated that treatment of primary human osteoblasts with estrogenresults in an upregulation of OPG production. This involvement of estrogen receptor activation is further verified by observations that the selective estrogen receptor modulator raloxifene, which is used to increase BMD can stimulate OPG production in osteoblasts [40].In addition to being central to regulating RANK– RANKL interactions in bone metabolism, OPG can also stimulate cell survival by acting as a receptor for TNF-related apoptosis-inducing ligand (TRAIL) [41]. OPG acts as a soluble decoy receptor, binding TRAIL and preventing its interaction with the functional death receptors, thus allowing cells to escape cell death. OPG may be involved in the survival of a number of tumor cell types via this mechanism [42]. This could be of particular importance regarding the ability of tumor cells to evade cell death since host immune cells present in the tumor microenvironment produce TRAIL, and in vivo data suggests this be important in mediating anti-tumor activity [43]. As such, the release of OPG by tumor cells is a potential mechanism of resistance by these cells to TRAIL-induced apoptosis [42]. The biological importance of OPG–TRAIL interactions is underscored by recent findings that in physiological conditions OPG can bind TRAIL with an affinity similar to that of RANKL [44].Precedent studies revealed that OPG protein is expressed by tumor cells and led to an investigation of the role of OPG in tumor biology. An increasing body of data has demonstrated that OPG modulates breast tumor behavior. Initially, previous research focused on OPG in the bone microenvironment as a potential inhibitor of RANKL-driven osteolysis. More recently, attention has shifted to include OPG expression and interactions in the primary breast tumor independent of RANKL. In the primary tumor, OPG may interact with another TNF superfamily member, TNF-Related Apoptosis-Inducing Ligand (TRAIL) to prevent apoptosis induction. Additional interest in OPG in breast cancer has been stimulated by the tumor-promoting role of its binding partner RANKL in association with BRCA1 gene mutations. Previous studies have summarized functional studies on OPG and breast cancer [45]. Precedent studies suggest the involvement of OPG in all of these three cancer types. In terms of tumor cell survival, Precedent studies have demonstrated both breast and prostate cancer cell lines to produce sufficient OPG to protect them against TRAIL-induced apoptosis [42]. Similar findings have been demonstrated in colon carcinoma cell lines [46]. This

suggests that OPG can have a direct role in promoting the survival of certain tumor types. With regards to breast and prostate cancer cells, this only applied to the poorly differentiated hormone-independent cell lines, however, the significance of this remains to be determined [42].

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