

# Variations in Serum Levels of Estradiol, Vitamin D and Calcium in Women with Breast Cancer on Tamoxifen

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## Abstract

**Background:** Tamoxifen works as a selective estrogen receptor modulator (SERM) by binding to estrogen receptors on mammary epithelium and inhibiting estrogen's proliferative activity on the mammary tissues. however, it has a little estrogen agonistic impact on bone, liver, and endometrial. **Aim:** This cross-sectional study aimed to investigate the variations in serum estradiol, vitamin D and calcium levels in postmenopausal breast cancer women on tamoxifen. **Methods:** This study is conducted at the oncology center in Kerbala/Iraq at Imam al-Hussain medical city during the period between November (2021) and May (2022), the patients were divided into groups according to their ages, BMI, duration of disease and duration of treatment. A blood sample from 100 breast cancer patients (being on tamoxifen for at least 3 months) were taken and laboratory tests done by measuring plasma levels of (estradiol, vitamin D and calcium) to assess its effect. Ethical approval and interviews with the patients were performed. **Results:** The mean age of 100 patients was 51 years. As serum calcium and vitamin D increased, serum estradiol level decreased with age and there were statistically significant changes with different age groups (P value < 0.001). Besides that, the increasing in BMI accompanied by increasing levels of serum calcium, estradiol and vitamin D with statistically significant change in serum vit. D between the obese and non-obese groups (P value = 0.002). Finally, Serum calcium, estradiol and vitamin D were decreased upon long term of disease and tamoxifen use (>5 years). **Conclusion:** Tamoxifen shows significant positive influence on hormonal level of estradiol and calcium with negative effect on vitamin D in female patients with breast cancer.

**Keywords:** Breast cancer, tamoxifen, vitamin D, calcium, estradiol.

## 1. Introduction

Breast cancer (BC) is the most common malignancy diagnosed in women globally, following lung cancer [1, 2]. Breast cancer ranks first among the top ten malignant neoplasms impacting the population in Iraq; it is the major frequent cancer among Iraqi women, particularly in the last decade. According to the most recent Iraqi Cancer Registry, breast cancer is accounting for nearly 30% of all recorded female malignancies [3].

Breast cancer is a pathologically heterogeneous illness that can be slow developing with a great prognosis or aggressively invasive with poor prognostic and predictive characteristics [4]. The clinical benefit of early diagnosis of primary and recurring breast cancer is significant in guiding decision-making for breast cancer therapy in terms of improving survival rates [5, 6].

Numerous risk factors have been identified, including family history, age, late menopause, early menarche, nulliparity, alcohol use, obesity, the use of hormonal-replacement therapy, and smoking are all related with an increased risk of breast cancer [7]. Furthermore, the mutation in BRCA1 and BRCA2 are the two primary tumor suppressor genes associated to breast cancer [8].

In 60–70% of breast cancer patients, estrogen receptor (ER) expression is dysregulated. Estrogen plays a direct role in cellular proliferation by regulating the expression of ER target genes in several tissues, such as the breast [9].

The primary circulating ovarian steroid, 17- $\beta$  estradiol (E2) is the most physiologically active hormone in breast tissue, stimulates breast growth between puberty and sexual maturity. During menopause E2 plasma levels drop by 90%. Despite significantly different circulating estrogen levels in pre- and postmenopausal women, the concentrations of E2 in breast cancer tissues do not differ between these two groups of women, indicating that its uptake from the circulation may not contribute significantly to the total content of this hormone in breast tumors, but rather that de novo biosynthesis, or peripheral aromatization of ovarian and adrenal androgens, is of great importance [10].

Hormones are responsible for around 78 percent of breast cancers. Stopping the function of estrogen in the breast is therefore a well-known treatment method for avoiding recurrence, and it may be accomplished by either inhibiting the hormone's activity on estrogen receptors or stopping its biosynthesis [11]. Because it is an estrogen-dependent tumor, its beginning and development are significantly connected with estrogen levels, despite the fact that people maintain a dynamic equilibrium at various physiological phases. Furthermore, persistent low E2 concentrations are sufficient to raise breast cancer risk in premenopausal women, despite the fact that high E2 levels are linked to breast cancer in postmenopausal women [12].

Tamoxifen, a nonsteroidal triphenylethylene derivative, has been shown to compete with estrogen in inhibiting ER activity,

which is linked to tumor cell proliferation. It is a selective estrogen receptor modulator (SERM) having antiestrogenic activity in breast tissues and agonistic activity in bone, with mixed effects in the uterus [13].

Tamoxifen was licensed by the U.S for the treatment of postmenopausal women with advanced breast cancer. In 1977, the Food and Drug Administration approved it for use as a post-surgery adjuvant therapy to remove micrometastasis from primary breast cancer. As a result, tamoxifen eventually became a primary hormone treatment in preventing ER-positive cancer recurrence [14, 15].

Vitamin D is a precursor to the hormone calcitriol (1,25-dihydroxyvitamin D<sub>3</sub>), which governs multiple functions in many human tissues [16]. Vitamin D may be created endogenously in the skin by sun exposure and, to a lesser extent, gained through food and supplementation. Vitamin D is well-known for its involvement in calcium homeostasis and in influencing bone mineralization [17, 18].

Aside from its advantages for bone health, subsequent studies have looked at its impact in the prevention and treatment of a number of illnesses such as cancer, autoimmune disorders, and cardiovascular disease [19-21]. According to laboratory results, vitamin D may have significant anticancer effects [22] which including anti-proliferative, pro-apoptotic, pro-differentiating, anti-inflammatory, anti-invasion, and anti-angiogenesis [23-25].

Previous research has revealed that the link between vitamin D and breast cancer risk is higher in premenopausal women than in postmenopausal women. Two cohort studies in postmenopausal women found no connection between vitamin D level and breast cancer risk [26, 27].

Serum calcium levels influence vital physiologic processes such as heart rate and nerve conduction and therefore are under tight physiologic control. The skeleton is the reservoir for calcium in blood. When levels of ionized calcium in serum drop below their set point, the calcium-sensing receptor on the parathyroid glands signals parathyroid cells to manufacture and release parathyroid hormone (PTH) into the circulation. PTH acts to conserve calcium by driving the conversion of 25-hydroxyvitamin D (25-OHD) to 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) in the kidney; reducing calcium excretion in the urine, and by liberating calcium from the skeleton into the circulation. The resulting increase in ionized calcium in blood restores calcium balance and may be associated with tumor protective effects by inhibiting further release of PTH that have anti-apoptotic effects and may promote invasiveness and stimulate tumor growth [28, 29].

Results from a prospective cohort study showed that serum calcium levels were inversely associated with breast cancer risk in premenopausal women, it also indicates that calcium levels are positively associated with breast cancer in overweight (BMI>25mg/kg)peri-/postmenopausal women [30]. The aim of this study is to investigate the variations in serum estradiol, vitamin D and calcium levels in postmenopausal breast cancer women on tamoxifen treatment.

## 2. Materials and Methods

This cross-sectional observational study was carried out at the Oncology Center in Kerbala at Imam AL-Hussein Medical City in Iraq. The study was approved by ethical and scientific

committee of college of pharmacy at Kerbala University, and each participant was given a written informed consent form for their participation.

The study was conducted on 100 female patients with hormone receptor (estrogen and/or progesterone) positive breast cancer depending on histopathology and immunohistochemistry report, treated with tamoxifen (20 mg) once daily for at least 3 months (nolvadex®) aged 45-65 years. Data were collected from female patients with breast cancer who came to the center for treatment and follow-up. In some situations, the data were collected from first-degree relatives or from patient's medical records in the center. The clinical information collected included patient age, weight, height, academic achievement, workplace, marital status, family history, pre-and postmenopausal and breast feeding. Another information taken include date of diagnosis, site and type of breast cancer, stage and grade, dose, duration and time of tamoxifen.

The women taking tamoxifen therapy concomitantly with other adjuvant endocrine therapies, adjuvant chemotherapy or radiotherapy (or both), and also females who have taken drugs that induce or inhibit CYP2D6 such as fluoxetine, pregnant or lactating women and females with history of thromboembolic events (deep vein thrombosis, pulmonary embolism) are excluded from this study.

Sample collection and biochemical analysis

A 3 ml of peripheral blood sample was taken from each female and placed in a gel tube that allowed to clot in a warm waterbath for 10 minutes. Subsequently the samples were centrifuged at 3000 rpm for 10 minutes to separate the serum that was used to measure the levels of the following biochemical parameters estradiol, vitamin D and calcium.

Both estradiol and 25-OH Vitamin D levels in the serum of breast cancer patients were determined by using a MAGLUMI 800 series (CLIA) fully auto chemiluminescence immunoassay analyzer (Snibe/China). This assay is a competitive binding immunoassay. The method was performed according the manufacturers instructions. Serum calcium Determined by (Mindray/China) according to (Arsenazo III method), as calcium ions and Arsenazo III combine to form a blue colored complex at a neutral solution. The absorbance increase is directly proportional to the concentration of calcium.

Statistical Analysis

Result of this study were subjected to statistical analysis using the Anova single factor and student t-test for comparison of means between studied groups and analyzed statistically through Statistical Package for Social Science (SPSS) version 28 USA for windows software. All the data were expressed as mean±standard deviation and percentage. For all statistical procedures P-value < 0.05 was considered significant.

## 3. Results

Demographic data of studied population

The studied population included 100 female patients with breast cancer. Their mean age involved in this study at time of enrolment was 51 year (range: 45-65). Ninety three percent were married and only (7%) single. The women with known family history of BC were (44%) in corresponding to (56%) had no family history. Patients who had cancer in left side were (45%), while (55%) of

them have right side breast cancer. In addition, lymph node involvement appeared in (64%) of patient and absent in the others. Ninety eight percent (98%) of patients have both ER/PR-positive and only (2%) of them were ER or PR positive, for patients who have positive HER-2 were (67%) compared with (33%) of women with negative test. The percentage of patients who undergo previous surgery, radiotherapy and chemotherapy were (94%), (79%) and (91%) respectively, as shown in table (1).

Table (1) Patients demographics and disease characteristics			
Variables		Statistical values	
Age (Years)		51.08 ± 4.85	
BMI (Kg/m <sup>2</sup> )		28.30 ± 5.57	
Duration of tamoxifen (Years)		3.41 ± 2.36	
Duration of disease (Years)		4.18 ± 2.50	
Marital status (%)	Married	93	
	Single	7	
Family History (%)	Yes	44	
	No	56	
Breast cancer Side (%)	Left breast	45	
	Right breast	55	
Lymph node Involvement (%)	Yes	64	
	No	36	
Patients who have previous surgery (%)	Yes	94	
	No	6	
Patients who have chemotherapy (%)	Yes	91	
	No	9	
Patients who have radiotherapy (%)	Yes	79	
	No	21	
Histochemical tests (%)	Human epidermal growth factor receptor-2 (HER-2)	Negative test	67
		Positive test	33
	Positive for Both Estrogen receptor (ER) & Progesterone receptor (PR)		98
	Positive for Either Estrogen receptor (ER) or Progesterone receptor (PR)		2

The mean level of serum calcium and estradiol were within the normal range while the mean level of vitamin D has shown a clear decline (11.8) than the normal range 30-100 ng/ml, as demonstrated in table (2).

Table (2): Laboratory characteristics of studied population		
Biomarkers	Statistical values	Reference Range
S. Calcium mg/dl	8.87 ± 2.58	8.1-10.4
S. Estradiol pg/ml	33.14 ± 11.25	10-66
S. Vit D ng/ml	11.8 ± 5.69	30-100

Results represent mean±SD

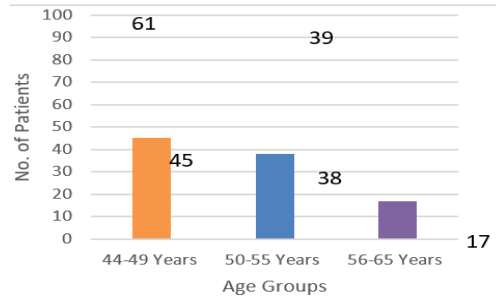


Figure1: BMI groups

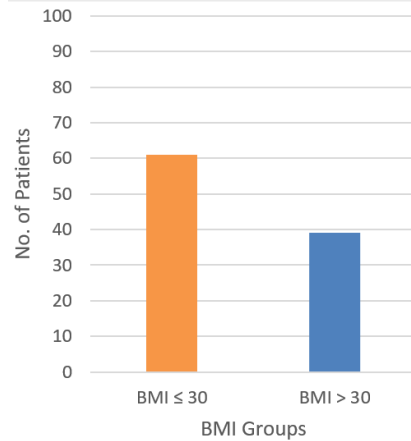


Figure2: Age groups

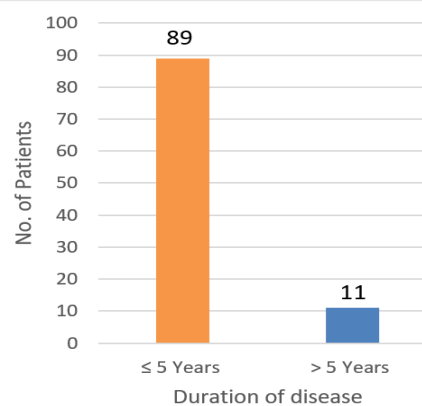


Figure3: Duration of disease

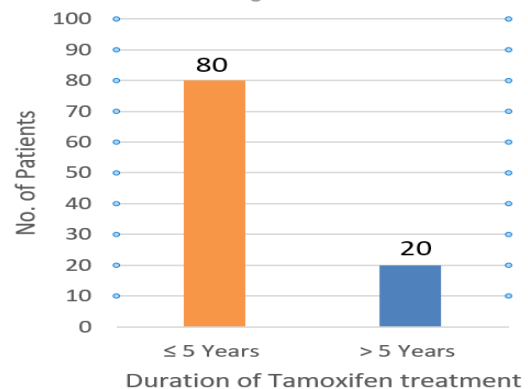


Figure4: Duration of Tamoxifen treatment

Results in Table 3 were shown an increased mean levels of serum calcium, estradiol and vitamin D with increasing body mass index (BMI). Although there was statistically significant change in serum levels of vitamin D (P value= 0.002) between the obese and non-obese groups, there is no significant change in the serum level of estradiol and Ca (P value>0.05) as shown below.

**Table 3: Association between Serum levels of estradiol, vitamin D and calcium with BMI categories of studied patients**

Biomarkers	BMI category		P value
	≤ 30 Kg/m <sup>2</sup> (non-Obese) n= (61)	> 30 Kg/m <sup>2</sup> (Obese) n= (39)	
S. Calcium mg/dl	8.55 ± 1.06	9.37 ± 3.89	0.410
S. Estradiol pg/dl	32.86 ± 11.22	33.59 ± 11.44	0.520
S. Vit D ng/dl	10.76 ± 5.32	13.45 ± 5.94	0.002*

Results represent mean±SD; Group 1: non-obese BMI ≤30 kg/m<sup>2</sup>, Group 2: Obese postmenopausal women BMI >30 Kg/m<sup>2</sup> with breast cancer taken tamoxifen 20 mg on daily dose. P≤0.05 considered significant\*.

A Kruskal-Wallis H test showed that there was a statistically significant difference in the levels of Calcium , S. Estradiol and S.Vit D3 among different Age groups, χ<sup>2</sup>= 4.471, p < 0.001, the mean levels of Calcium and S.Vit D3 were shown a positively increased levels with age while S. Estradiol was decreased with age as shown in Table 4.

Throughout the results, the quartiles from the estimated distribution of S. Estradiol and S.Vit D3 were a great variability with increasing age groups

**Table 4: Association between Serum levels of estradiol, vitamin D and calcium with age categories of studied patients**

Biomarkers	Age Category			P value
	44-49 years n= (45)	50-55 years n= (38)	56-65 years n= (17)	
S. Calcium mg/dl	8.55 ± 1.14	8.75 ± 1.18	10.00 ± 5.73	<0.001*
S. Estradiol pg/dl	34.53 ± 10.51	31.36 ± 11.94	33.48 ± 11.73	<0.001*
S. Vit D ng/dl	11.15 ± 5.44	12.06 ± 5.94	13.00 ± 5.92	<0.001*

Results represent mean±SD; Group 1: postmenopausal women aged 44-49 years, Group 2: aged 50-55 years; Group 3: postmenopausal women aged 56-65 years. P≤0.05 considered significant\*.

Results represent mean±SD; Group 1: non-obese BMI ≤30 kg/m<sup>2</sup>, Group 2: Obese postmenopausal women BMI >30 Kg/m<sup>2</sup> with breast cancer taken tamoxifen 20 mg on daily dose. P≤0.05 considered significant\*.

Patients with breast cancer were divided into two sub groups based on Duration of diagnosis: Group 1– Duration range ≤ 5 Years, Group 2- Duration range > 5 Years. Both S. Estradiol and S.Vit D were shown a decreased differences. Range levels of both markers were decrease parallely in patients who undergone a long duration of diagnosis. Unfortunately, these differences were not statically report since (p values were > 0.05) as shown in Table 5.

**Table 5: Association between Serum estradiol, vitamin D and calcium levels with duration of disease in studied patients**

Biomarkers	Disease duration		P value
	≤5 Years n= (70)	>5 Years n= (30)	
S. Calcium mg/dl	9.03 ± 3.01	8.51 ± 1.03	0.26
S. Estradiol pg/dl	33.70 ± 11.46	31.84 ± 10.85	0.58

S. Vit D ng/dl	12.23 ± 5.71	10.83 ± 5.63	0.20
Results represent mean±SD; Group 1: patients diagnosed with breast cancer below or within 5 years, Group 2: patients diagnosed with breast cancer for more than 5 years.			

Serum biomarkers levels was also examined based on the Duration of Tamoxifen treatment. Generally, patients with breast cancer were shown a decreasing range levels of Calcium , S. Estradiol and S.Vit D when comparing the duration of treatment which were less than 5 years with those who have treatment duration more than 5 years.

Furthermore, S.Vit D levels was shown a clear decreasing difference with increasing the duration of treatment. No significant difference was found with increasing the duration of Tamoxifen treatment as shown in Table 6.

**Table6: Association between serum estradiol, vitamin D and calcium levels with duration of tamoxifen treatment in studied patients**

Biomarkers	Duration of Tamoxifen treatment groups		P value
	≤5 Years n= (81)	>5 Years n= (19)	
S. Calcium mg/dl	8.96 ± 2.82	8.51 ± 1.06	0.36
S. Estradiol pg/dl	33.60 ± 11.44	31.18 ± 10.46	0.64
S. Vit D ng/dl	11.96 ± 5.69	11.16 ± 5.83	0.48
Results represent mean±SD; Group 1: patients taken tamoxifen on daily dose under or within 5 years, Group 2: patients taken tamoxifen daily over 5 years			

#### 4. Discussion

Breast cancer is the most commonly diagnosed malignancy worldwide, and the leading cause of cancer-related mortality in women, according to the American Cancer Society [31]. Since it is an estrogen-dependent tumor, its initiation and progression are highly correlated with estrogen levels, despite the fact that humans maintain a dynamic balance at various physiological stages [32, 33].

Tamoxifen is a nonsteroidal triphenylethylene compound that was the first SERM to be successfully used in the prevention and treatment of breast cancer by blocking the uptake of estrogen by the tumor cells [34]. It quickly became the treatment of choice for ER positive breast cancer, as well as for lowering the risk of breast cancer in high-risk patients [14, 35].

Hormone receptor positive tumor cells have receptors for estrogen or progesterone hormones, which can promote tumor growth in the breast. HER2 refer to human epidermal growth factor receptor 2. HER2 positive indicates that tumor cells produce a high level of a protein called HER2/neu, which has been linked to certain aggressive types of breast cancer[36]. The higher the ER and PR content in breast cancer, the higher response rate to hormonal therapy about 73%. ER expression is linked with age and menopausal status. It is more frequently detected in postmenopausal than in premenopausal women and in older than younger women with breast cancers [37]. Lymph node involvement in breast cancer

has long been recognized as an important prognostic factor. Positive axillary lymph nodes predict an increased risk of local and distant recurrence, that has a direct impact on mortality and worse prognosis. The survival rates are up to 40% lower in node-positive than node-negative patients.

The current study shows the variations in serum levels of vitamin D, calcium and estradiol in Iraqi breast cancer women on tamoxifen therapy. The results show that serum vitamin D and calcium were positively increased with age, while serum estradiol levels decreased with age. There was statistically significant change in mean serum level of estradiol, vitamin D and calcium when comparing the mean results with different age groups of postmenopausal women. A case control study carried by Gopinath et al. [38] on Indian women with test group (100) being on tamoxifen from 1-4 years and control group having breast carcinoma and not taking tamoxifen, these groups further divided into pre- and postmenopausal women. The results showed that there is no significant change in serum calcium between test and control group and so it did not have any unfavorable effect on bone. The tumor suppressor effect of tamoxifen occurs by inhibition of calcium calmodulin or calcium phospholipid activated protein kinase.

According to the body mass index, our study results revealed that increased mean results of serum calcium, estradiol and vitamin D with increasing BMI, which was in contrast to the study carried by AL-BIATI et al. [3]. Their study carried out at Baquba teaching hospital, Diyala, Iraq; showed that treatment of obese breast cancer women with tamoxifen decrease serum estradiol level in treated patients, with significant positive effect of tamoxifen on serum vitamin D level compared to negative effect of letrozole which results in significant reduction on serum vitamin D level. Other studies showed that overweight and obese people with extra adipose tissue, have a negative connection between vitamin D and BMI that may be explained by a volume-distribution effect with reduced bioavailability of fat-soluble vitamin D or it may become sequestered in adipose tissue [39].

The results of multicenter cohort study by Dignam et al. [40] on 3385 women with breast cancer, indicated that obesity was not associated with a material increase in recurrence risk or a change in tamoxifen efficacy. However, obesity is associated with less favorable disease characteristics upon diagnosis, such as bigger tumors and a greater number of affected lymph nodes. The major hypothesis emphasizes the effect of adipose tissue volume on hormone levels. High fat volume is linked with enhanced blood estrogen levels; this mediated both by androgen conversion by the aromatase enzyme in adipose fat and by a reduction in sex hormone-binding globulin, which decreases estrogen activity [41].

Sampling results in our study such as estradiol, vitamin D and calcium were compared according to duration of tamoxifen use and duration of breast cancer (below or above five-year interval), no significant difference was found between the groups ( $p$  value > 0.05), but it was found that serum levels were decreased with increased treatment or disease duration. The inverse result in Kim

et al. [42] showed that patients who received anti-hormonal therapy (tamoxifen) had significantly increased serum 25OHD levels at 6 months and 12 months when compared with baseline serum 25OHD, while decreased with chemotherapy.

Finding of Yetkin et al. [43] study stated that vitamin D when used in combination with tamoxifen reduce the proliferation of cancer cells and induce cell cycle arrest at G0/G1 phase. Depending on the expression results of p53, Bcl-2 and Bax, it was found that combination was more effective in directing the cells toward apoptosis. Therefore, to retain the anticancer effects, it is important to maintain patient serum 25(OH)D levels within the normal range [44].

## 5. Conclusion

In summary, results of this cross-sectional study demonstrated that treating postmenopausal female suffering from breast cancer with tamoxifen had significant positive influence on the hormonal level represented by estradiol (E2) and serum calcium, while having negative impact by lowering the circulating level of vitamin D (25-hydroxyvitamin D); accordingly, administration of vitamin D supplement for these patients may helped in improving prognosis and clinical outcome.

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