Diyala Journal of Medicine

ORIGINAL RESEARCH
Published: 25 April 2026
DOI: 10.26505/djm.v30i1.1364

Evaluation of the Effect of Vitamin D on Breast Cancer Development: its Association with Endocrine Functions and Bone Metabolism

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Received: 20 March 2025
Accepted: 06 November 2025
Published: 25 April 2026

Abstract

Background: Breast cancer remains one of the leading causes of morbidity and mortality among women worldwide, with incidence rates steadily increasing over the past decades. Despite significant advances in early detection and treatment modalities, identifying reliable biomarkers that can predict disease onset, progression, and patient outcomes remains a major clinical challenge. Among the various biological factors under investigation, vitamin D and calcium homeostasis have attracted considerable attention due to their critical roles in cell growth, differentiation, and immune regulation.

Objectives: Vitamin D, parathyroid hormone (PTH), estrogen, alkaline phosphatase, correlation levels between the parameters measured, and the receiver operating characteristic curve (ROC) were all examined in this study, which aims to shed light on the role of vitamin D in the development of breast cancer and its relationship to some parameters.

Patients and Methods: The patients were divided into three groups. The control group was G1; patients diagnosed early and receiving chemotherapy were G2; and patients receiving surgery or chemotherapy were G3.

Results: This study showed that, compared to the control group, the breast cancer groups had lower vitamin D levels, with statistically significant P-values for G1 and G2 for vitamin D and PTH, respectively. The fact that groups of people with breast cancer had significantly low vitamin D levels and high levels of parathyroid hormone supports the idea that vitamin D can fight cancer. Receiver operating characteristic (ROC) curve analysis underscored the diagnostic potential of vitamin D and PTH, both of which demonstrated excellent sensitivity and specificity.

Conclusion: Vitamin D and PTH were found to be factors that lead to tumor progression in breast cancer patients in this study. The idea that low vitamin D levels and high parathyroid hormone support the idea that vitamin D fights cancer.

Keywords: Breast cancer, Vitamin D, Parathyroid hormone, Calcium level.

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Introduction

There has been a steady rise in the number of diagnoses of breast cancer each year, and the disease has surpassed ovarian cancer as the second most prevalent cancer killer of women (1). Vitamin D is present in almost all human tissues and cells; it is a fat-soluble vitamin that helps maintain a healthy ratio of calcium to phosphorus. It plays a role in many biological processes, including those that cause cancer, and is the precursor of the steroid hormone 1,25-dihydroxyvitamin D (2). The correlation between vitamin D and cancer is becoming increasingly clear, making it all the more important to keep blood levels at optimal ranges for cancer prevention. A vitamin D deficiency, where serum 25-hydroxyvitamin D levels are less than 50 nmol/L, affects around 30% of adults (3). Its production entails exposure to the sun's ultraviolet-B (UVB) rays. The cholesterol precursor 7-dehydrocholesterol absorbs this radiation and transforms it into pre-vitamin D3. After that, it undergoes thermal isomerization to form vitamin D3 (4). Through the VDR, the liver enzymatically hydroxylates 25-hydroxyvitamin D (25(OH)D), which is subsequently further transformed in the kidney to 1,25-dihydroxyvitamin D2 or D3, also known as calcitriol, which has potent antiproliferative effects on breast cancer cells by inhibiting growth, cell differentiation, migration, invasion, and apoptosis (5). Epidemiological studies have linked low levels of calcitriol, the precursor to calcitriol, to an increased risk of breast cancer (6). While vitamin D is primarily responsible for maintaining normal calcium levels and osteosynthesis, it also helps keep the immune system, muscles, and nervous system in good working order (7). Vitamin D metabolites have anticancer effects on apoptosis, angiogenesis, and differentiation, the three key steps of breast tumor formation, according to multiple laboratory studies (8). Secondary sex traits, as well as the maturation and control of the reproductive system, are regulated by estrogen, the female sex hormone (9). There are three main subtypes of breast cancer tumors that are identified by the expression of specific genes: HER2 (encoded by ERBB2), PR (progesterone receptors), or ERα and ERβ (estrogen receptors) (10). Estrogens may be more carcinogenic, according to a number of epidemiological studies (11). This is supported by the fact that women with ovarian functional difficulties had a much lower incidence of breast cancer compared to those whose estrogen exposure was continuous (12). It is possible that ERRα worsens the breast cancer prognosis by increasing the disruption of VDR genomic activity (13). The hormone known as parathyroid hormone (PTH), parathormone, or parathyrin controls the gastrointestinal tract, kidneys, and bones to maintain a steady blood calcium level (14). A prohormone, this polypeptide contains 84 amino acids. A molecular weight of about 9,500 Dalton is assigned to it. To counteract its effects, the hormone calcitonin is produced (2). There is a negative correlation between vitamin D levels and parathyroid hormone (PTH), and both of these factors influence blood calcium levels (15). Parathyroid hormone (PTH) is secreted by the parathyroid gland in response to low calcium levels. It promotes the absorption of calcium from bone and the conversion of its stored form, 25-hydroxyvitamin D, into active vitamin D (1,25(OH)2D) (10). Many epidemiologic studies have shown that parathyroid hormone (PTH) increases the risk of breast cancer due to its carcinogenic and tumor-promoting properties, and since calcium is known to be an important intracellular messenger involved in cell signaling, apoptosis, and proliferation (16), it stands to reason that calcium could influence survival after breast cancer (17). Although it's present throughout the body, the ALP enzyme is most abundant in the intestines, kidneys, liver, and

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bones. If the liver sustains damage, the alkaline phosphatase enzyme will leak into the bloodstream, indicating an issue. Elevated ALP enzyme levels in the blood indicate a problem with the bones or liver (18). Some medical conditions affect bone formation and increase bone cell activity, which affects the interpretation of ALP analysis. As a result, ALP analysis is employed in the diagnosis of bone illnesses. Additionally, ALP analysis is employed for the detection of bone cancer types and the monitoring of vitamin D deficiency (19). This study aims to validate vitamin D readings by investigating the variables influencing stimulation in breast cancer patients and the link between vitamin D levels and those variables. Keeping an eye on the alkaline phosphatase (ALP) enzyme can teach us more about how the kidneys and liver work together to make active vitamin D.

Patients and Methods

Study design: The Department of Early Diagnosis of Breast Cancer at Medical City's Oncology Teaching Hospital in Baghdad, Iraq, provided all of the blood samples. Between January 2023 and January 2024, blood samples were taken. These analyses cover the newly diagnosed group, the control group, and the group receiving treatment for breast cancer. The first group comprised 40 blood samples from Iraqi women, and the second and third groups comprised 45 blood samples each. The first group represents the control group (G1). The second group includes the newly diagnosed group (G2). The third group (G3) consists of women who got the first and second dosages of chemotherapy. The women were between 25 and 45 years old before menopause.

Estimates of vitamin D, estradiol (E2), and parathyroid (PTH): Vitamin D (25-OH) ELISA kit was purchased from Bioassay Technology Laboratory, Shanghai, China. THE PTH ELISA kit was purchased from Elabscience, Wuhan, China. The Estradiol (E2) ELISA kit was purchased from R&D Systems (Minneapolis, USA). Alkaline Phosphatase (ALP) colorimetric assay kit was obtained from Abcam, Cambridge, UK. Five milliliters of blood were drawn from both healthy individuals and breast cancer patients using a syringe. After a predetermined amount of time has passed for the samples to coagulate in a gel tube, they are transferred to the separator. Next, the samples were centrifuged at 3000 rpm for 10 minutes. The serum is transferred to an Abendorf tube, a specialized storage container, once it has been separated. After being identified using the ELISA approach, components such as vitamin D, estradiol (E2), and parathyroid (PTH) can be evaluated by storing the samples at -20 °C.

Statistical Analysis

The data were calculated as the mean plus or minus the standard deviation (SD). Pearson's correlation coefficient was used to find the relationship between two continuous variables. Extremely significant results were defined as P-values below 0.001, while significant results were defined as P-values below 0.05.

Results

Levels of vitamin D, PTH, E2, and ALP in the control and breast cancer patients: Table 1 and Table 2 show the mean and SD of vitamin D, parathyroid hormone, E2, and alkaline phosphatase (ALP for the control group (G1), the early diagnostic group (G2), and the treatment group (G3) with respect to vitamin D, parathyroid hormone, estrogen, and alkaline phosphatase (ALP).

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Table 1. Compare the levels of vitamin D, PTH, E2, and ALP in the control and breast cancer patients.

Parameters	Control (mean±SD)	Ear. (mean±SD)	Chem. (mean±SD)
Vit D3 (ng/ml)	20.68±3.320	5.931±1.817	9.020±2.195
PTH (pg/ml)	67.03±12.67	165.2±19.05	139.6±19.34
E2 (pg/ml)	87.82±6.794	77.72±7.933	79.35±8.126
ALP (U/L)	101.6±8.026	108.0±7.827	106.3±7.807

Table 2. Cancer-diagnosed groups. (**** Extremely significant (P<0.0001), *** Highly significant (P<0.001), *Statistically significant (p<0.05), and ns (Non-significant)).

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold	Summary	Adjusted P Value
vit-D-G1. vs. vit-D-G2	14.74	13.54 to 15.95	Yes	****	<0.0001
vit-D-G1. vs. vit-D- G3	11.66	10.45 to 12.86	Yes	****	<0.0001
vit-D-G2. vs. vit-D- G3	-3.090	-4.298 to -1.881	Yes	****	<0.0001
PTH-G1 vs. PTH-G2	-98.18	-106.5 to -89.91	Yes	****	<0.0001
PTH-G1 vs. PTH-G3	-72.59	-80.86 to -64.31	Yes	****	<0.0001
PTH-G2 vs. PTH-G3	25.60	17.32 to 33.87	Yes	****	<0.0001
E2-G1 vs. E2-G2	10.10	6.446 to 13.76	Yes	****	<0.0001
E2-G1 vs. E2- G3	8.469	4.814 to 12.12	Yes	****	<0.0001
E2-G2 vs. E2- G3	-1.633	-5.288 to 2.023	No	ns	0.5419
ALp-G1 vs. ALp-G2	-6.367	-10.14 to -2.594	Yes	***	0.0003
ALp-G1 vs. ALP-G3	-4.653	-8.427 to -0.8793	Yes	*	0.0113
ALp-G2 vs. ALP-G3	1.714	-2.059 to 5.488	No	ns	0.5306

The results of the study showed that there was a highly significant difference in vitamin D levels between two groups of breast cancer patients and the control group. P-values were less than 0.0001 for G1 and G2 (14.74), less than 0.0001 for G1 and G3 (11.66), and less than 0.0001 for G2 and G3 (-3.090), respectively, (Figure 1). The PTH results showed that G1 and G2 (-98.18, P-value <0.0001), G1 and G3 (-72.59, P-value ~0.0001), and G2 and G3 (25.60, P-value <0.0001) showed highly significant variations in parathyroid hormone findings as seen in Figure 2. Serum ALP levels were significantly lower in G1 compared with both G2 (P ~ 0.0003) and G3 (P ~ 0.0113). No significant difference was observed between G2 and G3 (P ~ 0.5306) (Figure 3). The P-value of 0.5419 for the E2 data showed that the differences in estrogen levels between the G2 and G3 groups were not significant, while the P-value of less than 0.0001 showed that the differences between the G1 and G2 groups (10.10) and G1 and G3 (8.469) were highly significant (Figure 4).

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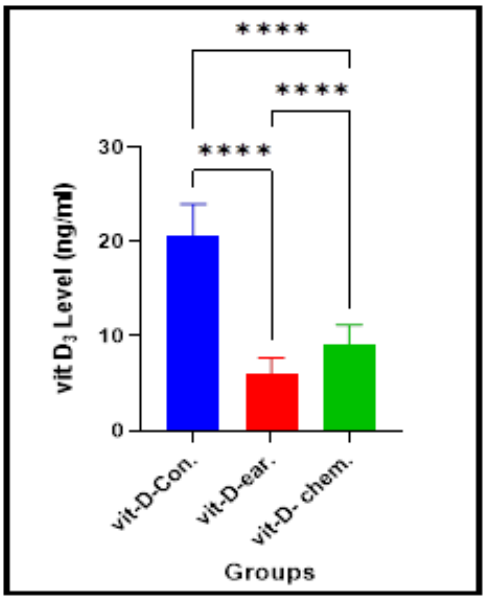

Figure 1. Diagram shows levels of vit D for patients (early and chemotherapy) and control groups (ng/ml).

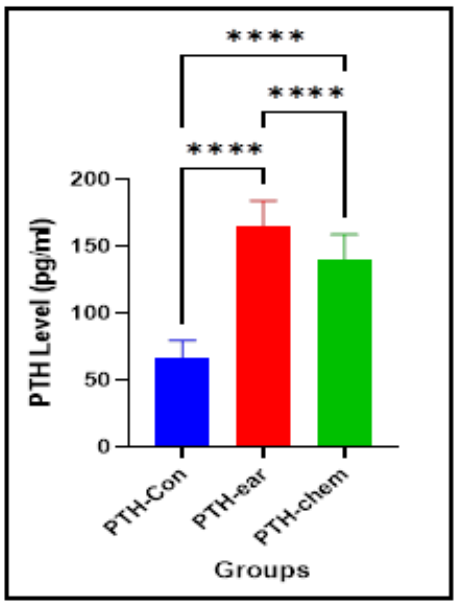

Figure 2. Diagram shows levels of PTH for patients (early and chemotherapy) and control groups (pg/ml).

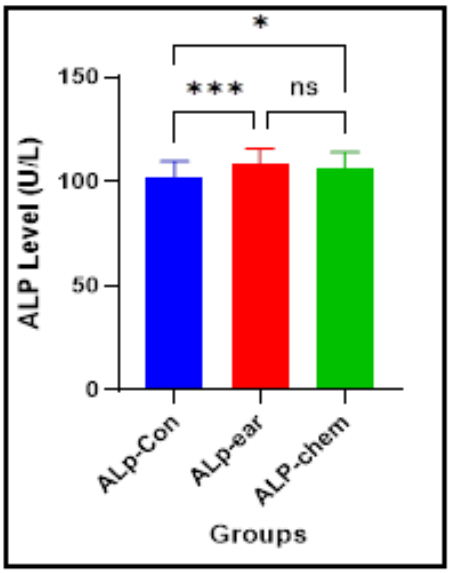

Figure 3. Diagram shows levels of ALP for patients (early and chemotherapy) and control groups (U/L).

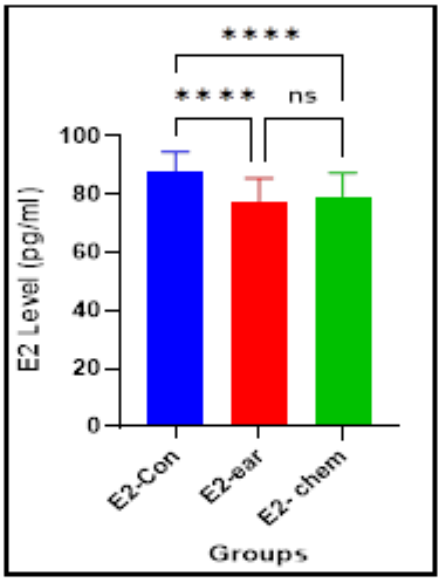

Figure 4. Diagram shows levels of E2 for patients (early and chemotherapy) and control groups (pg/ml).

The correlation between vitamin D, PTH, E2, and ALP variables was examined for BC patients (G3, G2) using Pearson correlation analysis, as seen in Table 3 and represented in Figures 5, 6, 7, and 8.

The area under the curve of the ROC (AUC) for (Vitamin D, PTH, E2, and ALP) was found to be (1.000, 1.000, 0.04110, and 0.7185), respectively. Sensitivity: (100, 100, 81, and 71) and Specificity: (97, 95, 75, and 59), respectively, as shown in Table 4

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Table 3. show correlations between variables in Breast Cancer with chemotherapy (G3) and early (G2) groups. ** Correlation is significant at the 0.01 level.

Correlation coefficients between variables G3				
	vit-D-G1	**PTH-G3**	**E2-G3**	**ALp-G3**
vit-D-G3.	1	-0.18	-0.27	-0.085
PTH-G3	-0.18	1	0.05	-0.08
E2-G3	-0.27	0.05	1	0.06
ALp-G3	-0.085	-0.08	0.06	1
Correlation coefficients between variables G2				
	vit-D-G2	**PTH-G2**	**E2-G2**	**ALp-G2**
vit-D-G2.	1	-0.12	-0.06	0.02
PTH-G2	-0.12	1	-0.33	0.05
E2-G2	-0.06	-0.33**	1	0.06
ALp-G2	0.02	0.05	0.06	1

Table 4. Shows the area under the curve of the ROC, Sensitivity, Specificity of vitamin D, PTH, E2, ALP for the G2 group.

	Area	**Std. Error**	**95% confidence interval**	**P value**	**Sensitivity**	**Specificity**
vit D3	1.000	0.000	1.000 to 1.000	<0.0001	100	97
PTH	1.000	0.000	1.000 to 1.000	<0.0001	100	95
E2	0.04110	0.04110	0.7622 to 0.9233	<0.0001	81	75
ALP	0.7185	0.05149	0.6175 to 0.8194	0.0002	71	59

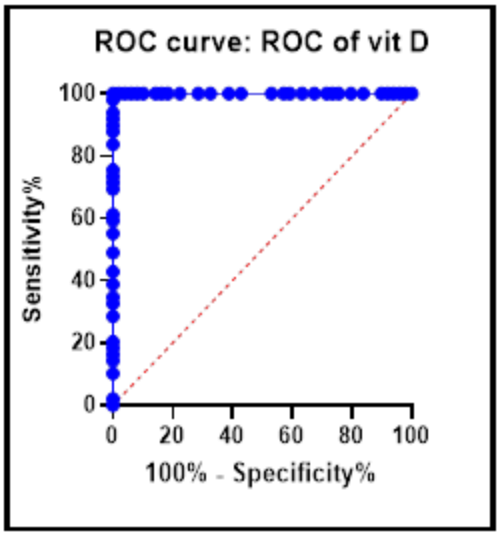

Figure 5. Receiver operating characteristic curve for vit D.

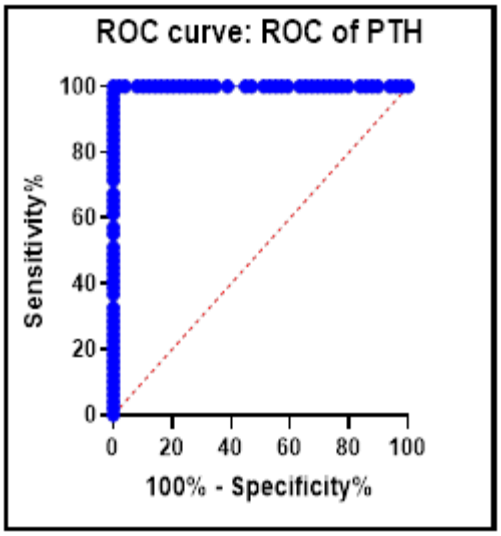

Figure 6. Receiver operating characteristic curve for PTH.

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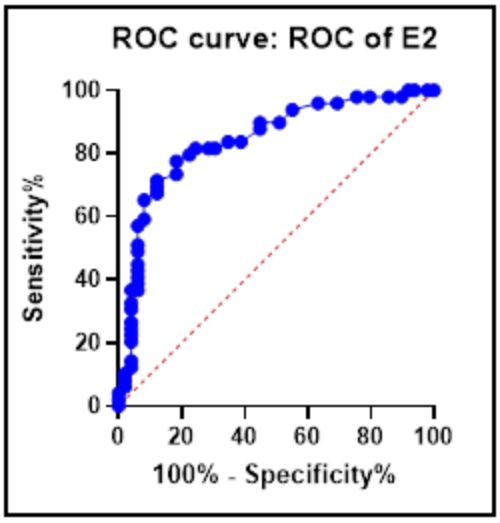

Figure 7. Receiver operating characteristic curve for E2.

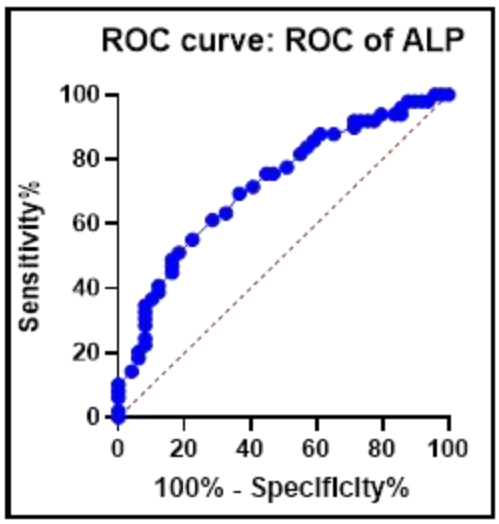

Figure 8. Receiver operating characteristic curve for ALP.

Discussion

Vitamin D levels were significantly decreased in the patient group compared to the control group. Vitamin D exerts its anti-proliferation effect by binding to the vitamin D receptor (VDR) present in various tissues and cells of the body. Many human genes contain vitamin D response elements (specific DNA sequences) that encode proteins important in regulating cell proliferation, differentiation, cell death, and angiogenesis. When serum vitamin D levels are suboptimal, these activities are impaired, and as a result, enhanced cell growth, neo angiogenesis, and cancer progression occur. Vitamin D's estrogenic action on hormone receptors is responsible for its ability to suppress breast cancer cell metastasis. The positive effects of low-dose vitamin D may be due to activation of the estrogen receptor (ER), according to some research. Breast cancer incidence in these women can be reduced by vitamin D because it lowers estrogen production and signaling and down-regulates estrogen receptor expression (20). Even while the study found that the levels were within normal ranges, it doesn't mean that estrogen isn't a factor in breast cancer. Elevated estrogen levels in postmenopausal women increase their risk of breast cancer (21). The increasing amounts of estradiol lend support to the idea that these hormones contribute to the progression of breast cancer (22). The results of the current study showed that the levels of estrogen hormone in women diagnosed with breast cancer are within the normal limits. This does not mean excluding the role of this hormone is causing the disease because it is one of the main reasons that increase the risk of breast cancer. Primary hyperparathyroidism is characterized by an increase in the size and hormone production of one or more parathyroid glands; symptoms include hypercalcemia and an elevated risk of breast cancer. As vitamin D levels and measures fall, these hormone levels rise, which is a sign of hyperparathyroidism (23). The results of the current study showed that the levels of parathyroid hormone increased in women with breast cancer compared to the control group as a response to the low levels of vitamin D in the body, and this rise increases the risk of breast cancer because the parathyroid hormone has carcinogenic and tumor-promoting effects linked to the risk of developing cancer. Patients with cancer that has spread to their bones or liver typically have elevated levels of alkaline phosphatase. Possible metastases include cancers

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of the bones, thyroid, colon, breast, prostate, and lungs. But, preexisting malignancies in particular organs and tissues can still cause ALP increases, even in the absence of metastases (24). Blood ALP levels rose dramatically with cancer stage, and total ALP was higher in breast cancer patients than in controls, according to the prior research. If the ALP level is high, it means the illness has spread to the liver or bones (18). These findings corroborated those of the current inquiry regarding breast cancer in women. Breast cancer patients' normal ALP levels in this study provide more evidence that the disease did not initially break down bone mass, and they also provide hope that ALP might be a biomarker for women whose cancer has progressed. This study found that there were statistically significant differences in the level of parameters (Vit D and PTH) in patients in the early stage compared to patients who underwent surgery and/or chemotherapy. This indicates the direct effect of these parameters on the development of the disease, as shown in Table 2. Specifically, the data showed a positive correlation between PTH and E2, and a negative correlation between vitamin D, E2, and ALP levels in the G3 group. In addition, the study found that in the G2 group, PTH and E2 were negatively associated with one another, whereas ALP levels were positively correlated with Vit D, PTH, and E2. Receiver operating characteristic (ROC) curve analysis underscored the diagnostic potential of vitamin D and PTH, both of which demonstrated excellent sensitivity and specificity. These results highlight their promise as accessible, cost-effective biomarkers for breast cancer risk assessment and monitoring.

Conclusion

In conclusion, this study provides evidence that vitamin D deficiency and elevated PTH are strongly associated with breast cancer development and progression, supporting their role as potential biomarkers. While estrogen and ALP levels were within normal ranges, their contributions cannot be disregarded, particularly in advanced disease. The results emphasize the importance of maintaining adequate vitamin D levels to mitigate cancer risk and progression. Future large-scale, longitudinal studies are warranted to validate these findings and to explore whether vitamin D supplementation could serve as an adjuvant therapeutic strategy in breast cancer management.

Source of funding: No source of funding.

Ethical clearance: Approval was obtained from the Institutional Review Board (I.R.B.) of College of Medicine for the collection of blood samples from the Early Diagnosis of Breast Cancer Department, Oncology Teaching Hospital, Medical City, Baghdad, Iraq (January 2023–January 2024).

Conflict of interest: None.

Use of Artificial Intelligence (AI): The authors state they did not use any generative AI tools for creating or editing the manuscript's language.

Acknowledgments: Researchers thank Oncology Teaching Hospital/Medical City/Baghdad/Iraq for providing facilities for sample collection and patient data collection.

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Diyala Journal of Medicine

ORIGINAL RESEARCH
Published: 25 April 2026
DOI: 10.26505/djm.v30i1.1364

تقييم تأثير فيتامين د على تطور سرطان الثدي: ارتباطه بوظائف الغدد الصماء واستقلاب العظام

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الملخص

الخلفية: لا يزال سرطان الثدي أحد الأسباب الرئيسية للمرض والوفيات بين النساء حول العالم، مع تزايد معدلات الإصابة به بشكل طردي على مدى العقود الماضية. وعلى الرغم من التقدم الكبير في الكشف المبكر ووسائل العلاج، إلا أن تحديد المؤشرات الحيوية الموثوقة التي قد تتنبأ ببداية المرض وتطوره ونتائجه على المريضات لا يزال يمثل تحديًا سريريًا كبيرًا. ومن بين العوامل البيولوجية المختلفة قيد البحث، حظي فيتامين د والكالسيوم باهتمام كبير نظرًا لأدوارهما الحيوية في نمو الخلايا وتمايزها وتنظيم المناعة.

الأهداف: دُرست في هذه الدراسة فيتامين د، وهرمون الغدة الدرقية (PTH)، والإستروجين، والفوسفاتيز القلوي، ومستويات الارتباط بين المعايير المقاسة، ومنحنى خاصية التشغيل المُستقبِل (ROC). تهدف هذه الدراسة إلى تسليط الضوء على دور فيتامين د في تطور سرطان الثدي وعلاقته ببعض المعايير.

المرضى والطرق: صنفت G1 كمجموعة تحكم أما المريضات اللاتي شُخِّصن مبكرًا وتم يخضعن للعلاج الكيميائي في المجموعة G2 أما المريضات اللاتي خضعن للجراحة أو العلاج الكيميائي في المجموعة G3.

النتائج: أظهرت هذه الدراسة أنه مقارنةً بالمجموعة الضابطة، انخفضت مستويات فيتامين د في مجموعات سرطان الثدي بشكل ملحوظ إحصائيًا. وكانت القيم الاحتمالية (P) ذات دلالة احصائية بين المجاميع G1 وG2 وG1، على التوالي، لفيتامين د وهرمون الغدة جار الدرقية. ويدعم انخفاض مستويات فيتامين د بشكل ملحوظ وارتفاع مستويات هرمون الغدة جار الدرقية لدى مجموعات المصابين بسرطان الثدي فكرة قدرة فيتامين د على مكافحة السرطان. أكد تحليل منحنى خصائص تشغيل المُستقبِل (ROC) على الإمكانات التشخيصية لفيتامين د وهرمون الغدة جار الدرقية، حيث أظهر كلاهما حساسيةً وخصوصيةً ممتازتين.

الاستنتاج: وُجد في هذه الدراسة أن فيتامين د وهرمون الغدة جار الدرقية (PTH) من العوامل المؤدية إلى تطور الورم لدى مريضات سرطان الثدي. وتدعم فكرة أن انخفاض مستويات فيتامين د وارتفاع هرمون الغدة جار الدرقية أن فيتامين د يمكنه محاربة السرطان.

الكلمات المفتاحية: سرطان الثدي، فيتامين د، هرمون الغدة الدرقية، مستوى الكالسيوم.

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تاريخ الاستلام: ٢٠ اذار ٢٠٢٥

تاريخ القبول: ٦ تشرين الثاني ٢٠٢٥

تاريخ النشر: ٢٥ نيسان ٢٠٢٦

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