Relationship Between Serum 25-Hydroxy Vitamin D Level and Breast Cancer Prognostic Factors

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Abstract- Vitamin D deficiency plays an important role in the development of various diseases, including cancer. Regarding the high prevalence of breast cancer and vitamin D deficiency in Iran, this study aimed to investigate the relationship between vitamin D deficiencies and prognostic factors in breast cancer. This descriptive-analytical cross-sectional study was performed from March 2015 to March 2017 at Imam Reza hospital in Kermanshah city, Iran. 145 breast cancer patients with pathologic confirmation and before the neoadjuvant or adjuvant treatments were included by simple and convenient sampling. Serum 25(OH) D levels were measured in all patients before receiving treatment. The collected data were analyzed by using SPSS software (version. 20), and the relationship between the levels of 25(OH) D and the studied factors was assessed by inferential statistical tests in each group. The results showed that there was a statistically significant direct relationship between serum vitamin D levels and some factors, including age, ER, and PR, but a significant inverse relationship was observed between serum vitamin D levels and the level of ki67 and metastasis. There was no statistically significant relationship between the mean serum level of vitamin D and tumor grade and P53 receptor, but high levels of vitamin D were associated with low-grade tumors and P53 negativity. According to the findings, lower levels of vitamin D can be associated with higher levels of ki67 and P53, higher-grade breast cancer, a higher rate of metastases, as well as lower percentage of hormone receptor positivity.

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Introduction

Breast cancer is the most common malignancy among women and is known as the main cause of cancer mortality in females worldwide (1-3). Breast cancer risk factors can be divided into two groups. The first group includes a wide spectrum of risk factors, which cannot be changed or changes that seems hard, like genetic factors. The second group includes potentially modifiable risk factors like lifestyle and environmental factors (4,5). Studies indicate that various risk factors, including vitamin D, could be associated with breast cancer progression (6). Recently, vitamin D has been noted as a breast cancer risk factor for cancer prevention. The serum vitamin D levels (≥45 ng/mL) may protect against breast cancer because breast cancer chemoprevention drugs such as tamoxifen, raloxifene, estrogen receptor modulators, and aromatase inhibitor have high toxicities and are not effective in the aggressive estrogen receptor-negative (ER−) cancers (7-9).

Vitamin D is a lipid-soluble substance that plays an important role in the metabolism and various functions of the body, including calcium absorption and bone metabolism, muscle function, cellular regulation, and immune system function. A major source of vitamin D is sunlight because the conversion of 7-dehydrocholesterol

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to pre-vitamin D_3 in the skin requires UV-B radiation. In the human body, vitamin D_3 is activated by two metabolic steps. First is converted into 25-hydroxyvitamin D (25(OH) D) form in the liver, which forms the major circulating metabolite, then the active form of 1 and 25(OH) D is synthesized in the kidney. The activation of 25(OH) D, in addition to the kidneys in other tissues, including the breast, prostate, and colon, also takes place (10,11). The active form of vitamin D plays an important role in maintaining and regulating the balance of serum calcium levels, cell growth and cellular differentiation, cell death, indivision, and angiogenesis of tumors. Hence, vitamin D deficiency can be associated with increasing the risk of certain cancers (9).

The best indicator of vitamin D status is the measurement of serum 25(OH) D level. The amounts of 1 and 25(OH) D in the breast tissue are dependent on the availability of 25(OH) D; a low level of 25(OH) D in circulating can disrupt the production of topical 1 and 25(OH) D in breast tissue. The main functions of 1 and 25(OH) D are applied by the vitamin D receptor, which is present in healthy and cancerous breast tissue; therefore, 1 and 25(OH) D can inhibit cell proliferation and promote cell differentiation in breast tissue cells. Vitamin D and its analogs lead to interruptions in the cell cycle, induction of apoptosis, reducing the expression of estrogen and progesterone receptors, and limit the response to cell proliferation (12).

Due to the vitamin D deficiency and high prevalence of breast cancer in Iran, as well as the lack of adequate studies in the country, this study aimed to investigate the relationship between vitamin D deficiencies and prognostic factors in breast cancer.

Materials and Methods

This descriptive-analytical cross-sectional study was performed from March 2015 to March 2017 at Imam Reza hospital in Kermanshah city, Iran. In the current study, the informed consent form was signed by all the enrolled patients in compliance with the Declaration of Helsinki principles. The checklist was used to record patients' personal and clinical data, and all data was kept confidential. Participation in this research was absolutely voluntary, and non-cooperation did not cause any problems in the treatment procedure. 145 breast cancer patients with pathologic confirmation before beginning the neoadjuvant or adjuvant treatments were included in the study by simple and convenient sampling. 25(OH) D as a circulating form is the most suitable indicator of vitamin D status that was measured as the main marker. Radiotherapy and chemotherapy are the factors that affect serum levels of 25(OH) D; therefore, 25(OH) D level was measured in all subjects before receiving treatment. Sampling was done in a single laboratory to prevent laboratory controversy.

Information on the subjects and test results were recorded on the specific checklist that was specifically designed for this study. The collected data were analyzed by using SPSS software (Version 20), and the relationship between the levels of 25(OH) D and the studied factors was assessed by inferential statistical tests in each group. The normality of the data was evaluated through the Kolmogorov-Smirnov (K-S) test. $P<0.05$ was considered statistically significant.

Results

In total, 145 patients with a mean age of 48.73±12.6 were examined. The subjects' age ranged between 22 and 78 (median 46 years). Serum 25(OH) D levels are shown in Table 1. The mean level of 25(OH) D was 25.61±22.13, with a median of 11.8. The level of 25(OH) D ranged from 0 to 100.

Table 1. Classification of patients based on vitamin D status

<10 Deficiency	10-30 Insufficiency	30-100 Sufficiency	>100 Toxicity	Total
63	49	29	4	145
43.4%	33.8%	20%	2.8%	100%

In the present study, invasive ductal carcinoma was reported as the most common type of carcinoma (93.1%). Invasive lobular carcinoma and medullary carcinoma were in the next categories, respectively. Most tumors were in stage IIB. This is while stages IIA and IV were in the next categories, respectively. Most patients with breast cancer had estrogen receptor-positive (ER+). In 28.3% of cases, this variable was reported negative, also called ER-. 71% of patients with breast cancer had progesterone receptor-positive (PR+), which was similar to estrogen receptor (ER) status. 29% of subjects had progesterone receptor-negative (PR-). The human epidermal growth factor receptor 2 (HER2) was negative in most patients (60.7%), and this receptor was positive

only in 39.3% of cases. The P53 receptor was positive in most patients (69%). But it was negative in 31% of cases. Lymphovascular space invasion (LVSI) and perineural invasion (PNI) receptors were positive in 60% and 59.3% of cases, respectively.

Spearman correlation test showed that serum vitamin D level was significantly correlated with age (ρ=0.349, *P*=0.000), which means the mean vitamin D level increased with increasing age (Table 2).

Table 2. Classification of subjects' age groups based on vitamin D level

Age groups	Number	Minimum	Maximum	Mean	Std dev	*P*
≤35	14	3	55.80	12.71	13.73	
36-45	58	3	70.90	15.58	15.33	
46-55	42	3	149.5	25.28	33.93	0.000
>55	31	3	114	34.37	27.60	
Total	145	3	149.5	22.13	25.64	

According to Table 3, high levels of vitamin D significantly increase the probability of ER positivity (*P*=0.02).

Table 3. Status of ER based on vitamin D level

Estrogen receptor	Number	Minimum	Maximum	Mean	Std dev	*P*
Negative	41	3	103	14.58	18.42	
Positive	104	3	149.5	25.10	27.49	0.02
Total	145	3	149.5	22.13	25.64	

As shown in Table 4, high levels of vitamin D significantly increase the probability of progesterone receptor (PR) positivity (*P*=0.004).

Table 4. Status of PR based on vitamin D level

Progesterone receptor	Number	Minimum	Maximum	Mean	Std dev	*P*
Negative	42	3	103	14.80	18.04	
Positive	103	3	149.5	25.12	27.68	0.004
Total	145	3	149.5	22.13	25.64	

According to Table 5, no statistically significant relationship was reported between the mean serum level of vitamin D and the HER2 (*P*=0.829).

Table 5. Status of HER2 based on vitamin D level

HER2	Number	Minimum	Maximum	Mean	Std dev	*P*
Negative	88	3	114	21.90	23.10	
Positive	57	3	149.5	22.49	29.35	0.829
Total	145	3	149.5	22.13	25.64	

There was no statistically significant relationship between the mean serum level of vitamin D and the P53 receptor (*P*=0.074), but in the group with negative P53, the serum vitamin D level was higher (Table 6).

According to Table 7, no statistically significant relationship was observed between the mean serum level of vitamin D and tumor grade (ρ=0.145, *P*=0.194), but higher vitamin D level was associated with lower tumor grade.

No statistically significant relationship between the mean serum level of vitamin D and tumor stage (*P*=0.244). Also, according to Spearman's correlation coefficient and Spearman's rank table, the relationship between the mean serum level of vitamin D level and

tumor size was not significant (*P*=0.06).

Table 6. Status of P53 receptor based on vitamin D level

P53 receptor	Number	Minimum	Maximum	Mean	Std dev	*P*
Negative	45	3	89.60	22.63	19.73	
Positive	100	3	149.5	21.90	27.99	0.074
Total	145	3	149.5	22.13	25.64	

Table 7. Tumor grade based on vitamin D level

Tumor grade	Number	Minimum	Maximum	Mean	Std dev	*P*
Grade 1	12	4.30	149	30.44	37.43	
Grade 2	83	3	149.5	23.21	27.01	0.194
Grade 3	50	3	87.4	18.34	19.06	
Total	145	3	149.5	22.13	25.64	

The relationship between the mean serum level of vitamin D and metastasis was statistically significant (*P*=0.017), so the serum level of vitamin D was higher in the group without metastasis (Table 8).

Table 8. Tumor metastasis based on vitamin D level

Tumor metastasis	Number	Minimum	Maximum	Mean	Std dev	*P*
Negative	29	15	14.19	2.6	29	
Positive	116	23.9	27.5	2.5	116	0.017
Total	145					

As shown in Table 9, there was no statistically significant relationship between the mean serum level of vitamin D and the LVSI receptor (*P*=0.355).

Table 9. Status of LVSI receptor based on vitamin D level

LVSI receptor	Number	Minimum	Maximum	Mean	Std dev	*P*
Negative	58	3	149.5	20.48	26.21	
Positive	87	3	140	23.23	25.35	0.355
Total	145	3	149.5	22.13	25.64	

No significant relationship (*P*=0.375) was reported between the mean serum level of vitamin D and the PNI receptor (Table 10).

Table 10. Status of PNI receptor based on vitamin D level

PNI receptor	Number	Minimum	Maximum	Mean	Std dev	*P*
Negative	59	3	149.5	21.07	27.53	
Positive	86	3	140	22.85	24.40	0.375
Total	145	3	149.5	22.13	25.64	

The mean serum level of Ki67, the factor in cell cycle regulation, was 25.45±22.14. This factor had a minimum of 0 and a maximum of 90. In most of the patients with the Ki67 greater than or equal to 2, the mean of vitamin D was 49.63 (ng/ml). For most subjects, the Ki67 level and the mean of vitamin D were reported to be 3-20 and 22.20 (ng/ml), respectively. In patients with the Ki67 above 20, the mean serum level of vitamin D was reported as 18.8 (ng/ml).

Kruskal-Wallis test proved the significant inverse relationship between the Ki67 molecular marker and vitamin D (*P*=0.007). This means that increasing the

serum level of vitamin D decreased the Ki67 level (Table 11).

Table 11. Vitamin D level and Ki67 classification

Ki67	Number	Mean	Std dev	*P*
<=2	6	50.41	49.63	0.007
3-20	86	22.20	22.80	
>20	53	18.81	25.11	
Total	145	22.13	25.64	

According to the other classification (Table 12), the results showed that the Ki67 was less than or equal to 20 in most of the patients. Mann-Whitney U test indicated that the level of vitamin D was significantly correlated with the Ki67 level (*P*=0.026). The Ki67 decreased with decreasing the serum vitamin D level.

Table 12. Vitamin D level and Ki67 classification

Ki67	Number	Mean	Std dev	*P*
<=20	92	24.04	25.89	0.026
>20	53	18.81	25.11	
Total	145	22.13	25.64	

Most of the patients had invasive ductal carcinoma, and metaplastic carcinoma was the least common type of tumor in the statistical population. Due to the lack of some pathologies, statistical analysis was not possible to determine the relationship between vitamin D levels with pathology.

Discussion

Breast cancer risk factors can be divided into two groups. The first group includes a wide spectrum of risk factors, which cannot be changed or changes that seems hard, like genetic factors. The second group includes potentially modifiable risk factors like lifestyle and environmental factors (4,5). Lifestyle modification, exercise, and the use of antioxidants are known as potentially modifiable risk factors associated with breast cancer.

In this study, the mean and standard deviation of serum 25(OH) D level was 25.6±0.22 with a median of 11.8 and a range from 3 to 149.5. Invasive ductal carcinoma was the most common type of carcinoma, with a rate of 93.1%; invasive lobular carcinoma and metaplastic carcinoma were in the next categories, respectively. ER was positive in most women with breast cancer but was negative in 41 cases. PR was positive in 71% of women but was reported negative in 29% of subjects. The HER2 was negative in most women (60.7%) and only reported positive in 57 subjects. The results showed that there was a statistically significant direct relationship between serum vitamin D levels and some factors, including age, ER, and PR, but a significant inverse relationship was observed between serum vitamin D levels and the level of ki67 and metastasis. There was no statistically significant relationship between the mean serum level of vitamin D and tumor grade and P53 receptor, but high levels of vitamin D were associated with low-grade tumors and P53 negativity. It seems that a low level of 25(OH) D is associated with a poor prognosis, while a high level of vitamin D can be considered a protective agent against cancer.

Various studies have been conducted to determine the relationship between plasma concentrations of 25(OH) D and the risk of breast cancer, which reported different results. According to the Anderson results, no associations were found between calcium or vitamin D intakes with breast cancer incidence. However, supplemental vitamin D intake was independently associated with reducing the risk of breast cancer (13). The results from the French E3N cohort study suggest that the benefits of vitamin D intake are modulated by ultraviolet (UV) exposure (14). McCullough reported the inverse association between the intake of calcium with the risk of postmenopausal breast cancer (15). The findings of Kim's study suggest that vitamin D deficiency is a risk factor for recurrence in breast cancer patients, especially those with hormone receptor-positive (16). Robien reported that daily vitamin D intake could be associated with a small decrease in breast cancer risk among postmenopausal women (17). The results of the Vrieling study suggest that lower serum 25(OH) D level in postmenopausal breast cancer patients is associated with poorer overall survival (18). Another study showed that adequate vitamin D levels might prevent breast

cancer development. The optimal level for breast cancer prevention was reported as >or=40 ng/mL (19).

The Ki67, as a proto-oncogene, is activated in the cell proliferation process and plays an important role in prognosis and response to chemotherapy. Investigating the Ki67 gene expression is recommended in patients with breast cancer by the immunohistochemistry method (20). In the present study, the mean of Ki-67 level, as a factor in cell cycle regulation, was 25.45, with a standard deviation of 14.22. The highest level and the lowest level of serum Ki-67 were 90 and 0, respectively.

Kawase's study showed a significant inverse association between the intake of vitamin D and calcium with the risk of breast cancer. The findings of Kawase's study suggest that calcium and vitamin D intakes decrease the risk of breast cancer and that association may differ based on receptor status and menopausal status (21). In Eliassen's study, there was no significant association between plasma 25 (OH) D levels and the risk of breast cancer. The results did not change when restricted to women who were premenopausal. Results were similar between ER+/PR+ and ER-/PR- tumors. This association did not vary by the season or patient's age in the blood collection time but did vary when stratified by body mass index (BMI) (22). Of note, the differences in the results of the mentioned studies may be due to the differences in the population studied or the method of measuring the concentration of 25(OH) D.

In recent years, expertise in breast cancer cytopathology and cytologic grading have developed. Tumor grading is routinely done on histologic specimens as a prognostic factor. The use of breast cytology is frequently limited to differentiating between benign and malignant lesions. However, this method can provide additional information about tumor grade and its prognosis. Complete information from cytological samples leads to better decisions and improves the treatment process. Therefore, this information can be assimilated into the pre-operative plan to determine the biological behavior of the tumor and avoid a blind therapeutic approach (23).

Our findings showed that vitamin D plays an important role in the prognosis of breast cancer. The deficiency of vitamin D was accompanied by a worse prognosis for breast cancer. The major estimated prognosis factors in the current study were hormone receptors negativity (ER-/PR-). A significant direct relationship was observed between the serum vitamin D level and ER and PR receptors. In the group with higher vitamin D levels, metastasis was significantly lower. The Ki67 molecular factor, which is a sign of tumor cell proliferation and tumor growth rate, was significantly lower in subjects with higher levels of vitamin D than those with lower serum vitamin D levels. There was no statistically significant relationship between the mean serum level of vitamin D and tumor grade and P53 receptor, but based on the results, lower levels of vitamin D are associated with high-grade breast cancer and high levels of P53. In this study, a statistically significant direct relationship was reported between serum vitamin D levels with age. Younger patients had lower levels of vitamin D. In other words, vitamin D deficiency can be associated with lower age of breast cancer incidence. These findings suggest that vitamin D deficiency is associated with aggressive cancers and the incidence of breast cancer in lower ages. Due to the severe deficiency of vitamin D in the country, precise planning to correct this deficiency in the community effectively and also carry out more extensive studies is needed.

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