Association of Vitamin D Deficiency and Inflammatory Cytokines with the Clinicopathological Features of Breast Cancer in Female Saudi Patients

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Abstract

Background: Breast cancer is considered to be the most common leading cause of cancer related death among women in Saudi Arabia. Many researches supposed a strong correlation between vitamin D and different types of cancer.

Patients and methods: We aimed to study the implication of serum vitamin D, calcium, interleukin 6 (IL6), tumor necrosis factor alpha (TNF-α) and chemerin on progression of breast cancer. One hundred female Saudi patients were included in the current research and we assessed their serum levels of vitamin D, calcium, IL-6, TNF-α and chemerin.

Results: vitamin D was significantly decreased in tumors with high grade ($P < 0.0001$), patients with obesity ($P = 0.013$), negative estrogen receptors (ER) ($P < 0.0001$), negative progesterone receptors (PR) ($P < 0.0001$) and positive HER2 receptors ($P < 0.0001$). It was also decreased in large tumors ($P < 0.0001$), patients with axillary lymph node involvement ($P < 0.0001$) and in the patients with advanced clinical stage ($P < 0.0001$). Moreover, higher levels of serum IL-6, TNF-α, chemerin were significantly related to breast cancer and its advanced stages.

Conclusion: vitamin D deficiency and elevated inflammatory cytokines (IL6, TNF-α and chemerin) are associated with breast cancer progression in female Saudi patients.

Introduction

Breast cancer has been recognized as the leading cause of cancer-related death in women worldwide, and it constitutes around one-third of all female malignancies [1]. In 2018, nearly 2,088,000 new breast cancer cases were diagnosed, and 6,260,000 deaths from breast cancer were recognized [2]. It is the most common malignant tumor in Saudi women with a prevalence rate of 21.8 %; it represents the ninth cause of death among them [3].

Vitamin D deficiency is considered communal health distress worldwide. In Saudi Arabia, vitamin D deficiency is around 60%. Its occurrence was reported in different ages and both gender among the Saudi population [4, 5]. Serum 25(OH)D levels were determined as the best biomarker to measure vitamin D status due to its greater half-life and stability in circulation versus the active form of vitamin D [6]. The deficiency of vitamin D was defined as serum 25(OH)D level of ≤ 20ng/ml, vitamin D insufficient as 20-30ng/ml, and vitamin D sufficient as >30ng/ml [7].

Substantial scientific evidence has been revealed a strong relationship between vitamin D and Cancer [8]. The protective role of vitamin D against breast cancer was supported by the results of a recent study that proved a significant relationship between low serum vitamin D and high risk of breast cancer [7]. The protective effect of calcium against breast cancer risk is thought to be majorly triggered by chemo-preventive action of the active form of vitamin D, which is the major regulator of calcium homeostasis [9].

Vitamin D deficiency may lead to uncontrolled metabolic syndromes that result in carcinogenesis [10]. The biologically active form of vitamin D ($1,25(OH)_2D_3$) was produced in the kidney by the action of 1-alpha hydroxylase (CYP27B1). CYP27B1 is produced by other tissues and immune cells that can regulate their local amounts of active vitamin D through it [11]. The expression of CYP27B1 in the immune cells is triggered either by the presence of the cytokines directly [12] or through immune regulatory mechanisms associated with vitamin D receptor activation [13].

The deficiency of vitamin D may result in immune cell dysfunction [14] and cytokine variability [15, 16]. It was noted that adequate levels of 25(OH)D are associated with increasing levels of IL-4 and IL-10 and decreasing the pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α. Vitamin D is down-regulating the expression and production of these pro-inflammatory cytokines [17].

The effect of pleiotropic cytokines on cancer development was established. The treatment efficacy and prognosis in malignant diseases were assessed by the disease stage and by the function of immune system pathways mediated by IL-6, IL-8, and TNF-α [18, 19].

The inflammatory pathogenic environment of breast cancer may represent an additional mechanism that may associate the chemerin expression with breast cancer [20]. Chemerin is an adipocytokine regulating different immunological and biological functions. Chemerin exerts most of its effects through the chemokine-like receptor 1 (CMKLR 1), expressed by many cells [21. 22]. It possesses variable links with inflammation and metabolism of the cells as well as cellular differentiation. Chemerin release is governed by various mediators as vitamin D, retinoid, and corticosteroids, in addition to the cytokines that regulate acute and chronic inflammation [23].

Chemerin was linked to the pro-inflammatory cytokines that mediate the cytotoxic cellular immunity as IL-6, Il-8, and TNF-α; they were considered as inflammatory promoters for breast carcinogenesis [24, 25].

Through the prevalent anticancer actions and specific signaling pathways of active vitamin D in breast cancer and according to epidemiological evidence, vitamin D may have a protecting role against breast cancer. Furthermore, this meta-analysis revealed a significant dose-response association between circulating vitamin D and overall survival in breast cancer patients [26].

In the currents study, we aimed to evaluate the association of vitamin D deficiency and some inflammatory cytokines, with the progression of breast cancer in Saudi female patients.

Patients And Methods

Study design and population

This is a case control study, was conducted at the College of Applied Medical Sciences, Taif University, Taif. It included one hundred Saudi female patients with early or locally advanced breast cancer. The patients were recruited from King Faisal Hospital in Taif city, during the period between January 2020 to September 2020. All patients were histopathologically confirmed and clinically staged based on the American Joint

Committee of Cancer (AJCC) staging system [27]. Their ages ranged between 20 and 80 years. One hundred healthy females free from any cancer diagnosis were included as controls. They were matched with patients by age and residence area and not receiving vitamin D supplementation.

Inclusion criteria: female patients early diagnosed or locally advanced breast cancer and not metastasized.

Exclusion criteria: we excluded the following: patients receiving any vitamin D supplementation or anti-cancer (hormonal, chemo- or radiotherapy), patients with other types of cancer or metastasis.

Before entry in the study, All patients had a careful history, physical examination, complete blood picture (CBC), liver and kidney function tests, chest X-ray, pelvic abdominal ultrasonography, echocardiogram, magnetic resonance imaging of both breasts, and isotopic bone scan. Hormone receptors estrogen, progesterone, and HER2 receptors were determined by immunohistochemistry using SP1, 1E2 and 4B5 (Ventana) antibodies, respectively. The weight (kilograms) and height (meters) were measured to calculate the body mass index (BMI) by the equation = Weight (kg)/ height (m^2).

Blood samples

We obtained 5 ml of venous blood from all included participants.
The samples remained at room temperature for 1 hour to clot in the serum separator tubes and centrifuged for 5 minutes at 3000 rpm. The sera were collected and stored at -20°C until analysis.
Informed written consent was taken from each participant in addition to the approval of the Research Ethics Committee of Taif University (approval no.
42 – 0010).

Methods

Estimation of serum 25(OH)D level by enzyme-linked immunosorbent assay (ELISA)

25(OH)D level was estimated in serum samples from all included females using Abcam human vitamin D ELISA kit, USA (Cat No: ab213966) conferring in the protocol manufacturers. The range of detection was 0.5 – 1010 ng/mL, and the sensitivity of the assay was 1.98 ng/mL Serum vitamin D level less than 20 ng/ml was considered deficient.

Estimation of serum interleukin-6 (IL 6), tumor necrosis factor (TNFα) and chemerin levels by ELISA

Serum levels of IL 6 were estimated in all participant samples by ELISA kit (MyBioSource, USA; Cat No MBS261259), with detection range 300 – 4.7 pg/ml and a sensitivity up to 1pg/mL. The serum TNF-α levels were assayed using the Abcam human TNF-α ELISA kit, UK (ab181421), with a sensitivity 4.32 pg/mL and detection range 15.63–1000 pg/ml. The serum chemerin concentration was quantified using Abcam human chemerin ELISA kit (ab155430) with sensitivity 0.5 ng/ml and detection range 0.51–50 ng/ml, according to the manufacturer's guidelines.

Estimation Of Serum Calcium

Serum calcium was estimated by calcium colorimetric assay kit, Abcam, USA (Cat No: ab102505) with detection range 0.4–100 mg/dL according to the manufacturer's protocol.

Statistical Analysis

Analysis of our data was performed by SPSS 10.00 software (SPSS Inc., Chicago, IL, USA). The one –way analysis of variance (ANOVA) and the Student's t-test were considered to evaluate the statistical significance between different variables. Pearson correlation coefficient was used to assess the association between vitamin D and other studied parameters. P values were considered statistically significant at < 0.05.

Results

The current study included 100 Saudi female patients with early or locally advanced breast cancer. Their age ranged between 20 and 80 years. There were 37 patients suffering from obesity. Fifty-three were post-menopausal. Infiltrative duct carcinoma (IDC) were histopathologically diagnosed in 84 patients. The Patient's characteristics are presented in Table 1.

Table 1
Patient's characteristics

Age	No.
Age group	
20–30	6
31–40	16
41–50	40
51–60	20
61–70	11
71–80	7
BMI	
Normal weight	29
Over weight	34
Patients with obesity	37
Menopausal status	
Premenopausal	47
Postmenopausal	53
Pathology	
IDC	84
ILC	16
Pathological grade	
I	24
II	27
III	24
IV	25
Estrogen receptors (ER)	
Positive	84
Negative	16
Progesterone receptors (PR)	
Positive	78

Age	No.
Negative	22
HER2 receptors	
Positive	26
Negative	74
T	
T1	25
T2	50
T3	25
N	
N0	33
N1	36
N2	31
TNM stage	
I	13
IIA	27
IIB	27
IIIA	33

The serum levels of Vitamin D, Calcium, IL-6, TNF-α and Chemerin in the patients and healthy controls

The serum level of vitamin D was reduced in the patients compared with controls. The mean level in the patients was 16.44 ± 4.73 ng/ml compared to 37.01 ± 3.69 ng/ml in the controls. This reduction was statistically significant (Student's t-test = 25.88, $P < 0.0001$).

The serum calcium was also reduced in patients relative to the controls with a mean value of 8.91 ± 1.46 mg/dl versus 10.85 ± 83 mg/dl. This difference was statistically significant ($t = 4.9$, $P < 0.0001$).

In the studied patients IL-6, TNF-α and Chemerin were significantly elevated in the serum compared with the healthy controls. The mean serum level of IL-6 was 51.66 ± 12.09 pg/ml in the patients compared to 4.65 ± 1.78 pg/ml in the controls ($t = 15.06$, $P < 0.0001$). The mean value of TNF-α was 42.15 ± 18.76 pg/ml the patients versus 5.54 ± 2.32 pg/ml in the controls ($t = 19.26$, $P < 0.0001$) and the Chemerin mean value was 308.2 ± 100.71 ng/ml in the patients compared to 177.9 ± 36.98 ng/ml in the controls ($t = 11.21$, $P < 0.0001$). (Fig. 1).

The relations between serum levels of Vitamin D, Calcium, and different clinical and pathological features

The serum level of vitamin D was analyzed according to the different clinical and pathological features. There was no statistically significant relationship between the serum vitamin D level and the patients' ages or their menopausal status, and the histopathological type of tumor. On the other hand, the serum level of vitamin D was significantly reduced in tumors with high grade ($P < 0.0001$), patients with obesity ($P = 0.013$), negative estrogen receptors (ER) ($P < 0.0001$), negative progesterone receptors (PR) ($P < 0.0001$), and positive HER2 receptors ($P < 0.0001$). It was also decreased in large tumors ($P < 0.0001$), patients with axillary lymph node involvement ($P < 0.0001$) and in the patients with advanced clinical stage ($P < 0.0001$) (Table 2). The reduced Vitamin D level was significantly associated with bad prognostic features in breast cancer. The study of the serum calcium levels in our included breast cancer patients revealed no significant relation with the different clinical and pathological features.

Table 2

The relations between the serum levels of vitamin D and calcium, and different clinical and pathological features

	No.	Vitamin D		P	Calcium		P
		Mean	SD		Mean	SD	
Age group*							
20–30	6	**14.97**	**5.76**	**0.768**	**8.13**	**1.65**	**0.485**
31–40	16	**16.69**	**4.87**		**7.99**	**1.52**	
41–50	40	**15.95**	**4.61**		**8.45**	**1.47**	
51–60	20	**17.53**	**4.79**		**8.86**	**1.1**	
61–70	11	**17.21**	**5.52**		**8.21**	**1.27**	
71–80	7	**15.55**	**3.967**		**7.97**	**1.73**	
BMI*							
Normal weight	29	**16.76**	**3.45**	**0.013**	**8.89**	**1.12**	**0.087**
Over weight	34	**15.54**	**4.34**		**8.39**	**1.38**	
Patients with obesity	37	**13.21**	**6.23**		**8.12**	**1.59**	
Menopausal Status**							
Premenopaus	47	**16.09**	**4.73**	**0.498**	**8.32**	**1.51**	**0.709**
Postmenopaus	53	**16.74**	**4.82**		**8.43**	**1.34**	
Pathology**							
IDC	84	**16.67**	**4.72**	**0.175**	**8.38**	**1.46**	**0.982**
ILC	16	**14.94**	**4.24**		**8.37**	**1.24**	
Histopathological grade*							
I	24	**21.7**	**3.62**	**< 0.0001**	**8.85**	**1.4**	**0.316**
II	27	**17.64**	**2.25**		**8.18**	**1.54**	
III	24	**14.7**	**3.19**		**8.24**	**1.25**	
IV	25	**11.7**	**2.97**		**8.26**	**1.43**	

*One way ANOVA, **Student t-test.

	No.	Vitamin D		P	Calcium		P
Estrogen receptors (ER)**							
Positive	84	**17.63**	**4.19**	**< 0.0001**	**8.4**	**1.45**	**0.594**
Negative	16	**10.15**	**1.58**		**8.19**	**1.197**	
Progesterone receptors**							
Positive	74	**18.08**	**4.001**	**< 0.0001**	**8.45**	**1.39**	**0.376**
Negative	26	**10.59**	**1.53**		**8.17**	**1.48**	
HER2 receptors**							
Positive	26	**10.87**	**1.55**	**< 0.0001**	**8.4**	**1.45**	**0.594**
Negative	74	**18.39**	**3.88**		**8.19**	**1.19**	
T*							
T1	25	**21.61**	**3.95**	**< 0.0001**	**8.88**	**1.38**	**0.115**
T2	50	**15.73**	**3.38**		**8.18**	**1.39**	
T3	25	**12.96**	**3.74**		**8.26**	**1.4**	
N*							
N0	36	**20.48**	**3.75**	**< 0.0001**	**8.32**	**1.37**	**0.334**
N1	31	**15.84**	**2.59**		**8.57**	**1.57**	
N2	33	**13.44**	**4.62**		**8.03**	**1.39**	
Stage*							
I	13	**20.48**	**3.74**	**< 0.0001**	**8.41**	**1.35**	**0.801**
IIA	27	**16.66**	**3.8**		**8.6**	**1.54**	
IIB	27	**14.76**	**2.93**		**8.29**	**1.49**	
IIIA	33	**9.75**	**1.38**		**8.26**	**1.32**	
*One way ANOVA, **Student t-test.							

The relations between the serum levels of IL-6, TNF-α, Chemerin, and different clinical and pathological features

Evaluation of the serum level IL-6 in breast cancer patients revealed that it was significantly increased in patients with old age ($P < 0.0001$), patients with obesity ($P < 0.0001$). It is increased infiltrative lobular carcinoma (ILC) ($P < 0.0001$), in tumors with high histopathological grade ($P < 0.0001$), tumors with negative estrogen and progesterone receptors ($P < 0.0001$), tumors with HER2 positive receptors ($P < 0.0001$). In

addition, it was elevated in patients with large size tumor ($P < 0.0001$), axillary lymph nodes infiltration ($P < 0.0001$), and advanced clinical stage ($P < 0.0001$).

TNF-α serum level was significantly increased in the premenopausal patients ($P = 0.029$), patients with obesity ($P = 0.0001$), in the ILC pathology ($P < 0.0001$), in high-grade tumors ($P < 0.0001$), tumors with negative estrogen and progesterone receptors ($P < 0.0001$), tumors with HER2 positive receptors ($P < 0.0001$). It was observed to significantly increase in patients with large size tumors ($P < 0.0001$), axillary lymph nodes involvement ($P < 0.0001$) and advanced tumor stage ($P < 0.0001$).

The serum level of the Chemerin was noted to be increased in patients with obesity ($P = 0.0001$), patients with ILC tumors ($P < 0.0001$), with undifferentiated tumors ($P < 0.0001$), with tumors with negative estrogen ($P < 0.0001$) and progesterone receptors ($P < 0.0001$), with positive HER2 receptors ($P < 0.0001$), with a large tumor size, axillary lymph nodes infiltration and advanced clinical stage ($P < 0.0001$) (Table 3).

Table 3
The relations between the serum levels of IL-6, TNF-α, Chemerin and different clinical and pathological features

	No.	IL-6		P	TNF-α		P	Chemerin		P
		Mean	SD		Mean	SD		Mean	SD	
Age group*										
20–30	6	24.56	19.65	< 0.0001	36.45	23.0	0.171	294.1	54.69	0.611
31–40	16	31.14	14.47		45.49	20.58		307.2	42.08	
41–50	40	41.64	13.22		45.99	16.24		292.1	47.91	
51–60	20	41.64	13.22		37.96	21.56		282.0	52.00	
61–70	11	50.28	19.94		43.07	14.69		296.0	41.17	
71–80	7	64.41	7.1		27.94	19.21		274.0	49.04	
BMI*										
Normal weight	29	29.51	12.32	< 0.0001	31.23	14.43	0.0001	231.11	47.56	0.0001
Over weight	34	43.76	15.74		38.43	16.22		271.64	50.32	
Patients with obesity	37	58.56	17.32		49.22	18.32		288.94	58.91	
Menopausal Status**										
Premeno	47	43.95	23.31	0.148	46.51	18.29	0.029	300.8	44.54	0.0606
Postmeno	53	37.09	23.61		38.28	18.68		283.1	48.75	
Pathology**										
IDC	84	34	19.84	< 0.0001	31.38	18.99	0.012	277	35.54	< 0.0001
ILC	16	73.42	10.32		44.2	18.24		369.2	14.18	
Histopathological grade**										
I	24	12.8	1.93	< 0.0001	27.15	13.77	< 0.0001	235.1	8.07	< 0.0001
II	27	27.44	6.28		30.65	16.03		268.7	14.03	
III	24	47.93	6.63		53.11	11.89		307.0	11.87	

*One way ANOVA, **Student t-test.

	No.	IL-6		P	TNF-α		P	Chemerin		P
IV	25	73.31	8.64		55.74	14.19		356.5	20.90	
Estrogen receptors**										
Positive	84	33.03	17.98	< 0.0001	39.26	18.21	< 0.0001	277.0	35.54	< 0.0001
Negative	16	78.55	5.64		59.88	12.15		369.2	14.18	
Progesterone receptors**										
Positive	74	28.91	14.89	< 0.0001	37.85	18.31	0.0004	269.5	30.74	< 0.0001
Negative	26	72.76	8.91		52.18	16.38		355.2	21.40	
HER2 receptors**										
Positive	26	72.76	8.911	< 0.0001	37.79	18.15	< 0.0001	269.5	30.74	< 0.0001
Negative	74	28.91	14.89		54.54	15.19		355.2	21.40	
T*										
T1	25	13	2.14	< 0.0001	17.63	3.69	< 0.0001	235.6	8.287	< 0.0001
T2	50	37.46	11.94		42.82	11.06		287.5	22.77	
T3	25	73.31	8.64		65.32	4.21		356.5	20.90	
N*										
N0	36	15.77	4.77	< 0.0001	20.69	5.85	< 0.0001	241.6	11.78	< 0.0001
N1	31	38.34	7.56		44.40	7.14		290.6	12.68	
N2	33	68.94	10.91		63.44	4.98		347.6	24.17	
Stage*										
I	13	11.50	0.72	< 0.0001	14.61	1.832	< 0.0001	229.2	5.996	< 0.0001
IIA	27	19.51	5.19		25.62	5.378		252.1	11.14	
IIB	27	39.99	6.63		45.91	6.339		293.4	11.18	
IIIA	33	65.53	8.86		63.44	4.979		347.6	24.17	
*One way ANOVA, **Student t-test.										

Correlations between vitamin D and Calcium, IL-6, TNF-α, and Chemerin

In the current study, we analyzed the relationship between the serum levels of vitamin D and calcium, IL-6, TNF-α, and chemerin. Pearson's correlation revealed a significant positive correlation between vitamin D deficiency and the serum levels of calcium ($r^2 = 0.298$, $P < 0.0001$), and significant negative correlation with IL-6 ($r^2 = 0.537$, $P < 0.0001$), TNF-α ($r^2 = 0.437$, $P < 0.0001$), and Chemerin ($r^2 = 0.484$, $P < 0.0001$). Figure 2.

Discussion

Breast cancer is the most prevalent malignant tumor in women [1]. Vitamin D has an anti-inflammatory effect on the micro-environment of malignant cells [28]. Many of the pro-inflammatory cytokines induced by inflammation in the tumors can accelerate tumor progression, enhance angiogenesis, and suppress cell apoptosis [1].

Prediction of prognosis is one of the cornerstones for improving the outcome of breast cancer patients. Treatment adapting is an essential component for curing patients with poor prognosis and increasing their overall survival. In the current study, we explored the relationship between the serum levels of 25(OH)D, calcium, IL-6, TNF-α, and chemerin with breast cancer. We also studied their relationship with different clinical and pathological parameters to evaluate their association with breast carcinoma progression.

The influences of vitamin D on the inflammatory mechanisms in cancer were studied, several pathways were implicated. These pathways included the relationship between immune and cancer cells by controlling and regulating cytokine levels, inhibiting the nuclear factor-kappa B (NF-k B) signalling pathways, up-regulation of Mitogen-Activated Kinase Protein 5 (MKP5), suppression of immune system cells, and inhibition of prostaglandins regulated mechanisms [29].

Analysis of our results showed a significant reduction in the serum levels of 25(OH)D and calci um in breast cancer patients compared to the controls (P 0.0001). Vitamin D reduction was significantly related to the adverse prognostic features in breast cancer as high histopathological tumor grade, large tumor size, positive axillary lymph nodes, negative estrogen and progesterone receptors, positive HER2 receptors, and advanced clinical stage (P 0.0001).

Our study supports the results of Narvaez et al. [30]; Imtiaz and Siddiqui [31] who reported that vitamin D deficiency was observed in breast cancer patients. Moreover, de Sousa et al. [32], Colston and Hansen, [33], and Shaukat et al. [34] reported that lower vitamin D levels are related to a higher risk of breast malignancies. Quiroz et al. [35] revealed that vitamin D inhibits breast carcinogenesis through enhancement of apoptosis, suppression of angiogenesis, and malignant cell division. In breast malignancies, vitamin D has a definite role through the estrogen receptors (ER) signaling pathways, as inhibition of aromatase enzyme expression in the mammary adipose tissues [36, 37]. Besides, it decreases the expression of ER in breast cancer cells [38]. Interestingly, in ER-negative tumors, Vitamin D can also; induce ER expression and the tumor response to antiestrogen drugs [39].

Therefore, vitamin D may allow a protective role against breast cancer, which could be explained by the ability of vitamin D to prevent the initiation of the malignant transformation by its anti-inflammatory, antioxidant defences, and by repairing the DNA damage [40, 41].

Our results revealed a significant decrease in the levels of serum calcium in our included patients. This observation was supported by Chen et al. that reported the protective function of calcium against breast cancer. This protective role could be explained by the chemo-preventive mechanism of active vitamin D, responsible for the regulation of calcium homeostasis [42].

One study reported that vitamin D and calcium supplementation reduce the percent of mammographic density (PMD) which is considered a risk factor for breast cancer, in premenopausal women [43]. Our results were also supported by Colston and Hansen, who observed an inverse relationship between the breast cancer risk and sunlight exposure that increases the dermal synthesis of vitamin D resulting in a reduction of the breast cancer risk [33]. On the other side, Chlebowski et al. [44] reported that there was no relation between 25(OH)D levels and breast cancer risk. Besides, vitamin D and calcium supplementation in postmenopausal women didn't reduce the risk of breast cancer among them.

In the malignant microenvironment, inflammation is considered a cornerstone as the immune cells, and cytokines have a direct influence on malignant cell growth and migration [45, 46]. In our patients, we noted a significant increase in the serum levels of IL- 6, TNFα, and chemerin compared to the controls (P ≤ 0.0001). We found a statistically significant link between these elevations and adverse prognostic parameters in breast cancer as infiltrative lobular carcinoma, high-grade tumors, negative hormone receptors (ER & PR) tumors, positive HER2 tumors, T2 and T3 tumors, axillary lymph nodes involvement (N2 & N3) and advanced TNM (IIB & IIIA) clinical stage (P ≤ 0.0001).

In agreement with our results, Shuchen et al. [47] concluded that the higher levels of serum IL-6 were significantly related to breast cancer and its advanced stages. Furthermore, these results were associated with a poor prognosis of breast cancer. On the other hand, the study of Ahmad et al. [48], revealed that high IL-6 and IL-10 levels were related to the features of good prognosis of breast cancer as small tumor size and low histopathological tumor grade. Some clinical studies reported high levels of serum TNF- α in patients with breast cancer compared to healthy women [49]. Similarly, high levels of TNF-α messenger RNA expression were found in primary breast cancer, relative to normal breast tissue [50]. Kesler et al. [51] reported that IL-6 and TNF-α levels were significantly increased in breast cancer patients compared to controls. Also, Yunfeng et al. [52] concluded that the higher levels of serum IL-6, IL-8, and TNF-α are significantly associated with the clinical stage of breast cancer and with ER and HER2 expression by the tumors. Therefore, these cytokines were considered to be possible prognostic biomarkers of breast cancer.

There are controversial data about the relationship of chemerin to cancer. In agreement with our results, Sarmadi et al. [53] revealed chemerin has been recognized in breast cancer tissues and the pro-inflammatory cytokines, IL-1β, TNF-α, IL-6, and especially interferon-γ were up-regulated the chemerin expression in cancer tissues. Wang et al. [54] reported that high chemerin levels were linked with aggressive features of squamous cell cancer of the tongue such as advanced stage and increased tumorigenesis. On the other hand, Serkan et al. [55] noted that serum chemerin level was not associated with the stage of breast cancer. Also, Parolini et al.[56] suggested that the protective role of chemerin against cancer may be through enrolment of natural killer (NK) cells. So, through increasing chemerin levels, the immune system identifies the cancer cells and combats them.

We analysed the correlation of serum levels of vitamin D with serum calcium, IL-6, TNFα, and chemerin in our series of patients. There was a significant positive correlation between serum 25(OH)D and calcium. Moreover, there was a significant negative correlation between serum 25(OH)D and IL-6, TNFα, and chemerin. BENETTI E et al. [57] suggested that vitamin D is a possible negative regulator of pro-inflammatory cytokines release. It suppresses the activation of nuclear factor- κB and therefore the transcription of its downstream pro-inflammatory cytokines.

Conclusion

We concluded that the vitamin D is the key regulator of pro-inflammatory cytokines (IL6, TNF-α and chemerin). Deficiency of vitamin D and increased inflammatory cytokines are associated with breast cancer progression.

Recommendation

Further studies are required to investigate the clinical significance of using vitamin D supplementation in early diagnosed or locally advanced breast cancer patients.

Declarations

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Conflict of interest:

None declared

Ethics

Informed written consent was taken from each participant in addition to the approval of the Research Ethics Committee of Taif University (approval no. 42-0010).

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Figures

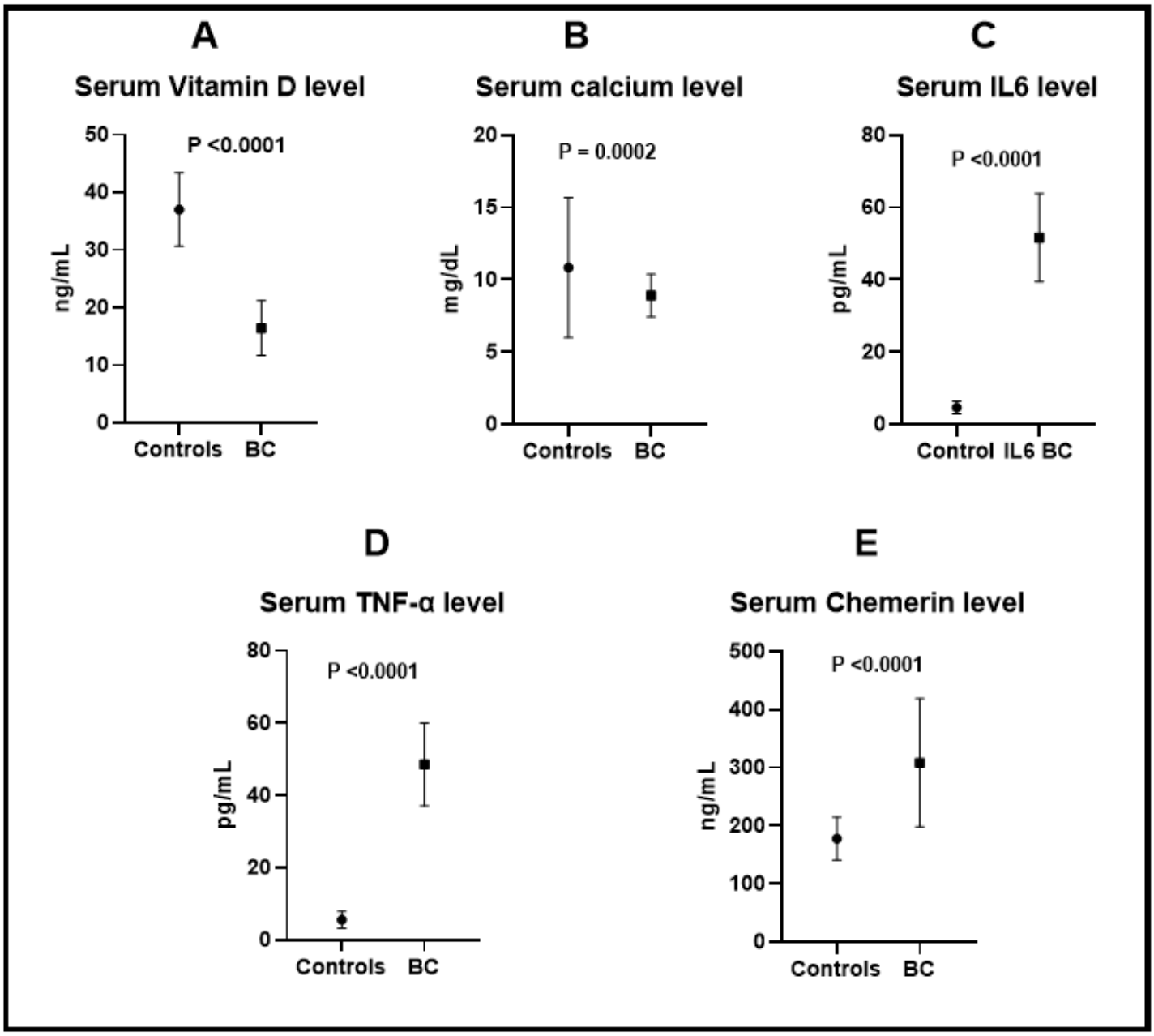

Figure 1

Represent in the serum levels of Vitamin D, Calcium, IL-6, TNF-α, and Chemerin in the breast cancer patients and the healthy controls (BC: breast cancer)

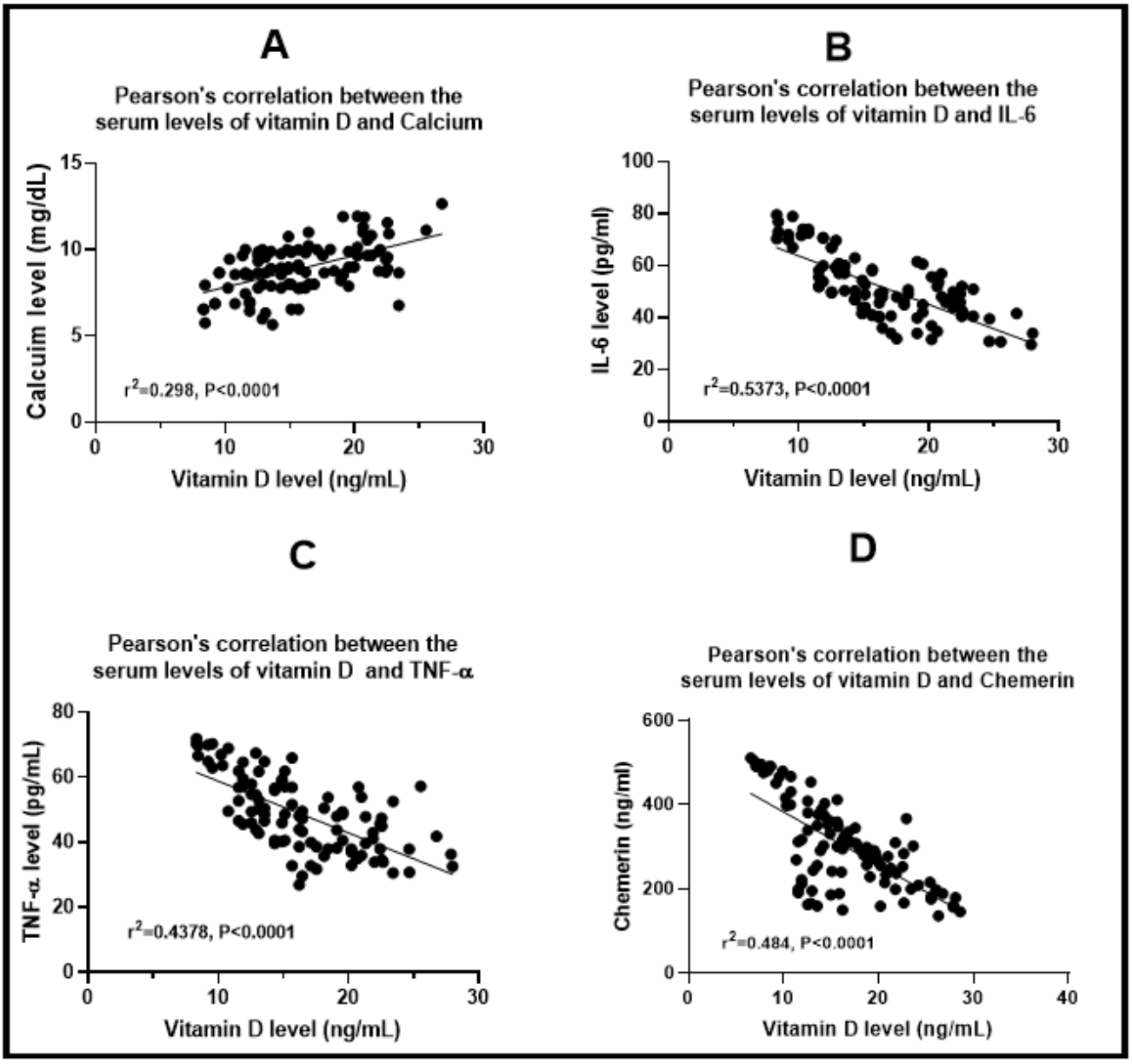

Figure 2

Pearson’s correlation between serum vitamin D levels and serum calcium (A), serum IL-6 (B), serum TNF-α (C), and serum Chemerin (D).