Original Article

Association between Serum 25-hydroxy Vitamin D Concentration and *TaqI* Vitamin D Receptor Gene Polymorphism among Jordanian Females with Breast Cancer

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Abstract

Background: Breast cancer is the most common type of cancer among females. Genetic polymorphisms might have a role in carcinogenesis. The aim of this study was to determine whether C to T base substitution within *TaqI* Vitamin D receptor (VDR) gene (rs731236) in exon 9 was a risk factor among patients with breast cancer.

Methods: Peripheral blood was drawn from 122 Jordanian breast cancer patients and 100 healthy Jordanian volunteers in Al-Basheer Hospital during the summer months (from June to November of 2013, 2014, and 2015). DNA was amplified using polymerase chain reaction (PCR), followed by *TaqI* restriction enzyme digestion. Quantification of serum 25-hydroxy Vitamin D (25[OH]D) level was determined by competitive immunoassay Elecsys.

Results: Genotypic frequencies for *TaqI* TT, Tt, and tt genotypes were 41%, 46%, and 13% for breast cancer compared to 42%, 50%, and 8% for control, respectively. Vitamin D serum level was significantly lower in the breast cancer patients (8.1 ± 0.3 ng/ml) compared to the control group (21.2 ± 0.6 ng/ml; $P = 0.001$). This study showed an inverse association between 25(OH)D serum level and breast cancer risk (odds ratio [*OR*], 22.72, 95% confidence interval [*CI*], 10.06–51.29).

Conclusions: An inverse association was found between 25(OH)D serum level and breast cancer risk. Statistical difference was also found between different VDR *TaqI* genotypes and circulating levels of 25(OH)D among Jordanian females with breast cancer.

Key words: Breast Cancer; Genotypes; *TaqI*; Vitamin D Receptor

Introduction

Nowadays, cancer turns out to be one of the most widespreading diseases. Breast cancer is the most common type of cancer among females, as well as the second leading cause of cancer mortality among women. In 2017, an estimated 255,180 new cases of invasive breast cancer are expected to be diagnosed in women in the U.S., along with 63,410 new cases of noninvasive (*in situ*) breast cancer.[1] In Jordan, breast cancer is the most common malignancy that affects women, accounting for 36.7% of all female cancers and it is the leading cause of cancer deaths among Jordanian women.[2] Risk factors associated with breast cancer include age, family history of breast cancer, ethnicity, weight, using hormone replacement therapy, and low Vitamin D intake.

Vitamin D regulates the growth and differentiation of various cell types, including the cancer cells. Vitamin D has also regulatory effects on cell death, tumor invasion, and angiogenesis.[3] Vitamin D hormone also has an important role in many metabolic pathways, including those involved in the immune responses. The actions of Vitamin D are mediated by the nuclear Vitamin D receptor (VDR), in which nearly every cell type in our bodies has receptors for Vitamin D. VDR is expressed in the normal mammary gland and plays a significant role in the development and function of the mammary gland.[4] VDR gene is located

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on chromosome 12cen-ql2, it contains 14 exons and spans approximately 75 kb of genomic DNA.[5]

Epidemiological studies demonstrated that decreased sunlight exposure, and diminished Vitamin D production by the skin, is correlated with higher breast cancer incidence and mortality.[6,7] Vitamin D status and the practice of moderate physical activity were considered protective factors for breast cancer.[8] Vitamin D supplementation, mostly taken daily and combined with calcium, was associated with a decreased postmenopausal breast cancer risk in menopausal hormone therapy users.[9] Experimental and clinical observations suggest that Vitamin D and its analogs might be effective in preventing the malignant transformation and/or the progression of various types of human tumors including breast cancer.[10] In addition, an association between low 25-hydroxy Vitamin D 25(OH)D levels and increased risk of triple-negative breast cancer was found[11] in Saudi Arabia, in which patients with 25(OH)D levels ≤25 nmol/L were 2.54 times more likely to present with triple-negative status compared to those with 25(OH)D levels >25 nmol/L.

VDR polymorphisms were associated with different cancers, and VDR polymorphisms might influence both breast cancer risk and prognosis. However, the associations between VDR gene polymorphisms and cancer risk showed controversial results according to the gene polymorphism, the race of patients, and cancer stage.[12] VDR gene has multiple gene polymorphisms in exon 2 and 3′UTR region; VDR-*FokI* (rs2228570), VDR-*BsmI* (rs1544410), VDR-*TaqI* (rs731236), and VDR-*ApaI* (rs7975232). These polymorphisms alter the polyadenylation of the VDR mRNA transcript and thus affect mRNA stability.[13] Previous studies showed no relationship between the breast cancer risk and *TaqI* gene polymorphism, while other studies showed a strong association between *TaqI* polymorphism, breast risk, and metastatic stage.[14,15] Up to our knowledge, gene polymorphism within VDR among Jordanian breast cancer females and its relation with Vitamin D activity had been never studied previously. Therefore, the aim of this study was to determine serum 25(OH)D level among Jordanian breast cancer females and to examine any association between VDR *TaqI* gene polymorphism, Vitamin D level, and breast cancer risk.

Methods

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki .Ethical approval was obtained from the Institutional Review Board (No: 14076) at the Hashemite University, and consent forms were signed by all participants before patients and control interviewing and sample collection.

One hundred and twenty-two breast cancer females were recruited from breast clinic at Al-Basheer Hospital, Amman, Jordan, during the summer time (from June to November of 2013, 2014, and 2015). Females were diagnosed by a specialized pathologist using tumor grading and staging system.[16] Blood samples were drawn at the time of diagnosis before surgery and before any chemotherapy treatment.

Age-matched, cancer-free female control volunteers, with no family history of any cancer (n = 100), were recruited as a control. Blood samples were collected (in the summer time from June to November of 2013, 2014, and 2015) from participants into two tubes; ethylenediaminetetraacetic acid (EDTA) tube and plain tube. DNA was extracted from EDTA tubes and stored at 4°C. Serum was separated from plain tubes, transferred to a microcentrifuge tube, and frozen at −60°C for Vitamin D determination.

DNA samples were amplified using a thermal cycler (BIO RAD iCycler, USA) by adding forward (5'-CAGAGCATGGACAGGGAGCAA-3') and reverse (5'-CACTTCGAGCACAAGGGGCGTTAGC-3') primers. Polymerase chain reaction (PCR) amplification reaction was carried out in 50 μl reaction volumes using Go Taq Green Master Mix and according to the manufacturer's instructions (Promega, USA). DNA samples were amplified using programmed PCR protocol: Initial denaturation step at 94°C for 3 min, followed by 30 cycles at 94°C for 45 s; 58°C for 60 s; and 72°C for 90 s, and then a reaction was carried out at 73°C for 5 min. Amplification products were electrophoresed on 2% agarose gel and stained with ethidium bromide; the amplified products were 501 bp fragment that contains the variant site. Small nuclear polymorphism *TaqI* in *VDR* gene was detected by restriction enzyme digestion using *TaqI* endonuclease digestion (New England Biolabs, USA). Digestion products were visualized under 2% agarose gel. The size of *Taq1* (TT) genotype was 494 bp, *Taq1* (Tt) genotype was 494 bp, 290bp, and 204bp and for *Taq1* (tt) genotype was 290 bp and 204 bp.

The serum level of 25(OH)D was determined according to Vitamin D Standardization Program[17] and the manufacturer's instructions by electrochemiluminescence method using Elecsys assay kit (Roche Diagnostics, France) for Beckman Coulter-Access/Access 2 Immunoassay System. The serum levels of 25(OH)D were classified into deficient (25[OH]D level ≤10.0 ng/ml), insufficient (25[OH]D level between 10 and 20 ng/ml), or optimal (25[OH]D level more than 20 ng/ml).

Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences version 17.0 and 20.0 (SPSS Inc., Chicago, IL, USA). Chi-square test was used to evaluate case-control differences for *TaqI* genotype distribution among test and control groups. A *t*-test was used to assess the significance of difference of mean 25(OH)D levels between test and control groups. Statistical significance was defined as $P < 0.05$.

Results

Table 1 shows that more than half of the breast cancer females (55.7%) were between 50 and 59 years old. Fifty-two and a half of them were in the menopausal stage and 49.2% were overweight. More than 60% of the breast cancer females had a unilateral tumor at the left side [Table 2] and more than 62% of them had Grade III.

According to the hormonal receptor status, 62.3% of the examined breast cancer females were estrogen receptor alpha positive, fifty-two and a half of them were progesterone receptor positive, and 36.1% of them were human epidermal growth factor receptor 2 (HER2) positive [Table 2].

VDR genotypic and allelic frequencies among breast cancer and control participants are shown in Table 3. The genotypes are in Hardy-Weinberg equation. There was no significant association of the VDR gene *Taq1* polymorphism with breast cancer risk among patients or healthy controls ($P = 0.460$). The frequency of TT, Tt, and tt genotypes was 41%, 46%, and 13%, respectively, for breast cancer patients compared with 42%, 50%, and 8%, respectively, for controls [Table 3].

Table 1: Age, menopausal, mammography testing, and BMI among breast cancer and healthy control groups, *n* (%)

Characteristics	Case group (*n* = 122)	Control group (*n* = 100)
Age groups		
40–49 years	34 (27.9)	18 (18)
50–59 years	68 (55.7)	50 (50)
60–69 years	20 (16.4)	32 (32)
Menopausal status		
Yes	62 (50.8)	48 (48)
Mammography testing		
Yes	122 (100)	12 (12)
BMI*		
Underweight	4 (3.3)	2 (2)
Normal weight	46 (37.7)	30 (30)
Overweight	60 (49.2)	56 (56)
Obese	12 (9.8)	12 (12)

*Underweight: BMI is <18.5 kg/m^2; normal weight: 18.5≤BMI≤24.9 kg/m^2; overweight: 25.0≤ BMI ≤29.9 kg/m^2; obese: BMI ≥30.0 kg/m^2. BMI: Body mass index.

Table 2: Neoplasm characteristics of Jordanian females with breast cancer, *n* (%)

Characteristics	Case group (*n* = 122)
Tumor side	
Unilateral right	40 (32.8)
Unilateral left	74 (60.7)
Bilateral	8 (6.6)
Grade	
1	8 (6.6)
2	38 (31.1)
3	76 (62.3)
Hormones receptor status	
Estrogen receptor positive	76 (62.3)
Estrogen receptor negative	18 (14.8)
Not examined	24 (19.7)
Progesterone receptor positive	64 (52.5)
Progesterone receptor negative	30 (24.6)
Not examined	28 (22.9)
HER2 positive	44 (36.1)
HER2 negative	38 (31.1)
Not examined	40 (32.8)

HER2: Human epidermal growth factor receptor 2.

The results of this study showed that the mean serum level of 25(OH)D for breast cancer patients (8.1 ± 0.3 ng/ml) was significantly lower than that in the control group (21.2 ± 0.6 ng/ml) (95% confidence interval [*CI*] 12.9–13.2, $P = 0.001$) [Table 4]. Breast cancer patients deficient for 25(OH)D (with <10.0 ng/ml) had 22.7-fold increased breast cancer risk [Table 5].

The study showed that there was statistically significant difference in the mean 25(OH)D levels among TT, Tt, and tt genotypes within both breast cancer patients ($P = 0.009$) and control group ($P = 0.027$) [Table 6]. TT, Tt, and tt genotypes had mean 25(OH)D level of 7.0 ± 3.5, 8.7 ± 3.0, and 9.7 ± 4.8 for breast cancer patients and 19.4 ± 5.7, 22.1 ± 6.3, and 25.0 ± 8.4 for controls, respectively.

Discussion

Breast cancer is the most common malignant affliction among women and the first cause of cancer deaths in women aged 15–54 years worldwide.[18] The origin of breast neoplasm is multifactorial; however, some factors may increase the risk of breast cancer including the age, family history, diet, presence of benign mammary disease, environment, and genetic factors related to Vitamin D level and VDR polymorphisms.[19] Vitamin D deficiency has become a major concern after the discovery of the monumental amplitude of populations blighted with it and its varied health consequences. Reports showed that most world's population are not getting sufficient amount of Vitamin D due to the current lifestyle and environmental factors that limit sunlight exposure.[20] Breast cancer research

Table 3: Association of VDR genotypic and allelic frequencies among breast cancer patients and controls with Hardy-Weinberg equilibrium

Items	Frequency, *n* (%)		χ^2	*P*
	Case (*n* = 122)	Control (*n* = 100)		
Genotype				
TT	50 (41.0)	42 (42)	1.54	0.460
Tt	56 (45.9)	50 (50)		
tt	16 (13.1)	8 (8)		
Allele				
T	156 (63.9)	134 (67)	0.46	0.500
t	88 (36.1)	66 (33)		

VDR: Vitamin D receptor.

Table 4: Mean serum levels of 25(OH)D in breast cancer patients and controls

Groups	*n*	Mean ± SE (ng/ml)	95% *CI*	*P*
Breast cancer patients	122	8.1 ± 0.3	12.9–13.2	0.001
Controls	100	21.2 ± 0.6		

25(OH)D: 25-hydroxy Vitamin D; SE: Standard error; *CI*: Confidence interval.

Table 5: Association between 25(OH)D levels and breast cancer risk

25(OH)D status*	Breast cancer (n = 122), n (%)	Controls (n = 100), n (%)	*OR*	95% *CI*
Deficient	81 (66.4)	8 (8.0)	22.72	10.06–51.29
Insufficient	40 (32.8)	70 (70.0)	0.21	0.12–0.37
Optimal	1 (0.8)	22 (22.0)	0.03	0.004–0.22

*Deficient: 25(OH)D <10 ng/ml; Insufficient: 25(OH)D between 10 and 25 ng/ml; Optimal: 25(OH)D >25 ng/ml. *OR*: Odds ratio; *CI*: Confidence interval; 25(OH)D: 25-hydroxy Vitamin D.

Table 6: Mean serum levels of 25(OH)D for each *TaqI* genotype

Breast cancer patients (n = 122)				Controls (n = 100)			
VDR *TaqI* genotype	*n*	Mean ± SE (ng/ml)	*P*	VDR *TaqI* genotype	*n*	Mean ± SE (ng/ml)	*P*
TT	50	7.0 ± 3.5	0.009	TT	42	19.4 ± 5.7	0.020
Tt	56	8.7 ± 3.0		Tt	50	22.1 ± 6.3	
tt	16	9.7 ± 4.8		tt	8	25.0 ± 8.4	

VDR: Vitamin D receptor; SE: Standard error; 25(OH)D: 25-hydroxy Vitamin D.

in the Middle East is extremely limited and genetics studies about cancer within Jordan are scarce except for few studies that screen gene polymorphism.[21-24]

To the best of our knowledge, this is a very rare study that showed the association between VDR *TaqI* genotypes or allelic frequencies among breast cancer patients and controls. This study showed no significant association between VDR *TaqI* genotypes or allelic frequencies among breast cancer patients and controls. Similar results were reported by Hou *et al.* and Yang *et al*.[25,26] among both population in Taiwan (China) and Caucasian breast cancer women. The same results were also reported by the meta-analysis from pooling 39 studies that showed no significant associations between VDR *TaqI* polymorphisms and breast cancer risk.[27]

This study showed that most breast cancer participants were Vitamin D deficient (66.4%) and this result is consistent with the previous study that is carried out by Mallah *et al*.[28] By making revisions as followed in line with the results of this study, we found a significant difference in Vitamin D serum level between breast cancer females and controls, in which breast cancer patients had remarkably lower levels despite the sufficient sun exposure supplied by Amman-Jordan's latitude 31°59'N as described by Weinstock and Moses.[29] Vitamin D deficiency might be attributed to other contributing factors including darker skin tone of the Middle Eastern population, use of sun blocks as well as avoiding performing activity in sunny areas and the dietary regimen. Two pathways for Vitamin D biosynthesis and action have been proposed in mammary carcinogenesis. The first one involves $1,25(OH)_2D$ and the second involves 25(OH)D. In the first circulating pathway, $1,25(OH)_2D$ reaches the breast tissue to exert its anticarcinogenic effect. While in the other pathway, circulating 25(OH)D reaches the breast tissue and is catalyzed to $1,25(OH)_2D$ by the 1-α-hydroxylase in the breasts. All produced $1,25(OH)_2D$ might bind to VDR and therefore regulate cell proliferation, differentiation, and apoptosis.

Data of the present study showed a low frequency of patients with tt genotypes among breast cancer while a study performed by Mishra *et al*.[13] showed no association between VDR *TaqI* genotypes and disease outcome. Moreover, this study showed a significant difference between Vitamin D level among different genotypes and within breast cancer patients and control groups [Table 4], in which the rare genotype (tt) had the highest Vitamin D level compared to the other patterns, followed by the heterozygous genotype (Tt). Meanwhile, the wild-type genotype (TT) scored the lowest Vitamin D level which is compatible with results that were found by Janowsky *et al*.[30] One of the limitations of this study is the limited number of patients enrolled in this study. Hence, further large studies, particularly referring to larger sample size, more gene polymorphisms, and gene-gene and gene-environment interactions, are recommended.

In conclusion, this study found that circulating level of 25(OH)D was significantly lower among breast cancer patients indicating an inverse relationship between 25(OH)D level and breast cancer risk. Furthermore, a significant association between different *TaqI* genotypes and circulating levels of 25(OH)D was found.

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Conflicts of interest

There are no conflicts of interest.

References

1. Available from: http://www.breastcancer.org/symptoms/understand_bc/statistics. [Last accessed on 2017 Jan 07].
2. Atoum MF, Tanashat RQ, Mahmoud SA. Negative association of the HLA-DQB1*02 allele with breast cancer development among Jordanians. Asian Pac J Cancer Prev 2013;14:7007-10. doi: 10.7314/APJCP.2013.14.11.7007.
3. Nair R, Maseeh A. Vitamin D: The "sunshine" vitamin. J Pharmacol Pharmacother 2012;3:118-26. doi: 10.4103/0976-500X.95506.

4. Beaudin SG, Robilotto S, Welsh J. Comparative regulation of gene expression by 1,25-dihydroxyvitamin D3 in cells derived from normal mammary tissue and breast cancer. J Steroid Biochem Mol Biol 2015;148:96-102. doi: 10.1016/j.jsbmb.2014.09.014.
5. Crofts LA, Hancock MS, Morrison NA, Eisman JA. Multiple promoters direct the tissue-specific expression of novel N-terminal variant human Vitamin D receptor gene transcripts. Proc Natl Acad Sci U S A 1998;95:10529-34. doi: 10.1073/pnas.95.18.10529.
6. Guo B, Jiang X, Hu X, Li F, Chen X. Association between Vitamin D receptor gene polymorphisms and breast cancer in a Chinese population. Int J Clin Exp Med 2015;8:8020-4.
7. Moukayed M, Grant WB. The roles of UVB and Vitamin D in reducing risk of cancer incidence and mortality: A review of the epidemiology, clinical trials, and mechanisms. Rev Endocr Metab Disord 2017;1-16. doi: 10.1007/s11154-017-9415-2.
8. Oliveira Sediyama CM, Dias MM, Pessoa MC, Queiroz AR, Suhett LG, Freitas RN, *et al.* Lifestyle and Vitamin D dosage in women with breast cancer. Nutr Hosp 2016;33:584. doi: 10.20960/nh.584.
9. Cadeau C, Fournier A, Mesrine S, Clavel-Chapelon F, Fagherazzi G, Boutron-Ruault MC. Interaction between current Vitamin D supplementation and menopausal hormone therapy use on breast cancer risk: Evidence from the E3N cohort. Am J Clin Nutr 2015;102:966-73. doi: 10.3945/ajcn.114.104323.
10. Grant WB. 25-hydroxyvitamin D and breast cancer, colorectal cancer, and colorectal adenomas: Case-control versus nested case-control studies. Anticancer Res 2015;35:1153-60.
11. Abulkhair O, Saadeddin A, Makram O, Gasmelseed A, Pasha T, Shehata H, *et al.* Vitamin D levels and breast cancer characteristics: Findings in patients from Saudi Arabia. J Steroid Biochem Mol Biol 2016;164:106-9. doi: 10.1016/j.jsbmb.2015.11.003.
12. Wang LY, Zhang P, Wang HF, Qin ZW, Wei KB, Lv XA. Association of Vitamin D receptor gene polymorphisms with end-stage renal disease and the development of high-turnover renal osteodystrophy in a Chinese population. Genet Mol Res 2016;15;1-8. doi: 10.4238/gmr.15026825.
13. Mishra DK, Wu Y, Sarkissyan M, Sarkissyan S, Chen Z, Shang X, *et al.* Vitamin D receptor gene polymorphisms and prognosis of breast cancer among African-American and Hispanic women. PLoS One 2013;8:e57967. doi: 10.1371/journal.pone.0057967.
14. McKay JD, McCullough ML, Ziegler RG, Kraft P, Saltzman BS, Riboli E, *et al.* Vitamin D receptor polymorphisms and breast cancer risk: Results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. Cancer Epidemiol Biomarkers Prev 2009;18:297-305. doi: 10.1158/1055-9965.EPI-08-0539.
15. Abd-Elsalam EA, Ismaeil NA, Abd-Alsalam HS. Vitamin D receptor gene polymorphisms and breast cancer risk among postmenopausal Egyptian women. Tumour Biol 2015;36:6425-31. doi: 10.1007/s13277-015-3332-3.
16. Cowherd SM. Tumor staging and grading: A primer. Methods Mol Biol 2012;823:1-18. doi: 10.1007/978-1-60327-216-2_1.
17. Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM; Vitamin D Standardization Program (VDSP). Vitamin D status as an international issue: National surveys and the problem of standardization. Scand J Clin Lab Invest Suppl 2012;243:32-40. doi: 10.3109/00365513.2012.681935.
18. Available from: http://www.nationalbreastcancer.org/breast cancer facts. [Last accessed on 2017 Jan 07].
19. Colagar AH, Firouzjah HM, Halalkhor S. Vitamin D receptor poly(A) microsatellite polymorphism and 25-hydroxyvitamin D serum levels: Association with susceptibility to breast cancer. J Breast Cancer 2015;18:119-25. doi: 10.4048/jbc.2015.18.2.119.
20. Azarpeykan S, Dittmer KE, Gee EK, Marshall JC, Wallace J, Elder P, *et al.* Influence of blanketing and season on Vitamin D and parathyroid hormone, calcium, phosphorus, and magnesium concentrations in horses in New Zealand. Domest Anim Endocrinol 2016;56:75-84. doi: 10.1016/j.domaniend.2016.03.003.
21. Atoum MF, Tchoporyan MN. Association between circulating Vitamin D, the Taq1 Vitamin D receptor gene polymorphism and colorectal cancer risk among Jordanians. Asian Pac J Cancer Prev 2014;15:7337-41. doi: 10.7314/APJCP.2014.15.17.7337.
22. Atoum MF, AlKateeb D, AlHaj Mahmoud SA. The Fok1 Vitamin D receptor gene polymorphism and 25(OH)D serum levels and prostate cancer among Jordanian men. Asian Pac J Cancer Prev 2015;16:2227-30. doi: 10.7314/APJCP.2015.16.6.2227.
23. Atoum MF. ACC interleukin-10 gene promoter haplotype as a breast cancer risk factor predictor among Jordanian females. Onco Targets Ther 2016;9:3353-7. doi: 10.2147/OTT.S101628.
24. Atoum MF, Alzoughool F. Reduction in breast cancer susceptibility due to XbaI gene polymorphism of alpha estrogen receptor gene in Jordanians. Breast Cancer (Dove Med Press) 2017;9:45-9. doi: 10.2147/BCTT.S125652.
25. Hou MF, Tien YC, Lin GT, Chen CJ, Liu CS, Lin SY, *et al.* Association of Vitamin D receptor gene polymorphism with sporadic breast cancer in Taiwanese patients. Breast Cancer Res Treat 2002;74:1-7. doi: 10.1023/A:1016048900049.
26. Yang B, Liu S, Yang X, Wang Y, Zhao X, Zheng D, *et al.* Current evidence on the four polymorphisms of VDR and breast cancer risk in Caucasian women. Meta Gene 2013;2:41-9. doi: 10.1016/j.mgene.2013.09.008.
27. Zhang K, Song L. Association between Vitamin D receptor gene polymorphisms and breast cancer risk: A meta-analysis of 39 studies. PLoS One 2014;9:e96125. doi: 10.1371/journal.pone.0096125.
28. Mallah EM, Hamad MF, Elmanaseer MA, Qinna NA, Idkaidek NM, Arafat TA, *et al.* Plasma concentrations of 25-hydroxyvitamin D among Jordanians: Effect of biological and habitual factors on Vitamin D status. BMC Clin Pathol 2011;11:8. doi: 10.1186/1472-6890-11-8.
29. Weinstock MA, Moses AM. Skin cancer meets Vitamin D: The way forward for dermatology and public health. J Am Acad Dermatol 2009;61:720-4. doi: 10.1016/j.jaad.2009.04.016.
30. Janowsky EC, Lester GE, Weinberg CR, Millikan RC, Schildkraut JM, Garrett PA, *et al*. Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk. Public Health Nutr 1999;2:283-91.