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Research Article

STUDY OF RELATIONSHIP BETWEEN VITAMIN D RECEPTOR GENE POLYMORPHISM EXPRESSION (BSM- I & FOK- I), SERUM LEVELS OF VITAMIN D AND THE RISK OF BREAST CANCER IN EGYPTIAN FEMALES; CORRELATION WITH THE CLINICOPATHOLOGICAL FEATURES OF THE DISEASE

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ABSTRACT
Breast cancer (BC) represents the most common cancer among women, and its rates are increasing in developing countries, including Egypt. We aimed in this study to investigate whether the different specific vitamin D receptor (VDR) gene polymorphism expression are associated with breast cancer risk in Egyptian women. In addition, to study the correlation between serum level of 1α, 25-dihydroxyvitamin D3, VDR polymorphism and different clinicopathological features of BC. The study was conducted on forty five females; thirty women with different stages of breast cancer, and fifteen normal healthy females were included as a control group. Fresh blood samples were obtained from all subjects for measurement of Vitamin D levels and peripheral blood mononuclear cells (PBMCs) were isolated for DNA analyses for VDR gene BsmI and FokI polymorphisms expression using real-time PCR technique. The present study demonstrated that a significantly increased risk of breast cancer was observed with Bsm-I Bb genotype and increased risk of breast cancer was observed with Fok-I Ff genotype. No significant differences between the mean values of vitamin D and the three categories of Fok-I (FF, Ff, ff). But, data showed high significant differences between mean values of vitamin D at the three categories of Bsm-I. Vitamin D levels were significantly higher in the control group than breast cancer group**.** VDR polymorphisms have potential effects on Vitamin D deficiency and possible differential effects of breast cancer risk, low vitamin D levels were associated with breast cancer patients.

Keywords: *Vitamin D, Breast Cancer, Receptor Gene Polymorphism Expression, Egyptian Females*

INTRODUCTION
Breast cancer (BC) represents the most common cancer among women, and its rates are increasing in developing countries, including Egypt (Ferlay *et al.*, 2015). In Egypt, breast cancer is the most common cancer among women, representing 33.5% of newly diagnosed cancer cases (El-Deeb *et al.*, 2016).
In Alexandria, Egypt breast cancer accounts for 45.4% of all malignancies in females during the year 2012 (Alexandria Cancer Registry Annual Report, 2012).
Risk factors for breast cancer have always been categorized into modifiable and non-modifiable risk factors (Nomura *et al.*, 2016).
Modifiable risk factors include use of oral contraceptives, hormone replacement therapy, obesity, alcohol consumption and decreased physical activity (Nomura *et al.*, 2016) while non-modifiable risk factors include old age identified as the strongest risk factor (Phipps and Li, 2010), family History of BC (Spector *et al.*, 2011), benign breast lesions (Bostean *et al.*, 2013; McPherson *et al.*, 2000) and increased density of breasts (Green, 2016).
Vitamin D is a fat soluble vitamin that is required by the healthy skeleton for normal development (Stroud *et al.*, 2008).

Since its discovery in most tissues and cells of the body vitamin D receptor (VDR) gene has been known to have important functions as increasing intestinal calcium absorption, insulin secretion and aiding in phosphate homeostasis (Stroud *et al.*, 2008).

The deficiency of VDR gene has been implicated as a suspected risk factor for cardiovascular diseases, hypertension, fractures of the hip, and its related deficiency to several malignancies as breast, colon and prostate cancer (Holick, 2007).

Vitamin D and the VDR gene have been shown in experimental studies to be important in the etiology of BC through multiple pathways (Chen *et al.*, 2010).

Vitamin D pathway negatively impact cell growth regulation and proliferative activity as shown in experimental studies (Holick, 2007; Chen *et al.*, 2010). Other experimental studies showed that 1, 25(OH) 2D, the metabolically active form of vitamin D, exerts its main actions through the VDR gene (Perez-Lopez *et al.*, 2009). Both normal and malignant breast tissue contain VDR gene that responds to 1, 25(OH) 2D and these breast cells express the enzyme 25-hydroxyvitamin D 1-α-hydroxylase (Perez-Lopez *et al.*, 2009).

1, 25(OH) 2D reduces the potential for the malignant cell to survive as evidenced by previous studies (Perez-Lopez *et al.*, 2009).

Administering vitamin D compounds in animals have been shown to reduce incidence of BC (Holick, 2007) and VDR gene knockouts in animals have shown to increase number of tumors (Freedman *et al.*, 2009).

Optimum levels of serum vitamin D in the body have never been identified; however, vitamin D deficiency is defined by most as a serum level of less than 20 Nano gram per milliliter (ng/ml) (Holick, 2007).

Breast cancer cells exhibit decreased VDR expression compared to normal breast cells as evidenced by previous research (Mishra *et al.*, 2013; Lopes *et al.*, 2010). The change in VDR expression and activity may lead to deregulation of vitamin D uptake, metabolism and serum levels of biologically active vitamin D (Alimirah *et al.*, 2011).

Breast cancer cells show decreased levels of VDR which might be attributed to VDR gene polymorphisms, and/or DNA methylation (Marik *et al.*, 2010).

Vitamin D as evidenced participates in cell growth regulation, apoptosis, cell differentiation and also it plays a major role in the suppression of cancer cell invasion, angiogenesis and metastasis (Lopes *et al.*, 2012).

This study aimed to investigate whether the different specific vitamin D receptor (VDR) gene polymorphism expression are associated with breast cancer risk in Egyptian women. In addition, to study the correlation between serum level of 1α, 25-dihydroxyvitamin D3, VDR polymorphism and different clinicopathological features of BC.

Patients and Methods

The present study was conducted on forty five Egyptian female volunteers who were divided into two groups:

Group (1): Thirty women with different stages of breast cancer was recruited from Clinical and Experimental Surgery Department, Medical Research Institute, Alexandria University, Alexandria, Egypt.

Group (2): Fifteen ages matched normal healthy individuals was a control group.

A written informed consent was taken from all subjects in the study after approval by the ethical committee of Medical Research Institute.

All individuals in the study were subjected to full History taking, clinical examination and radiological investigations in the form of chest X-ray, abdominal ultra Sound (U/S), bone scan and CT when needed.

Histopathological examination was done for all patients of group (1).

Immunological Investigations were done in all subjects participating in the study in the form of:

1. Measurement of Vitamin D Level: Vitamin D level will be measured utilizing a competitive Enzyme Immuno-Assay (EIA) technique with a selected monoclonal antibody recognizing 1α, 25-dihydroxyvitamin D3 (Rizk *et al.*, 2014).

2. Isolation of Peripheral blood mononuclear cells (PBMCs) from EDTA venous whole blood sample using Ficoll-Hypaque density gradient centrifugation (Fuss *et al.*, 2009)
3. DNA extraction from all subjects in the study was done using the DNA extraction kit according to manufacturer's instructions (Mishra *et al.*, 2013).
4. VDR Gene polymorphism expression: VDR-BsmI (rs1544410) and VDR-FokI (rs2228570) was monitored by Real Time-polymerase chain reaction (RT- PCR) using forward and reverse primers (Kaleta *et al.*, 2013).

Statistical Analysis

Data were fed to the computer using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test. Quantitative data were described using mean and standard deviation for normally distributed data while abnormally distributed data was expressed using range. Comparison between two independent population were done using independent t-test while more than two population were analyzed using F-test (ANOVA). Correlations between two variables were assessed using Pearson coefficient. Statistical significance was defined as a p value < 0.05 and all tests were two-sided.

RESULTS AND DISCUSSION

Results

I. Clinicopathological Features of the Patients

There was no statistical significance difference between the mean age values of the two studied groups as well as menopausal status and family history as shown in table I.

Table I: Age, Menopausal Status and Family History in all Subjects

Age (Years)	**Breast Cancer (n=30)**	**Control (n=15)**
Range	21-75	26-62
Mean	51.3	47.8
Std. Deviation (SD)	12.86	9.30
Menopausal Status		
Pre-menopausal	13(43.3%)	10(66.7%)
Post-menopausal	17(56.7%)	5(33.3%)
Family History for Breast Cancer		
Positive	14(46.7%)	2(13.3%)
Negative	16(53.3%)	13(86.7%)

As regards histopathological tumor type, tumor grades and hormonal status there was no statistical difference as shown in table II.

II. Immunological Investigations:

Vitamin D levels of all subjects under study are shown in table III showing statistical difference between 2 groups being higher in the control group.

III. Genotyping Analysis

1. VDR Bsm-I SNP

The b allele of Bsm-I polymorphism was detected in 45 % of the breast cancer patients versus 33 % of the controls. Also, the B allele of Bsm-I polymorphism was detected in 55 % of the breast cancer patients versus 67 % of the controls and because of four cases of (bb) genotype among breast cancer group with no such corresponding categories among control group, such subdivision will lead to a non-logical result so instead of this, we ignored the four cases of (bb) and consider our subdivision to be (Bb and BB) genotypes for Bsm-I. According to this, we would have the following 2x2 cross table IV showing causative relationship between VDR gene (Bsm-I) with the BC cases and control group.

Table II: Histopathological Tumor Type, Tumor Grades and Hormonal Status of all Cancer Patients

Histological Tumor Type	**Breast Cancer (n=30)**
Ductal carcinoma in situ (DCIS)	17(56.7%)
Infilterating Ductal Carcinoma (IDC)	13(43.3%)
Other types	0
unknown	0
Tumor Grade	
I	15(50%)
II	5(16,7%)
III	6(20%)
IV	0
Unknown	3(13.3%)
ER/PR Expression	
Positive	13(43.3%)
Negative	13(43.3%)
Unknown	4(13.3%)
HER-2 Expression	
Positive	10(33.3%)
Negative	16(53.3%)
Unknown	4(13.3%)

Estrogen Receptor/ Progesterone Receptor (ER/PR)
Human Epidermal Growth Factor Receptor 2 (HER-2)

Table III: Vitamin D Levels in all Subjects

Vitamin D Levels (ng/ml)	**Breast Cancer (n=30)**	**Control (n=15)**	**P-Value***
Range	3-16	15-24	
Mean	9.13	17.83	0.034*
Std. Deviation (SD)	3.95	4.62	

* Significant at $p < 0.05$ level

Table IV: Causative Relationship between VDR Gene (Bsm-I) with the BC Cases and Control Group

	Breast Cancer	**Control**	**Total**	**Odd's Ratio (OR)**	**95% Confidence**	**p- value***
Bb	19 (73%)	10 (67%)	29		(0.34 -5.41)	0.01*
BB	7 (27%)	5 (33%)	12	1.36		
Total	26(100%)	15(100%)	41			

* Significant at $p < 0.05$ level

2. VDR Fok-I SNP

The f allele of Fok-I polymorphism was detected in 30 % of the breast cancer patients versus 16.7 % of the controls. The F allele of Fok-I polymorphism was detected in 70 % of the breast cancer patients versus 83.3 % of the controls and Because of one case of (ff) genotype among Breast Cancer group with no such corresponding categories among Control group, such subdivision will lead to a non-logical result, so instead of this, we ignored the only case of (ff) genotype and consider our subdivision to be (Ff and FF) genotypes for Fok-I. According to this, we would have the following 2x2 cross table v showing causative relationship between VDR gene (Fok-I) with the BC cases and control group.

Table V: Causative Relationship between VDR Gene (Fok-I) with the BC Cases and Control Group

	Breast Cancer	Control	Total	Odd's Ratio (OR)	95% Confidence	P- Value
Ff	16 (55%)	5 (33%)	21	2.46	(0.67 - 9.02)	0.01*
FF	13 (45%)	10 (67%)	23			
Total	29	15	44			

* Significant at $p < 0.05$ level

To analyze the correlation between Fok-I SNP and breast cancer risk, the FF genotype was assigned as reference genotype according to the previous studies (Kaleta *et al.*, 2013; Lim *et al.*, 2015).
When the Ff genotypes were compared with the reference genotype, a significantly (p value = 0.01) increased risk of breast cancer was observed with Fok-I Ff genotype (Table V). The corresponding odds ratio was 2.46 (CI 0.67–9.02) for Fok-I Ff genotype.
IV Comparison between Immunological Investigations, Genotyping Analysis and Clinicopathological Parameters of Breast Cancer Patients
1) The relation between vitamin D and clinicopathological features of breast cancer patients are summarized in table VI

Table VI: Vitamin D and Clinicopathological Features of Breast Cancer Patients

	Number of Cases	Vit. D (ng/ml)	
		Mean ±SD	P- Value
Age (Years)			
≥50	18	8.83±4.19	0.196
<50	12	9.58±3.70	
Menopausal Status			
Pre	13	9.5 ± 3.6	0.162
Post	17	8.9±4.3	
Family History			
+ve	14	9.93±3.63	0.433
-ve	16	8.44±4.21	
Tumor Type			
DCIS	16	9.5±3.5	0.308
IDC	14	8.7±4.51	
Tumor Grade			
I	15	10.67±4.97	0.052
II	5	8.8±3.7	
III	6	7.2±4.21	
ER/PR			
+ve	13	8.38±3.69	0.21
-ve	13	9.31±4.61	
HER-2			
+ve	10	9.69±4.43	0.433
-ve	16	9.31±4.73	

*Significant at $p < 0.05$ level

2) Relation between VDR SNP (Fok-I) frequencies, VDR SNP (Bsm-I) frequencies and clinicopathological features of breast cancer patients
Table VII summarizes relation between VDR SNP (Fok-I) genotypes (FF, Ff, ff) frequencies, VDR SNP (Bsm-I) frequencies and clinicopathological parameters in breast cancer patients.

Table VII: Genotype Frequencies for (Fok-I) and (Bsm-I) Genes and Clinicopathological Features of Breast Cancer Patients

	Number of Cases	Genotype Frequencies for (Fok-I) Gene						Genotype Frequencies for (Bsm-I) Gene					
		FF		Ff		ff		BB		Bb		bb	
		No	%	No	%	No	%	No	%	No	%	No	%
Age (Years)													
≥50	18	8	27	9	30	1	3	6	20	9	30	3	10
<50	12	5	17	7	23	0	0	1	3	10	33	1	3
p- value		P 0.318						p <0.005*					
Menopausal Status													
Pre	13	5	17	8	27	0	0	2	7	10	33	1	3
Post	17	8	27	8	27	1	3	5	17	9	30	3	10
p- value		p 0.160						p 0.044*					
Family History													
+ve	14	2	7	12	40	0	0	4	13	9	30	1	3
-ve	16	11	37	4	13	1	3	3	10	10	33	3	10
p- value		p <0.005*						p 0.148					
Tumor Type													
DCIS	16	3	10	12	40	1	3	4	13	10	33	2	7
IDC	14	10	33	4	13	0	0	3	10	9	30	2	7
p- value		p <0.005*						p 0.916					
Tumor Grade													
I	15	8	27	6	20	1	3	2	7	11	37	2	7
II	5	4	13	1	3	0	0	1	3	3	10	1	3
III	6	1	3	5	17	0	0	4	13	1	3	1	3
P value		p <0.005*						p <0.005*					
ER/PR													
+ve	13	7	23	5	17	1	3	3	10	9	30	2	7
-ve	13	6	20	7	23	0	0	4	13	6	20	2	7
p- value		p 0.128						p 0.046*					
HER-2													
+ve	10	5	17	4	13	1	3	3	10	6	20	1	3
-ve	16	8	27	8	27	0	0	4	13	9	30	3	10
p- value		p 0.0.067)						p 0.063					

Significant at $p < 0.05$ level

Discussion

Breast cancer is the most commonly diagnosed female-specific cancer and shows an increasing trend in diagnosed cases worldwide. An estimated one in eight women will develop breast cancer in her lifetime (Nomura *et al.*, 2016). BC is the most common female cancer in Egypt, and the incidence rate among Egyptian women is $48.8/10^5$ (Ibrahim *et al.*, 2014).

Since introduction of researches on the role of vitamin D in BC, evidence showed the protective effect of vitamin D against breast cancer and that vitamin D deficiency appears to be a risk factor for development and progression of BC (Colagar *et al.*, 2015).

BC development in animals can be reduced by using vitamin D compounds as evidenced by previous researches (Freedman *et al.*, 2009; Zerwekh, 2004).

The effects of the metabolically active form of vitamin D (1α, 25-dihydroxyvitamin D3) are mediated through the vitamin D receptor (VDR) which is a ligand-dependent transcription factor that belongs to the family of nuclear receptors (Rashid *et al.,* 2015).

Several VDR variants have been identified that may influence breast cancer risk. The most frequently studied SNPs are BsmI and FokI. Whether Bsm-I influences the expression or activity of the VDR protein is unclear. The FokI site is located in the 5´promoter region and changes the first of the two possible translation initiation sites, resulting in VDR proteins of different lengths. Many studies performed on Caucasian populations regarding the association of the BsmI and FokI SNPs with breast cancer risk have yielded inconsistent results (McKay *et al.,* 2009).

Limited financial resources for this study allowed us to evaluate only two polymorphisms in this study. We selected polymorphisms related to VDR (Fok1 rs2228570) and (Bsm1 rs1544410) genes.

The results obtained in the present study revealed that the mean±SD age value of controls was 47.80 ±9.30 years while it was 51.30 ±12.86 years in patients having breast cancer. No significant difference was found between the mean values of the two studied groups regarding the age. The majority of the normal subjects (66.7%) were at pre-menopause, while most patients with breast cancer (56.7%) were at post-menopause. Histopathological examination of malignant tissue sections revealed that 56.7% were ductal carcinoma in situ (DCIS) type and 43.3% infilterated ductal carcinoma (IDC). According to tumor grade, it was found that 50% of breast cancer patients were in grade I, 16.5% were in grade II and 20% were in grade III. The immunohistochemical (IHC) analysis showed that tumors with positive ER/PR expression were observed in 43.3% and Her-2 expression in 33.3% of patients with breast cancer. Our study also revealed that 46.7% of breast cancer patients had family history to breast cancer.

The results of the present study revealed that the mean values of vitamin D were significantly (p=0.034) decreased in patients with breast cancer (9.13±3.95) compared to normal controls (17.83±4.62). Analysis of different clinicopathological features of breast cancer patients showed non-significant differences in the mean values of vitamin D regarding age, menopausal status, family history, tumor type, ER/PR and Her-2 expression.

Whereas, our data showed a decreasing pattern in the mean values of vitamin D with advancing grade of breast cancer (10.67±4.97 for grade I, 8.67±3.7 for grade II and 7.2±4.21 for grade III). This result indicate that there is a significant difference (p=0.05) between the mean values of vitamin D for the three tumor grades. So, we can say that the less concentration of vitamin D, the more advanced grade of the breast cancer.

Inverse relationship between risk of breast cancer and vitamin D status has been repeatedly reported in the literature. Moreover, the aggressiveness of the disease was inversely correlated with 25(OH) D concentrations and low vitamin D levels were associated with increased risk of breast cancer death (Lopes *et al.,* 2012; Autier *et al.,* 2014; Grant, 2015).

Abdelgawad *et al.,* (2015) identified that 25-OH vitamin D was deficient in 67% and 49.0% of the breast cancer and the normal control groups in Egyptian females respectively, with the median level significantly lower in the breast cancer group.

In agreement with our results, Abulkhair *et al.,* (2016) reported that patients from Saudi Arabia with triple negative breast cancer were predominantly seen in the low vitamin D group. A similar finding was reported in the literature among non-Hispanic white women and women with African ancestry (Yao *et al.,* 2013).

Similarly, Park *et al.,* (2015) found significant protective association between serum 25(OH) D and breast cancer risk among both pre- and post-menopausal Korean women indicating that women with insufficient level of vitamin D (25(OH)D less than 20 ng/mL) had approximately 27 % higher risk of breast cancer compared to those with sufficient level of vitamin D (25(OH)D more than 20 ng/mL).

Another study by Lim *et al.,* (2015) determined the association between alterations in the 25(OH) D status during follow-up and the prognosis of Korean breast cancer patients. The results demonstrated that the 25 (OH) D statuses at diagnosis and at the 1-year follow-up are significantly associated with the survival of breast cancer patients.

From our finding, and other studies in the literature we can suggest that vitamin D deficiency increases the risk of developing cancer and that avoiding deficiency and adding vitamin D supplements might be an economical and safe way to reduce cancer incidence and improve cancer prognosis and outcome (David *et al.*, 2014).

In our study, breast cancer patients and healthy women group were analyzed for VDR gene Bsm-I and Fok-I polymorphisms. The allele frequencies of the VDR Bsm-I and Fok-I SNP were obtained.

To analyze the correlation between Bsm-I SNP and breast cancer risk, the BB genotype was assigned as reference genotype according to the previous studies (Hou *et al.*, 2002; Trabert *et al.*, 2007). When the Bb genotypes were compared with the reference genotype, a significantly increased risk of breast cancer was observed with Bsm-I Bb genotype (Table IV). Our data suggested that the b allele may contribute in susceptibility to breast cancer, either in heterozygote or homozygote state.

Regarding the relation between Bsm-I genotypes frequencies and different clinicopathological features, statistical analysis indicates significant differences between Bsm-I genotypes frequencies regarding to patient age, menopausal status, tumor grade, and ER/PR status. Also, our results showed a significant differences between Bsm-I genotypes frequencies for premenopausal and postmenopausal patients. Statistical analysis indicated significant differences between Bsm-I genotypes frequencies for the three grades of tumor.

Our results revealed also a significant differences between Bsm-I genotypes frequencies for ER/PR status and no significant difference between Bsm-I genotypes frequencies and family history for breast cancer, tumors histopathological classification, as well as Her-2 status.

Our finding showed a clear increase in the mean value of the Vitamin D with the advanced dominant of Bsm-I genotypes. Statistical analysis of our data indicated a high significant differences between these mean values of Vitamin D at the three categories of Bsm-I genotypes. That means a clear increase in the mean value of the vitamin D with the advanced dominant of Bsm-I categories (BB, Bb) compared with recessive category (bb).

Many previous studies assessing correlation of Bsm-I genotypes to breast cancer have reported controversial results (Shahbazi *et al.*, 2013; Ingles *et al.*, 2000). Since Bsm-I have various geographical distributions, the selected subjects and controls have a great impact on the results by population stratification. Most of the studies which were performed on Caucasian women reported increased risk of breast cancer with the BsmI bb genotype, whereas, BsmI BB genotype was correlated to breast cancer among Hispanic and Taiwanese population (Lopes *et al.*, 2012; Shahbazi *et al.*, 2013; Ingles *et al.*, 2000; Pena-Chilet *et al.*, 2013).

Ogunkolade *et al.*, (2002) found that the Bsm-I exhibits strong linkage disequilibrium with other polymorphisms, located at the 3' untranslated region (3' UTR) of the VDR gene. This haplotype may affect mRNA stability and processing as well as regulation of VDR transcription and translation.

Our results studying the f allele of Fok-I polymorphism indicated that on comparison of Ff genotypes with the reference genotype, a significant increased risk of breast cancer was observed with Fok-I Ff genotype. Regarding to the relation between Fok-I genotypes frequencies and different clinicopathological features, statistical analysis indicated significant differences between Fok-I genotypes frequencies regarding family history for breast cancer, tumor type and grade. While there was no significant differences were found regarding to age, menopausal status, ER/PR status and Her-2 status.

In agreement to our results, two comprehensive case–control study provided evidence that ff genotype of FokI was associated with a higher breast cancer risk (Sinotte *et al.*, 2008; Chen *et al.*, 2005). Meta-analyses assessments have demonstrated that FokI ff genotype is linked to susceptibility to breast cancer (Raimondi *et al.*, 2009; Tang *et al.*, 2009). Nemenqani *et al.*, (2015) demonstrated that Fok-I ff. genotype and f allele have an important role in breast cancer risk in Saudi patients. Nevertheless, there are other reports that have not supported this correlation, and in contrast, some researches even showed lower risk of breast malignancy in the subjects with ff genotype (Abbas *et al.*, 2008; John *et al.*, 2007).

In the present work, the results indicated that no significant differences between the mean values of vitamin D and the three categories of Fok-I (FF, Ff, ff). But, data showed high significant differences

between mean values of vitamin D at the three categories of Bsm-I. That is meaning a clear increase in the mean value of the vitamin D with the advanced dominant of Bsm-I categories (BB, Bb) compared with recessive category (bb). Also, data showed a decreasing pattern in the mean value of vitamin D with advancing stages of Breast Cancer.

The present study demonstrated that a significantly increased risk of breast cancer was observed with Bsm-I Bb genotype. The data suggested that the b allele may contribute in susceptibility to breast cancer, either in heterozygote or homozygote state.

When the Ff genotypes were compared with the reference genotype, a significant with increased risk of breast cancer was observed with Fok-I Ff genotype.

Conclusion

1. Our results support that VDR polymorphisms have potential effects on Vitamin D deficiency and possible differential effects of breast cancer risk.
2. Low vitamin D levels were associated with breast cancer patients.
3. Our findings showed a clear increase the Vitamin D levels with the advanced dominant of Bsm-I genotype (BB) compared with (Bb) and recessive (bb) genotypes.
4. The present study demonstrated that Egyptian women with the Bsm-I Bb or bb genotype as well as Fok-I Ff or ff were at higher risk for breast cancer.

Conflict of Interest Statement

We declare no conflict of interest as authors

REFERENCES

Abbas S, Nieters A, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ *et al.*, (2008). Vitamin D receptor gene polymorphisms and haplotypes and postmenopausal breast cancer risk. *Breast Cancer Research* **10**(2) R31.

Abdelgawad IA, El-Mously RH, Saber MM, Mansour OA and Shouman SA (2015). Significance of serum levels of vitamin D and some related minerals in breast cancer patients. *International Journal of Clinical Experimental Pathology* **8** 4074-82.

Abulkhair O, Saadeddin A, Makram O, Gasmelseed A, Pasha T, Shehata H *et al.*, (2016). Vitamin D Levels and Breast Cancer Characteristics: Findings in Patients from Saudi Arabia. *Journal of Steroid Biochemical Molecular Biology*. S0960-0760 **15** 30129-1.

Alexandria Cancer Registry Annual Report (2012). Alexandria, Egypt, Medical Research Institute, Alexandria University.

Alimirah F, Peng X, Murillo G and Mehta RG (2011). Functional significance of vitamin D receptor FokI polymorphism in human breast cancer cells. *Public Library of Science One* **6**(1) e16024.

Autier P, Boniol M, Pizot C and Mullie P (2014). Vitamin D status and ill health: a systematic review. *The Lancet Diabetes Endocrinology* **2** 76-89.

Bostean G, Crespi CM and McCarthy WJ (2013). Associations among family history of cancer, cancer screening and lifestyle behaviors: a population-based study. *Cancer Causes Control* **24** 1491–503.

Chen P, Hu P, Xie D, Qin Y, Wang F and Wang H (2010). Meta-analysis of vitamin D, calcium and the prevention of breast cancer. *Breast Cancer Research Treatment* **121** 469-77.

Chen WY, Bertone-Johnson ER, Hunter DJ, Willett WC and Hankinson SE (2005). Associations between polymorphisms in the vitamin D receptor and breast cancer risk. *Cancer Epidemiological Biomarkers Preview* **14** 2335–9.

Colagar AH, Firouzjah HM and Halalkhor S (2015). Vitamin D Receptor Poly (A) Microsatellite Polymorphism and 25-Hydroxyvitamin D Serum Levels: Association with Susceptibility to Breast Cancer. *Journal of Breast Cancer* **18**(2) 119-25.

Feldman D, Krishnan AV, Swami S and Feldman BJ (2014). The role of vitamin D in reducing cancer risk and progression. *Nature Reviews Cancer* **14**(5) 342–57.

El-Deeb MM, El-Sheredy HG and Mohammed AF (2016). The Role of Serum Trace Elements and Oxidative Stress in Egyptian Breast Cancer Patients. *Advanced Breast Cancer Researches* **5** 37-47.

Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Mathers C, Pakrin DM *et al.*, (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer* **136**(5) E359-86.

Freedman OC and Goodwin PJ (2009). The role of vitamin D in breast cancer recurrence. *American Society of Clinical Oncology Educational Book* **1** 79-83.

Fuss IJ, Kanof ME, Smith PD and Zola H (2009). Isolation of whole mononuclear cells from peripheral blood and cord blood. *Current Protocols in Immunology* **Chapter 7** Unit 7.1.

GLOBOCAN (2012). *Map Production: IARC, World Health Organization*. Available: http://globocan.iarc.fr/old/FactSheets/cancers/breast-new.asp [Accessed 4 December 2015].

Grant WB (2015). 25-Hydroxyvitamin D and Breast Cancer, Colorectal Cancer, and Colorectal Adenomas: Case-Control versus Nested Case-Control Studies. *Anticancer Research* **35** 1153-60.

Green VL (2016). Mammographic Breast Density and Breast Cancer Risk: Implications of the Breast Density Legislation for Health Care Practitioners. *Clinical Obstetrics and Gynecology* **59**(2) 419-38.

Holick MF (2007). Vitamin D deficiency. *New England Journal of Medicine* **357** 266-81.

Hou MF, Tien YC, Lin GT, Chen CJ, Liu CS, Lin SY *et al.*, (2002). Association of vitamin D receptor gene polymorphism with sporadic breast cancer in Taiwanese patients. *Breast Cancer Research Treatment* **74** 1–7.

Ibrahim A, Khaled H, Mikhail N, Baraka H and Kamel H (2014). Cancer Incidence in Egypt: Results of the National Population-Based Cancer Registry Program. *Journal of Cancer Epidemiology* **2014** ID 437971.

Ingles SA, Garcia DG, Wang W, Nieters A, Henderson BE, Kolonel LN *et al.*, (2000). Vitamin D receptor genotype and breast cancer in Latinas (United States). *Cancer Causes Control* **11** 25–30.

John EM, Schwartz GG, Koo J, Wang W and Ingles SA (2007). Sun exposure, vitamin D receptor gene polymorphisms, and breast cancer risk in a multiethnic population. *American Journal of Epidemiology* **166** 1409–19.

Kaleta B, Bogaczewicz J, Robak E, Sysa-Jwdrzejowska A, Wrzosek M, Szubierajska W *et al.*, (2013). Vitamin D Receptor Gene BsmI Polymorphism in Polish Patients with Systemic Lupus Erythematosus. *International Scholarly Research Notices: Endocrinology* **2013** 427818.

Lim ST, Jeon YW and Suh YJ (2015). Association between Alterations in the Serum 25-Hydroxyvitamin D Status During Follow-Up and Breast Cancer Patient Prognosis. *Asian Pacific Journal of Cancer Preview* **16** 2507-13.

Lopes N, Carvalho J, Duraes C, Sousa B, Gomes M, Costa JL *et al.*, (2012). 1 Alpha, 25-dihydroxyvitamin D3 induces de novo E-cadherin expression in triple-negative breast cancer cells by CDH1-promoter demethylation. *Anti-Cancer Research* **32** 249–57.

Lopes N, Paredes J, Costa JL, Ylstra B and Schmitt F (2012). Vitamin D and the mammary gland: a review on its role in normal development and breast cancer. *Breast Cancer Research* **14** 211-7.

Lopes N, Sousa B, Martins D, Gomes M, Vieira D, Veronese LA *et al.*, (2010). Alterations in Vitamin D signalling and metabolic pathways in breast cancer progression: a study of VDR, CYP27B1 and CYP24A1 expression in benign and malignant breast lesions. *BioMed Central Cancer* **10** 483.

Lowe LC, Guy M and Mansi JL (2005). Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK caucasian population. *European Journal of Cancer* **41** 1164-9.

Marik R, Fackler M, Gabrielson E, Zeiger MA, Sukumar S, Stearns V *et al.*, (2010). DNA methylation-related vitamin D receptor insensitivity in breast cancer. *Cancer Biological Therapy* **10**(1) 44-53.

McKay JD, McCullough ML, Ziegler RG, Kraft P, Saltzman BS, Riboli E *et al.*, (2009). Vitamin D receptor polymorphisms and breast cancer risk: results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. *Cancer Epidemiological Biomarkers Prevention* **18** 297–305.

McPherson K, Steel CM and Dixon JM (2000). ABC of breast diseases: Breast cancer- epidemiology, risk factors and genetics. *British Medical Journal* **321** 624-8.

Mishra DK, Wu Y, Sarkissyan M, Sarkissyan S, Chen Z, Shang X *et al.*, (2013).Vitamin D receptor gene polymorphisms and prognosis of breast cancer among African-American and Hispanic women. *Public Library of Science One* **8**(3) e57967.

Nemenqani DM, Karam RA, Amer MG and Abd El Rahman TM (2015). Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer. *Gene* **558** 215-9.

Nomura SJ, Inoue-Choi M, Lazovich D and Robien K (2016). WCRF/AICR recommendation adherence and breast cancer incidence among postmenopausal women with and without non-modifiable risk factors. *International Journal of Cancer* **138**(11) 2602-15.

Ogunkolade BW, Boucher BJ, Prahl JM, Bustin SA, Burrin JM, Noonan K *et al.*, (2002). Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. *Diabetes* **51** 2294–300.

Park S, Lee DH, Jeon JH, Ryu J, Kim S, Kim JY *et al.*, (2015). Serum 25-hydroxyvitamin D deficiency and increased risk of breast cancer among Korean women: a case–control study. *Breast Cancer Research Treatment* **152** 147–54.

Pena-Chilet M, Ibarrola-Villava M and Martin-Gonzalez M (2013). rs12512631 on the group specific complement (vitamin D-binding protein GC) implicated in melanoma susceptibility. *Public Library of Science One* **8** e5960.

Perez-Lopez FR, Chedraui P and Haya J (2009). Review article: Vitamin D acquisition and breast cancer risk. *Reproductive Science* **16** 7-19.

Phipps A and Li C (2010). *Breast Cancer Biology and Clinical Characteristics.* In: Li C, edition, (Springer New York, USA) 21-46.

Raimondi S, Johansson H, Maisonneuve P and Gandini S (2009). Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. *Carcinogenesis* **30** 1170–80.

Rashid MU, Muzaffar M, Khan FA, Kabisch M, Muhammad N, Faiz S *et al.*, (2015). Association between the BsmI Polymorphism in the Vitamin D Receptor Gene and Breast Cancer Risk: Results from a Pakistani Case-Control Study. *PLoS ONE* **10**(10) e0141562.

Rizk MM, Zakaria NH and Elshazely WG (2014). Study of Vitamin D Receptor (VDR) Gene Polymorphisms among Egyptian Cohort Patients with Different Stages of Colorectal Cancer. *Journal of Cancer Therapy* **5** 253-63.

Shahbazi S, Alavi S, Majidzadeh KA, GhaffarPour M, Soleimani A and Mahdian R (2013). BsmI but not FokI polymorphism of VDR gene is contributed in breast cancer. *Medical Oncology* **30** 393-8.

Sinotte M, Rousseau F, Ayotte P, Dewailly E, Diorio C, Giguere Y *et al.*, (2008). Vitamin D receptor polymorphisms (FokI, BsmI) and breast cancer risk: association replication in two case-control studies within French Canadian population. *Endocrine Related Cancer* **15** 975–83.

Spector D, Deroo LA and Sandler DP (2011). Lifestyle behaviors in black and white women with a family history of breast cancer. *Preventive Medicine* **106** 96–102.

Stroud ML, Stilgoe S, Stott VE, Alhabian O and Salman K (2008). Vitamin D - a review. *Australian Family Physician* **37**(12) 1002-5.

Tang C, Chen N, Wu M, Yuan H and Du Y (2009). Fok1 polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis. *Breast Cancer Research Treatment* **117** 391–9.

Trabert B, Malone KE, Daling JR, Doody DR, Bernstein L, Ursin G *et al.*, (2007). Vitamin D receptor polymorphisms and breast cancer risk in a large population-based case-control study of Caucasian and African-American women. *Breast Cancer Research* **9** 84.

Yao S and Ambrosone CB (2013) Associations between vitamin D deficiency and risk of aggressive breast cancer in African-American women. *Journal of Steroid Biochemical Molecular Biology* **136** 337-41.

Zerwekh JE (2004). The measurement of vitamin D: Analytical aspects. *Annals of Clinical Biochemistry* **41** 272-81.