Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

ASSOCIATION BETWEEN VITAMIN D STATUS AND VITAMIN D RECEPTOR GENETIC POLYMORPHISMS, AND BREAST CANCER RISK: A CASE CONTROL STUDY

HUMA NAQEEB*,
PhD-scholar, Department of Human Nutrition, The University of Agriculture Peshawar.

Dr. SAMI SIRAJ,
Associate Professor, Institute of Basic Medical Sciences, Khyber Medical University (KMU), Peshawar

Dr. ZIA-UD-DIN,
Associate Professor, Human Department of Human Nutrition, The University of Agriculture Peshawar.

*Corresponding Author

ABSTRACT

Vitamin D has anticarcinogenic properties and acts through vitamin D receptor (VDR) to carry out its functions. This study explored the independent and combined effects of vitamin D statusand VDR genetic polymorphisms on breast cancer (BC) risk in Pakistani population.This case–control study recruited 100 cases with histologically confirmed breast cancer and 100 age-matched controls. Serum 25(OH) vitamin D level was measured using Enzyme-linked immunosorbent assay (ELISA) kit. VDR genotype was conducted for Apa I (rs7975232), Taq I (rs731236) and *Fok I* (rs2228570), using the tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) and confirmation by Sanger sequencing. Logistic regression was usedto calculate odds ratio and 95% confidence interval after adjusting for various confounders.On average cases possessed a lower mean$\pm$Standard Deviation, serum vitamin D compared to control (cases: 15.1±9.5 ng/dl, vs controls: 19.6±9.3 ng/dl, $p<0.01$). Vitamin D deficiency was higher among cases than the control group (76% vs. 60%, $p<0.05$). Significant differences in serum vitamin D deficiency were evident between the groups. No significant association was found between Apa I, Fok 1 and breast cancer risk. Slightly significant association was found between Taq 1 and BC risk. The frequency of heterozygous (GA) genotype at Taq1 rs731236 G>A polymorphism was significantly higher in BC patients than in control subjects (37% versus 25% respectively, adjusted OR= 2.1, 95% CI: 1.09-4.1, $p<0.05$).A significant association exists between vitamin D deficiency and GA genotype of Taq I (rs731236) may be a susceptibility risk factor for BC development in the Pakistani population.

Keywords Vitamin D · Vitamin D receptor · Genetic polymorphisms · Breast cancer

1. INTRODUCTION

Breast cancer is the leading cause of mortality and morbidity in women all over the world. This disease is a major public health concern in Pakistan, the reported cases of BC was 25,928 which account for 14.5% of all type of cancer in Pakistan(1). BRCA1, BRCA2, TP53, CHEK2, and RAD51C variants contribute for about 20% of hereditary breast cancer in Pakistan (2). At this time, there are no data on the disease's contribution from low-penetrance mutations.Genetics play a role in the etiology and progress of BC. In

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

recent years, evidence from different studies emphasizes linking vitamin D deficiency and vitamin D receptor (VDR) gene polymorphism among many populations worldwide, given the strong link with breast cancer (3). Environmental factors such as latitude, season, time of day, atmospheric components, clothing, and sunscreen use, as well as biological factors such as skin color, age, gender, physical activity, fat malabsorption, obesity, genetic variants, and chronic illnesses like cancer, can all influence vitamin D synthesis and bioavailability(4-5). About 70-97% of the average population of Pakistan has deficient vitamin D levels both in men and women, with a higher prevalence in the urban (6). The calcitriol receptor (VDR) is an intracellular hormone receptor called the vitamin D receptor (VDR) located in the nucleus in all tissues and organs. The biologically active form of 25-dihydroxy vitamin D3 binds to VDR and leads to specific gene expression, and to regulate the functions of over 200 genes. Vitamin D is activated by forming a complex with the VDR and involved in several processes in the endocrine system including, the immune system, cell death, cell growth, and division/proliferation, as well as mediating vitamin D's anticancer activities in figure 1(7).

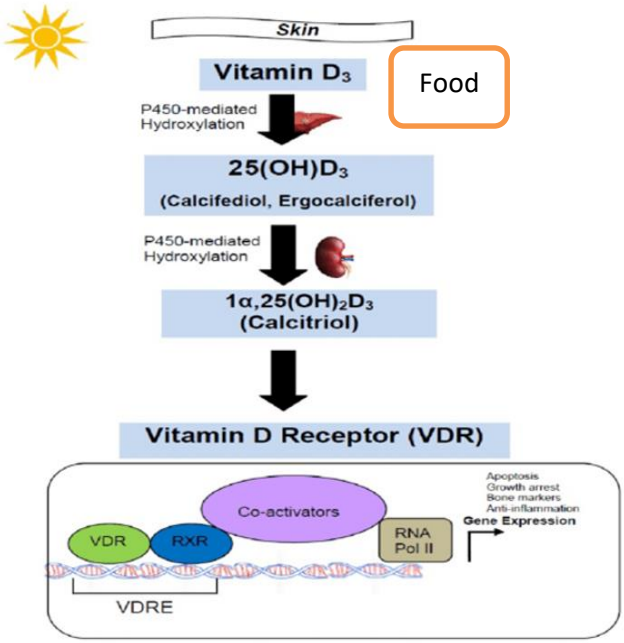

Figure 1. Possible pathways of association of VDR gene polymorphisms and breast cancer

There is a close link between nutrition and genetic factors that define the prevalence and severity of breast cancer. However, the possible association between nutrition and genetics in the context of breast cancer is not well established, particularly in developing societies like Pakistan. Thus, the aim of the current study was to examine the prevalence and severity of vitamin D deficiency and the association of VDR polymorphism with risk of breast cancer in Pakistan.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

2. RESEARCH METHODS AND MATERIELS

*2.1 Study population and design.*A case-control study was conducted at Out Patients Department (OPD) of the Oncology Units after seeking ethical permission from the concerned organizations. Based on gender, oncologist-confirmed histology, and the presence of initial breast cancer, newly diagnosed (in the last one month) women were selected randomly. Same area residence, age-match (± 2) years were the prerequisites of the hospital and visitor-based controls. Both cases and controls were exposed to the same risk factors, and the controls were remarkably similar to cases in every aspect except for the presence of the disease. Consent from the subjects was taken in order to successfully commence the study.

2.2 Sample size. The sample size was calculated for cases and controls based on prior sample size calculation recommendations (8) and estimates that the probability of exposure among controls is 70% and the correlation coefficient for exposure between matched cases and controls is 0.2. The true odds ratio for disease in exposed subjects versus unexposed subjects was 3, and the total sample size required for the present case-control study was 200 total participants (n= 100 cases and n= 100 controls). This odds ratio equals 1 with an 80% power, assuming a Type I error probability of 5% for this test of the null hypothesis.

Ethics Approval. The ethics committee of IRNUM (Institute of Radiotherapy and Nuclear Medicine-Peshawar and KTH (Khyber Medical Hospital) grant the approval for commence of this research study. All participants involved provided written informed consent form before participation in the research.

2.3 Sample collection. Respondents were interviewed on structured questionnaires, to collect data on socio-demographic variable such as age, education status, occupation status, and marital status. Data regarding reproductive characteristic such as age at first menarche, age at first live pregnancy, parity, breast-feeding practice, family history of BC, along with physical activitywere collected. Women anthropometric measurements including weight, height, waist and hip circumferences were taken using standardized tools (9). Body mass index was calculated using weight and height measurements while waist to hip ratio was calculated from respondent waist and hip measurements (10).

2.4 Blood sample collection and DNA isolation

Peripheral blood samples (5 ml) for all cases and controls were collected into an EDTA vials and genomic DNA was extracted from peripheral blood lymphocytes using standard phenol–chloroform extraction method. Blood was first digested with lyses buffer I (30 mMTris, 5 mM EDTA and 50 mMNaCl) and lyses buffer II (20% SDS, 100 mg/ml proteinase K) followed by the extraction with Tris saturated phenol and chloroform-isoamyl alcohol (24:1) and finally recovered by ethanol precipitation. These genomic DNA were then used for genotyping of ApaI, TaqI and Fok1 polymorphism in VDR gene.

2.5 Genotyping of ApaIrs 7975232, TaqIrs 731236 polymorphism and Fok1rs2228570.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

The DNA segment surrounding ApaI site was amplified by PCR using 50 ng of DNA in a final volume of 20 ml containing 1x PCR buffer, 0.2 mMdNTPs, 3 mM MgCl2, 0.5 mM forward primer 5/-GTGGGATTGAGCAGTGAG-3/),0.5mM reverse primer (5/-ATCATCTTGGCATAGAG- 3/), and 0.8 units of Taq DNA polymerase (MBI fermentas).

The PCR reaction was carried out in a Eppendorf thermocycler with an initial denaturation step of 5 min at 94 ^{0}C, followed by 30 cycles at 94 ^{0}C for 45 s, annealing at 56 ^{0}C for 45 s, and extension at 72 ^{0}C for 45 s with final extension at 72 ^{0}C for 1 min. For TaqI site, the PCR reaction was carried out at 94 ^{0}C for 5 min, followed by 30 cycles at 94 ^{0}C for 45 s, 60 ^{0}C for 45 s and 72 ^{0}C for 45 s and one final cycle of extension at 72 ^{0}C for 1 min containing 20 ml reaction mix including 50 ng of genomic DNA, 1x Taq polymerase buffer, 2 mM MgCl2, 0.2 mMdNTPs, 0.5 mM of forward primer (5/-CAGAGCATGGACAGGGAGCAAG- 3/) and 0.5 mM of reverse primer (5/-TGGATCATCTTGGCATAGAGCAGG-3/) and 0.8 units of Taq DNA polymerase.The Fok 1 genotyping only underwent Sanger sequence by third party. The digested fragments of Apa1and Taq1 were electrophoretically (100 V) run on gel electrophoresis and visualized with ethidium bromide whereas the digested fragments of Apa1 and TaqI were resolved in 3% agarose gel stained with ethidium bromide.ApaI and TaqI genotypes were defined by capital letters (A and G, respectively) in the absence of the restriction site and by small letters (a andg) where the restriction site was present. For sequencing, purified products of DNA samples were used to genotype VDR gene, were sequenced by a third party (China) using automatic Sanger sequencing techniques. Comparison was undertaken by using online software programs nucleotide Basic Local Alignment Search Tool (nBLAST). Comparison gave the exact genotypes, and the results of allele specific PCR were confirmed by sequencing.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

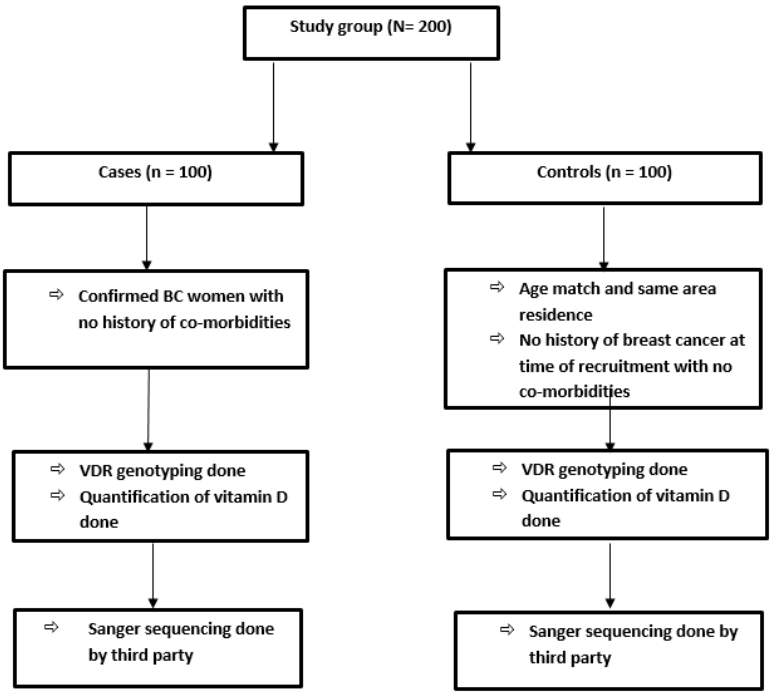

Figure 2. Study flow chart depicting study group and participation recruitment

2.6 Statistical analysis. Data was incorporated and statistical analyses were performed using SPSS software version 20 (IBM). The two groups were compared in terms of demographic, reproductive history, anthropometric, serum vitamin D status and the presence of VDR gene polymorphism. At the beginning of the study t test (for quantitative variables) and chi-square (for qualitative variables) methods were used. Then, the relationship between BC and the risk allele of rs9939609 polymorphism was investigated using the logistic regression method. The results were expressed as odds ratio (OR) with 95% confidence intervals (CI). By considering the significance level of $P < 0.05$. Percentage frequencies of genotypes for Hardy-Weinberg equilibrium was calculated using Microsoft Excel 2010. Polymorphism was tested for deviation from Hardy-Weinberg equilibrium using chi-square 70 test and “Oege Online Hardy-Weinberg Equilibrium Calculator”

3. RESULTS

Data was checked for normality, and entry errors using descriptive statistics such as histogram, mean, frequency, etc. before analysis. Table – 1 shows results on descriptive characteristics of the population by case/control status. Mean differences in age, age at first menarche, marital status, parity, menopause status and family history of BC of cases and controls were non-significant. Data regarding education status, occupation status, age at first pregnancy, breast-feeding practices and physical activity showed significant result. It depicts results on the current anthropometric status of the studied groups, BC

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

patients had higher mean BMI (Asian cut-off values) and waist to hip ratio (WHR) ($p<0.05$), as compared to healthy controls.

Table 1

Descriptive characteristics of the population by case/control status

Characteristics		**Mean ±SD/N%**			
Variable		Total	Case	Control	P value[1]
Age		200	45±6.3.3	45±2.7	NS
Education level	Illiterate	100	59(59%)	41(41%)	<0.05
	Educated	100	36(36%)	64(64%)	
Occupational status	Housewives	100	53(53%)	47(47%)	<0.05
	Working	100	33(33%)	67(67%)	
	Age at menarche (year)	200	12±0.05	13±0.06	NS
Marital status	Single	20	9 (9%)	11 (11%)	NS
	Married	180	91(91%)	89 (89%)	
Age of 1st pregnancy (year)		84	21±0.23	20±0.16)	<0.01
Parity (Number)		84	4±0.12	4.1±0.12	NS
Breastfeeding practices	No / rare	23	14(14%)	9(9%)	0.05
	Yes	177	86(86%)	91(91%)	
Menopausal Status	Premenopausal <45 year	100	49(49%)	51(51%	NS
	Postmenopausal >45years	100	51(51%)	49(49%)	
Family history of breast cancer	No	172	84(84%)	88(88%)	NS
	Yes	28	16(16%)	12 (12%)	
Physical activity	Not at all	160	86(86%)	74(74%)	<0.05
	< 30 minutes	23	7(7%)	16(16%)	
	≥ 30 minutes	17	7(7%)	10(10%)	
Weight indices	Body Mass Index (BMI)	200	24.4±5.0	23.7±4.1	<0.05
	Waist to Hip Ratio (WHR)	200	0.80±0.0	0.79±0.0	<0.05

[1] At α=0.05, To compare group variations, student t tests or chi-square tests were used.

Findings from the present study suggest that vitamin D deficiency is common among Pakistani females with breast cancer and healthy women, with 76% and 60% respectively. About 18% of cases and 23% of healthy women had insuffient vitamin level,

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

while only 6% of women with BC and 17% of healthy women had sufficient level of vitamin D level. In conclusion, low vitamin D levels increase the risk of breast cancer in this study.

Table 2

Nutritional state in cases and controls based on biochemical assessments

Variable	Total	Mean±SD N (%)		P-Value
		Cases (n=100)	Control (n=100)	
Serum Vitamin D (ng/dl)	100	15.1±9.4	19.6±9.3	<0.01
Deficient >20ng/dl	136	76(76%)	60(60%)	<0.05
Insufficient 21-29ng/dl	41	18(18%)	23(23%)	
Sufficient >30ng/dl	23	6(6%)	17(17%)	

SD=Standard Deviation; N (%)= Number of Women (Percentage)

There were no significant differences in the frequency of GA and AA genotypes between healthy subjects and breast cancer patients, in Apa1 and Fok 1. As related in Taq 1, there was a significant increase ($p<0.05$) in the prevalence of the heterozygous AG genotype in BC patients in comparison to healthy subjects (37% versus 25%, respectively, adjusted OR=2.1, Cl=1.09-4.1, $p<0.05$).TheHardy Weinberg equation (HWE) was not consistent with VDR gene, as data was collected from a specific population with a small sample size. These findings suggest that the genotypes of the VDR gene have no role in the incidence of BC in Pakistani women, and the development of breast cancer in women could be better clarified with a large study.

Table 3

The frequency of genotypes and alleles at VDR gene in women with breast cancer and healthy control

rs 9939609	Cases (%) (n=100)	Control (%) (n=100)	X^2 *P Value*	*Adjusted OR(95%CI)
GG	39(39%)	56(56%)	4.1 0.00	Ref
GA	39(39%)	31(31%)		1.8(0.96-5.3)
AA	22(22%)	13(13%)		2.4(1.09-5.3)**
rs731236			9.1 0.00	
AA	34(34%	49(49%)		Ref
AG	37(37%)	25(25%)		2.1(1.09-4.1)*
GG	29(29%)	26(26%)		1.6(0.8-3.1)
rs2228570			18.6 0.00	
GG	29(29%)	34(34%)		Ref
GA	18(18%)	11(11%)		0.7(0.2-3.1)
AA	3(3%)	5(5%)		1.9(0.7-4.7)

*Adjusted for Age, Menopause status, parity and family history of BC & BMI, ** P value <0.0

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

In table 4,the serum vitamin D concentrations were slightly lower ($p<0.05$) in breast cancer patients with mutant homozygous AA genotype of rs7975135 SNP in VDR gene) as compared to healthy subjects. Similarly, in Taq1 SNPrs 731336 both heterozygous and mutant homozygous had serum vitamin D level were lower in BC cases as compared to healthy women. In Fok1 SNP,rs 2228570, the serum Vitamin D level lower in mutant homozygous of BC cases as compared to healthy controls. The findings of this study showed that in breast cancer and healthy Pakistani women serum vitamin D concentrations were affected by genotypes of the rs731236, rs2228570, and rs7975232 SNPs of the VDR gene.

Table 4 :

Serum Vitamin D level as affected by the genotypes of rs731236, rs2228570, and rs7975232 SNPs of VDR gene in Pakistani women with BC and controls

VDR SNP,s	Genotype	Serum Vitamin D (ng/dl)		p-value[1]
		Cases	Control	
Apa1 rs7975135	CC	15±10.9	17.5±6.9	NS
	CA	14.7±7.9	21±10.6	0.056
	AA	14.9±10.3	19.9±10	<0.05
Taq1 rs 731336	AA	16.6±12.3	18.6±9.8	NS
	AG	14.6±8.3	21.6±7.6	<0.05
	GG	12.6±6.3	19.6±9.4	<0.05
Fok1 rs 2228570	GA	11.3±5.6	20±10.3	<0.01
	GA	11.6±6.3	20±11.2	<0.05
	AA	10.8±4.8	20±10.7	NS

[1] At α=0.05, to compare group variations, student t tests or chi-square tests were used.

4. DISCUSSION

The identification of 1,25(OH)2-D3 and VDR as components of a signalling network affecting breast tissue proliferation, differentiation, and apoptosis raises the idea that Vitamin D may protect against mammary transformation and that common VDR gene variations are linked to breast cancer risk.The aim of this study was to examine the vitamin D status, *VDR* genetic polymorphisms on BC risk in a Pakistani population. The results showed vitamin D status showed a statistically significant interaction with breast cancer risk. In parallel, that none of the two examined SNPs of VDR gene was associated with breast cancer, except the Taq1 SNP may associate with BC risk, would be clarified with large sample size. The current study findings agree to others who concluded that there is no connection between VDR gene/polymorphisms/SNPs variations and ApaI and breast cancer(12-14). The systematic meta-analysis reviewed by Iqbal, (15) found that VDR ApaI polymorphisms were associated with breast cancer, and thus not in agreement with this study findings. Nemenqani et al. (16) reported that VDR Apa1 polymorphisms correlated significantly with tumor differentiation, with VDR ApaI polymorphic alleles more

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

likely to be found in anaplastic tumors (Aa or aa). The vitamin D3 receptor (VDR) as a nuclear receptor complexed with its ligand 1-, 25-dihydroxycholecalciferol (1, 25(OH) 2 D3) has the potential to modulate gene expression. The VDR (Apa1) is normally expressed in the mammary gland and it inhibits estrogen-driven proliferation and maintains differentiation, and it may play a role in the negative growth regulation of mammary epithelial cells. As a result, VDR polymorphisms may influence vitamin D metabolism and 25(OH) D levels which known to be preventive role for developing breast cancer reported by El-Shorbagy et al. (17). Regarding our observations relating to the rs731236 SNP of the VDR gene, this finding suggests that this particular SNP may have a potential prognostic value for breast cancer patients with Taq1 SNPs. 18 proposed that the TaqI variant of VDR positively correlated with estrogen receptor-positive tumors, but not estrogen receptor-negative tumors. Other research contrasted with the findings of the present report with El-Shorbagy et al. (17), who found no significant variations between women with breast cancer patients and controls regarding the VDR gene (rs731236 T>C) polymorphism. Song et al. (19) was the first to show that the VDR TaqI variant was not linked to breast cancer. However, it was linked to a significant increase in the risk of lymph node metastasis, showing solid signs of breast cancer progression. Similarly, a research published by Li et al. (20), there was no significant increase in breast cancer risk in women homozygous or heterozygous for the t allele in Taq1 SNP of VDR gene. In contrast, several epidemiological and laboratory studies indicate that the VDR FokI polymorphism genotypes, particularly the ff genotype, correlate with an elevated risk of developing breast cancer (21). Consistent with this result, Kazemi et al. (22) reported/demonstarted that breast cancer studies in China and Iran, on a population of women have found that there hasn't been any evidence of a correlation amid the FokI polymorphism and the threat of breast malignancy. Genetic factors, environmental conditions, disease progression, estrogen and estrogen receptor levels, laboratory designs, and genotyping techniques, all play a crucial role in the generation of inconsistent results relating to studies focusing onVDR gene polymorphisms, as reported in case-control trials (23). The researcher Matini et al. (24)stated that VDR is a ligand that mediates the regulatory effects of 1,25(OH)2D3, because VDR function is important for 1,25(OH)2D sensitivity in cancer cells, loss of VDR function during carcinogenesis results in a loss of 1,25D's anti-proliferative effects. Furthermore, VDRs are responsible for the transcriptional regulation of a number of hormone-responsive genes, which affects breast cancer, which is known to be strongly influenced by the hormonal milieu and variation in genes that are responsive to such hormones (24).

However, there were some limitations in our present study. First, further studies on a larger sample size will be required on VDR gene polymorphism. Second, nutrient calcium and vitamin D intake, and its correlation with genetic polymorphism were not determined.

Conclusion

In conclusion, the current study shows a significant association between vitamin D deficiency and the risk of breast cancer among Pakistani females. We also report that GA

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

genotype of Taq1 SNP may be susceptibility risk factors for BC development. Larger population- based studies are warranted to confirm these results.

Recommendation

More studies required to screening other SNPs of VDR that correlate with breast cancer incidence.

Conflict of interest statement

None.

Acknowledgments

We thank the patients and their families, without whose cooperation this study would not have been possible.

Acknowledgments

We gratefully acknowledge the contribution of the study participants; without them the study would not have been possible.

Author's contribution

The author's responsibilities were as follows: HN collected the data, did the experiments, and wrote the paper. SS constructed the project design, supervised the study.

ZUD was responsible analyze the data and critically review the paper.

Funding

None

References

1. Hussain, I., A. Majeed, I. Masood, W. Ashraf, I. Imran, H. Saeed, A. Ur Rehman, F.K. Hashmi, F. Saleem and M. Akbar. A national survey to assess breast cancer awareness among the female university students of Pakistan. 2022. PloS one. (17): e0262030.
2. Rashid MU, Muhammad N, Faisal S, Amin A, Hamann U. Constitutional CHEK2 mutations are infrequent in early-onset and familial breast/ovarian cancer patients from Pakistan. BMC Cancer 2013; 13 (6):312–8.
3. Suksawatamnuay, S., Sriphoosanaphan, S., Aumpansub, P., Aniwan, S., Thanapirom, K., Tanasanvimon, S., Thaimai, P., Wiangngoen, S., Ponauthai, Y., Sumdin, S. and Angspatt, P.Association between Vitamin D Receptor Single-Nucleotide Polymorphisms and Colorectal Cancer in the Thai Population: A Case-Control Study. BioMed research international, 2020.
4. Mousavi, S.E., H. Amini, P. Heydarpour, F.A. Chermahini and L. Godderis. Air pollution, environmental chemicals, and smoking may trigger vitamin D deficiency: Evidence and potential mechanisms. Environment international. 2019. (122): 67-90.
5. Tsiaras, W.G. and M.A. Weinstock. Factors influencing vitamin D status. Acta dermato-venereologica. 2011. (91): 115-124.
6. Rasheed, S., M. Shafiq, O. Sanaullah and S. Chiragh. Comparison of vitamin d and lipid profile between rural and urban population. Pakistan Journal of Physiology 2018. 14: 32-35.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

7. Zmijewski, M.A. and C. Carlberg. Vitamin D receptor (s): In the nucleus but also at membranes? Experimental Dermatology. 2020. (29): 876-884.
8. Dupont WD Power calculations for matched case-control studies. Biometrics, (1988). 1157-1168.
9. World Health Organization. WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. World Health Organization; 2006.
10. Waist circumference and waist-hip ratio. Report of a WHO Expert Consultation. Geneva: World Health Organization. 2008 Dec;2008:8-11..
11. Cox DG, Kraft P. Quantification of the power of Hardy-Weinberg equilibrium testing to detect genotyping error. Human Heredity. 2006;61(1):10-4.
12. Chakraborty, A., Mishra, A.K., Soni, A., Regina, T., Mohil, R., Bhatnagar, D., Bhatnagar, A., Chintamani, C., Sharma, P.C. and Saxena, S. Vitamin D receptor gene polymorphism (s) and breast cancer risk in north Indians. 2009. Cancer detection and prevention, 32(5-6), pp.386-394.
13. Hamid, S.O.Y. Assessment of Serum vitamin D levels and Vitamin D Receptors Gene Polymorphisms Among Sudanese Females with Breast Cancer Attending Radio Isotope Center Khartoum, Sudan University of Science & Technology. 2017. PhD thesis.
14. Mishra, D.K., Y. Wu, M. Sarkissyan, S. Sarkissyan, Z. Chen, X. Shang, M. Ong, D. Heber, H.P. Koeffler and J.V. Vadgama. Vitamin D receptor gene polymorphisms and prognosis of breast cancer among African-American and Hispanic women.2013. PloS one 8: 57967.
15. Iqbal, M.U.N. and Khan, T.A. Association between vitamin D receptor (Cdx2, Fok1, Bsm1, Apa1, Bgl1, Taq1, and Poly (A)) gene polymorphism and breast cancer: a systematic review and meta-analysis. 2017.Tumor Biology, 39(10), p.1010428317731280.
16. Nemenqani, D.M., R.A. Karam, M.G. Amer and T.M. Abd El Rahman. Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer. 2015. Gene (558): 215-219.
17. El-Shorbagy, H.M., N.H. Mahmoud and S. Sabet. Association of vitamin D receptor gene polymorphisms with breast cancer risk in an Egyptian population. 2017. Tum. Bio. (39): 1-9.
18. Kazemian, E., M.E. Akbari, N. Moradi, S. Gharibzadeh, A.M. Mondul, Y. Jamshidi-Naeini, M. Khademolmele, K.R. Zarins, N. Ghodoosi and A. Amouzegar. Vitamin D Receptor Genetic Variation and Cancer Biomarkers among Breast Cancer Patients Supplemented with Vitamin D3: A Single-Arm Non-Randomized Before and After Trial. 2019. Nutrients (11): 1264-1273.
19. Song, D., Y. Deng, K. Liu, L. Zhou, N. Li, Y. Zheng, Q. Hao, S. Yang, Y. Wu and Z. Zhai. Vitamin D intake, blood vitamin D levels, and the risk of breast cancer: a dose-response meta-analysis of observational studies. Aging (Albany NY). 2019. (11): 12708-127721).
20. Li, J., B. Li, Q. Jiang, Y. Zhang, A. Liu, H. Wang, J. Zhang, Q. Qin, Z. Hong and B.-a. Li. Do genetic polymorphisms of the vitamin D receptor contribute to breast/ovarian cancer? A systematic review and network meta-analysis. 2018a. Gene (677): 211-227.
21. Hoseinkhani, Z., M. Rastegari-Pouyani, F. Tajemiri, K. Yari and K. Mansouri. Association of vitamin D receptor polymorphisms (FokI (Rs2228570), ApaI (Rs7975232), BsmI (Rs1544410), and TaqI (Rs731236)) with gastric cancer in a Kurdish population from west of Iran. Reports of Biochemistry & Molecular Biology. 2021. (9): 435-439.
22. Kazemi, S.M., A. Esmaieli-Bandboni, Z.V. Malekshahi, M.S. Sardood, M. Hashemi, K. Majidzadeh, M. Kadkhodazadeh, R. Esmaili and B. Negahdari. Vitamin D receptor gene polymorphisms and risk of breast cancer in Iranian women. Annals of Medicine and Surgery. 2022. (73): 103150.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online): 0493-2137
E-Publication: Online Open Access
Vol:55 Issue:07:2022
DOI 10.17605/OSF.IO/V59UY

23. Dogra, A.K., A. Prakash, S. Gupta, M. Gupta and S.A. Bhat. Genetic variations of vitamin D receptor gene and steroid receptors status in breast cancer risk: An updated review. 2022. Adv. in Bio. Sci. and Tech. (4): 1-11.

24. Matini, A.H., N. Jafarian-Dehkordi, B. Bahmani, M. Sharifi, D. Jahantigh and T. Mazoochi. Association of ApaI and TaqI polymorphisms in VDR Gene with Breast Cancer. 2020. APJCP (21): 2667.