RESEARCH PAPER

Association between Taq1 rs: 731236 SNP of VDR gene and risk factor among Sudanese patients with breast cancer

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Abstract

Introduction: Breast cancer is the most frequent cancer in women globally as well asSudan, it has risen to become the second commonest cause of death in women.Vitamin D receptor (VDR) is a ligand-dependent transcription factor. Vitamin D-ligandedVDR, has anti-proliferative properties in a variety of tumor types by inducing cell cycle arrest, senescence, differentiation, and death. The role of VDR TaqI polymorphism is currently unknown. However, studies suggest that these polymorphisms may affect messenger RNA (mRNA) stability.

Methods: This case control 198 participants, (97) breast cancer patients and another 101control group.Data wascollectedby a questionnaire. 2 ml of venous blood was collected and stored at -20 till DNA extraction. Phenol chloroform method was used for DNA extraction. VDR TaqI was examined and genotyped using CTPP-PCR after designing of suitable primers and PCR condition. Then data was analyzed statistically using the SPSS program (version 21) and the SNPStats online tool.

Results:the three genotypes reported in this study for TaqI SNP (CC, TC, and TT) were evenly distributed throughout cases and controls, so according to this findings there is no statistically significant association of this SNP with breast cancer risk (p. value 0.650) (OR(95%CI) 1.39 (0.64-3.00), 1.00 (0.54-1.87), 1.00).

Conclusions: This study found no association of TaqI polymorphism and breast cancer riskfactors

Keywords: Taq1; Breast cancer; rs:731236; VDR SNP; Sudanese

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Introduction

Breast cancer is the most common malignancy in women; diagnoses are rapidly increasing each year, and it has risen to become the second commonest cause of death in women.(1). Breast cancer strikes 43.1/100,000 people worldwide, according to Globocan (2018). BC is the most frequent cancer in Sudan, according to data from a hospital-based registry.(2). According to the Khartoum Cancer Registry, the state of Khartoum has a BC rate of 25.1 per 100,000(3). Apart from age, reproductive variables (e.g., null parity, older age at first birth), exogenous hormone use, and a family history of breast cancer all raise the

risk of breast cancer in women aged 50 and over. (4). Only 10–20 percent of breast cancers with a documented family history have a causal mutation of the breast cancer susceptibility genes BRCA1 and BRCA2 (5). The vitamin D receptor (VDR)is belongs to the nuclear receptor family and is a ligand-dependent transcription factor. (6)The VDR receptor gene spans 60 kb of DNA and is found on the long arm of chromosome 12 (12q12–14). (7).Vitamin D-ligandedVDR, has anti-proliferative properties in a variety of tumor types by inducing cell cycle arrest, senescence, differentiation, and death.(8). The expression of VDR is reduced in breast cancers(9)The SNP VDR TaqI (rs731236) is a substitution of T for C in exon 9, resulting in a synonymous alteration at codon 352. It is situated near the 3' untranslated region (UTR).(10)The precise role of VDR TaqI is currently unknown. However, studies suggest that these polymorphisms may affect messenger RNA (mRNA) stability.(11).

Methods

Study subjects:

This case control analytic study conduct in National Cancer Institute University of Gezira. The study involved 198 participant (97) breast cancer patients their ages between 25 and 80 years, and another (101) apparently healthy females with the same (age rang and environmental factors) as control group.

Ethical consideration:

Ethical permission was taken from ministry of health, Gezira state. An inform consent was designed, and taken from each participant in the study.

Inclusion and Exclusion criteria:

Diagnosed Breast cancer female patients age 25 – 80 years old, attending national cancer institute Gezira state, when the Patients with secondary Breast cancer were excluded

Sample collection and DNA extraction:

Two ml of venous blood was collected from each participant by using a sterile syringe and proper sample collection procedure, then discarded into a labeled EDITA blood container andstored at -20 until extraction.DNA was extracted by manualphenol:chloroformmethod**,** concentration measured at 260 nm and purity determined by measuring the A260/A280 ratio.

VDR TaqI was examined and genotyped using CTPP-PCR; in this approach, allele-specific DNA products are generated by putting suitably Newly designed two-pair primers (four primers) into a conventional PCR tube, generating distinct bands size to allow discrimination between variants.

SNP RS:	PRIMER SEQUENCE	PRODUCT SIZE
Taq1 (rs731236)	CPXFw: 5 - AGGTGCGCCCATGGAAGGA -3 AP236C: 5- CAGGACGCCGCGCTGCTC -3 AP236T: 5- CAGGACGCCGCGCTGCTC -3 CPXRev: 5 – TGGATAGGGGAGGTGGCAG -3	382 bp (C allele) 271 bp (T allele) Common band 617 bp

Total volume of PCR reaction mixture was 15-µL containing 5 µL of 50 ng template DNA, 0.5 µL of common primers (CPXFw, CPXRev) and 1 µL allele specific primer (AP236C , AP236T) (10 pmol), and 7 µL master mix Taq polymerase. The following condition was used for the PCR: initial denaturation at 95°C for 3 min, then denaturation at 94°C for 40 s, annealing at 40°C 60 s, 72°C for 40 s extension for 40 cycles, and final elongation at 72°C for 3 min, with a final hold at 4°C. Utilizing UV transillumination, the

PCR products were seen on a 2 percent agarose gel with ethidium bromide.The following are the varied sizes of DNA fragments:The C allele 382 bp and the T allele 271 bp, whereas the Common band (internal control) 617 bp.

Statistical analysis:-

The data was analyzed statistically using the SPSS program (version 11) and the SNPStats online tool. To detect association between VDR polymorphism and the risk of breast cancer, we use odds ratios and 95 % confidence intervals. The significance level was set at P >0.05

Results

Overall, 97 breast cancer patients attending National Cancer Institute were enrolled. Breast cancer patients were in the age group of <45 years representing 57%, and 43% in age group > 45years. A majority of patients had rural residences (70.1%). Only 27.8% of them were currently unmarried being divorced, widowed or separated while the remaining 72.2% were married. The distribution of patient according to occupation had 78.4 was housewife, while the remaining were employer, worker and farmer (Table 1). The Gynecological characteristics of cases include the age at menarche, parity and the menopausal status. 36 (72%) of cases their ages at menarche were < 14 Years and 14 (28%) > 14 Years, regarding parity 23% (18) had not child, 61 % (48) had children from 1 – 5 child, and 16% (13) had more than 5 child. 48 % (43) were postmenopause and 52% (47) were at the Premenopausal period (Table 2). Pathological features of tumor include lymph nodes invasion, expression of estrogen receptor (ER), progesterone receptor (PR), histological type and grade of tumor. Regarding tumor grade, 35 (36.1%) grade III, Grade II 28 (28.9 %), and 3 (3%) with Grade I at presentation (Table 3). The frequency of T allele (wild type allele) in cases and control was 119 (61%), 116 (57%) and C allele 75 (39%), 86 (43%) respectively (Figure 1). The count and percentage of CC, TC, and TT genotypes among cases and controls is illustrated in Table (4). Their frequencies were 17 (18%), 23 (23%), 41 (42%), 40 (40%), 39 (4%), 38 (38%) for case and control respectively. there is no significant deference between cases and controls in both genotypes distribution (p= 0.650). As shown in Table (5) there is no significant variation in distribution of different VDR rs.731236 genotypes TT, TC and CC with in different age groups p = 0.240. Age at menarche p = 0.074. BMI p. value 0.600. Residence p = 0.940. Menopause status p = 0.140. Race p = 0.210. There was insignificant difference of (TT, TC and CC) genotypes distribution among clinical characteristics of cases. Tumor grade, grade I: 2 (7.1%), 0 (0%), 1 (12.5%), grade II: 8 (28.6%), 16 (53.3%), 4 (50.0%) and grade III: 18 (64.3%), 14 (46.7%), 3 (37.5%), respectively to genotypes above. Estrogen receptor status: ER Positive 10 (40%), 12 (48%), 3 (12%), ER Negative 16 (39%), 21 (51.2%), 4 (9.8%) respectively. Progesterone receptor expression: PR Positive was 9 (32.1%), 15 (53.6%), 4 (14.3%). PR Negative was 17 (43.6%), 18 (46.1%), 4 (10.3%) respectively (Table 6).

Table 1 Scio -demographic characteristic:

CHARACTERISTIC	N (%)
Age group	
> 45 years old	42 (43)
<45 years old	55 (57)
Residences	
Rural	68 (70.1)
Urban	29 (29.9)
Occupation	
Employer	15 (15.5)
Worker	4 (4.1)
House wife	76 (78.4)
Farmer	1 (1)
Marital status	
Single	11 (11.3)
Married	70 (72.2)
Widowed	13 (13.4)
Divorced	3 (3.1)

Table 2 Gynecological characteristics

CHARACTERISTIC	N %
Age at menarche	
< 14 Years	36 (72)
> 14 Years	14 (28)
Parity	
0	18 (23)
1- 5	48 (61)
>5	13 (16)
Menopause	
Postmenopausal	43 (48)
Premenopausal	47 (52)

Table 3 Pathological characteristics:

CHARACTERISTICS	N (%)
ER	
Positive	25 (25.8)
Negative	41 (42.3)
PR	
Positive	28 (28.9)
Negative	39 (40.2)
Histological Grade	
Grade I	3 (3.1)
Grade II	28 (28.9)
Grade III	35 (36.1)
Unknown	27 (27.8)

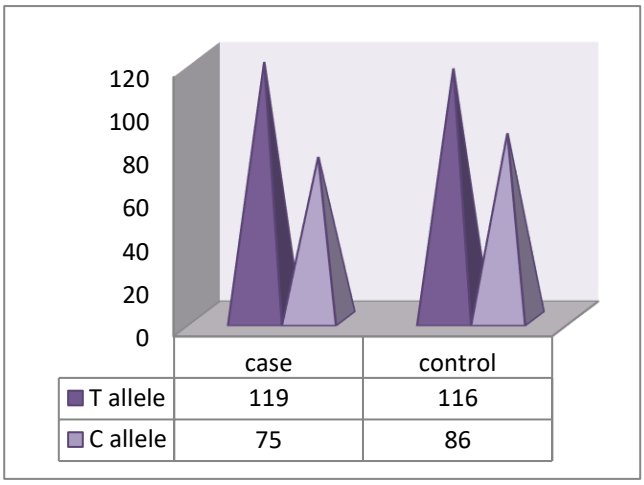

Figure 1 Frequency of rs.731236 alleles in cases and controls

Table4 Distribution genotypes among cases and controls:

GENOTYPE	CASES N%	CONTROL N%	OR(95%CI)	P. VALUE
C/C	17 (18%)	23 (22.7%)	1.39 (0.64-3.00)	0.650
T/C	41 (42%)	40 (39.6%)	1.00 (0.54-1.87)	
T/T	39 (40%)	38 (37.7%)	1.00	

Table5:Association genotypes with patient's characteristics:

CHARACTERISTICS	GENOTYPE T/T N%	GENOTYPE T/C N%	GENOTYPE C/C N%	P. VALUE
Age **less than45** **more than45** **OR**	26(46) 13 (31) 1.00	20 (36) 21 (5) 2.13(0.86-5.29)	9(16) 8 (19) 1.84 (0.57-5.99)	0.240
Age at menarche **less than14** **More than14** **OR**	5 (21) 13 (5) 1.00	14 (58) 8 (31) 0.22 (0.06-0.85)	5 (21) 5 (19) 0.40(0.08-2.15)	0.074
BMI **High BMI** **NormalBMI** **OR**	20 (36) 19 (45) 1.00	24 (44) 17 (4) 0.74(0.30-1.80)	11 (2) 6 (14) 0.56 (0.17-1.85)	0.600

Residence **Rural** **Urban** **OR**	28 (41) 11 (38) 1.00	28 (41) 13 (45) 1.19 (0.45-3.12)	12 (18) 5 (17) 1.08 (0.30-3.86)	0.940
Menopause **Post menopause** **Pre menopause** **OR**	13 (3) 24 (51) 1.00	22 (51) 16 (34) 0.40(016-1.02)	8 (19) 7 (15) 0.48(014-1.66)	0.140
Race **African** **Non- African** **OR**	13 (45) 26 (38) 1.00	14 (48) 27 (4) 0.92(0.36-2.34)	2 (7) 15(22) 3.30(0.64-16.97)	0.210

Table 6: Frequency and association of genotypes with clinical characteristics.

CHARACTERISTICS	GENOTYPE T/T N%	GENOTYPE T/C N%	GENOTYPE C/C N%	P. VALUE
Tumor grade **Grade I** **grade II** **grade III**	2 (7.1) 8 (28.6) 18 (64.3)	0 16 (53.3) 14 (46.7)	1 (12.5) 4 (50.0) 3 (37.5)	0.173
Estrogen receptor **Positive** **Negative** **OR**	10 (40) 16 (39) 1.00	12 (48) 21 (51.2) 0.94 (0.32-2.76)	3 (12) 4 (9.8) 1.43 (0.25-8.13)	0.890
Progesterone receptor **Positive** **Negative** **OR**	9 (32.1) 17 (43.6) 1.00	15 (53.6) 18 (46.1) 1.72 (0.57-5.15)	4 (14.3) 4 (10.3) 2.59 (0.49-13.79)	0.450

Discussion

Vitamin D receptor (VDR) is a nuclear receptor superfamily ligand-dependent transcription factor. (12).The VDR gene is located on chromosome 12q13 and has around 470 single-nucleotide polymorphisms (SNPs) that might alter VDR structure and function..(10). The VDR TaqI SNPs (rs731236), which cause a silent codon change, are considered to be essential in post-transcriptional control of gene expression. (13).The purpose of this study is to determine the prevalence of the VDR TaqI SNP in Sudanese females and it is association with breast cancer.This case control research was conducted at the National Cancer Institute at the University of Gezira; one hundred and ninety eight participants were enrolled in study.The control group consisted of 101 women who appeared to be in good health, while the case group consisted of 97well diagnosed breast cancer female patients. The mean age of Caseswas 45 with rang of 25 to 80 years old. The bulk of the cases were under 45 years old, which is a warning sign that breast cancer prevalence is more common at younger ages and this may be the result of different dietary patterns, which depend directly on starches, fats, and preservatives, as well as the widespread use of cosmetics and harmful weight gain products. Furthermore, the rate of awareness about the importance and how to conduct self-examination of breast cancer is higher which increases rates Diagnostics in this category. Majority of cases were married, housewife, and rural resident. Married females at more risk of developing hormonal disturbances thus may be due to the usage of contraceptive control drugs. In Sudan, where urban inhabitants suffer from poor hygiene and little knowledge about breast cancer early detection and the benefit of early medical care, as well as both poor economic status,and health services in those regions should be taken into account regarding breast cancer risk.

The frequency of wide type allele (T) gave almost the same frequency in case and control.

Moreover, the frequency of minor allele C was a pit higher in control than in case. This could be attributed to the socio-demographic characteristics of study population. The three genotypes reported in this study (CC, TC, and TT) were evenly distributed throughout cases and controls, so according to this findings there is no statistically significant association of this SNP with breast cancer risk (p. value 0.650) (OR(95%CI) 1.39 (0.64-3.00), 1.00 (0.54-1.87), 1.00).These findings agree with number of studies include(14), (10)(15)(16)which indicates that no association of breast cancer this SNP. While dis agree with study done by ((11) in which they found strong association with breast cancer. This variation between results may be attributed to the variations between the study population in terms of the environment and the nature of life;also we should not forget the ethnic diversity. Insignificant variation also was observedin genotypes distribution among both patients' characteristics (age, age at menarche, BMI, residence, menopause and patients race). And clinical characteristics (ER, PR and tumor grade) p = > 0.050.

Conclusion

The frequency of minor allele C was a pit higher in control than in case. TT, TC and CC genotypes were evenly distributed among cases and controls, being the heterozygous genotype TC the most frequent in both case and control group. Finally there was no association of this SNP with breast cancer risk among Sudanese females. This SNP showed in significant variation in the distribution in through patient's socio-characteristics and clinical characteristics

Conflict of interest: The authors declare no conflict of interest.

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References

1 1. de La Puente-Yagüe M, Cuadrado-Cenzual MA, Ciudad-Cabañas MJ, Hernández-Cabria M, Collado-Yurrita L. Vitamin D: And its role in breast cancer. The Kaohsiung Journal of Medical Sciences. 2018;34(8):423-7.

2. Eltayeb MA, Faggad A, Abbadi OS, Elhassan MMA. Characteristics of Breast Cancer at First Presentation in Sudanese Patients Attending the National Cancer Institute–University of Gezira (NCI–UG). Archives of Breast Cancer. 2020:104-10.

3. Saeed IE, Weng HY, Mohamed KH, Mohammed SI. Cancer incidence in Khartoum, Sudan: first results from the Cancer Registry, 2009–2010. Cancer medicine. 2014;3(4):1075-84.

4. Oh H, Eliassen AH, Beck AH, Rosner B, Schnitt SJ, Collins LC, et al. Breast cancer risk factors in relation to estrogen receptor, progesterone receptor, insulin-like growth factor-1 receptor, and Ki67 expression in normal breast tissue. NPJ breast cancer. 2017;3(1):39.

5. Jian W, Shao K, Qin Q, Wang X, Song S, Wang X. Clinical and genetic characterization of hereditary breast cancer in a Chinese population. Hereditary cancer in clinical practice. 2017;15(1):19.

6. Rashid MU, Muzaffar M, Khan FA, Kabisch M, Muhammad N, Faiz S, et al. Association between the BsmI Polymorphism in the Vitamin D receptor gene and breast cancer risk: Results from a Pakistani case-control study. PLoS One. 2015;10(10).

7. Khan MI, Bielecka ZF, Najm MZ, Bartnik E, Czarnecki JS, Czarnecka AM, et al.

Vitamin D receptor gene polymorphisms in breast and renal cancer: current state and future approaches. International journal of oncology. 2014;44(2):349-63.
8. Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. Nature reviews cancer. 2014;14(5):342-57.
9. Lopes N, Sousa B, Martins D, Gomes M, Vieira D, Veronese LA, et al. 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 Vitamin D pathways unbalanced in breast lesions. BMC cancer. 2010;10(1):1-10.
10. 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. 2014;2:41-9.
11. El-Shorbagy HM, Mahmoud NH, Sabet S. Association of vitamin D receptor gene polymorphisms with breast cancer risk in an Egyptian population. Tumor Biology. 2017;39(10):1010428317727738.
12. Khan TA, Maqbool SA. Vitamin D receptor Cdx-2 polymorphism and premenopausal breast cancer risk in southern Pakistani patients. PLoS One. 2015;10(3):e0122657.
13. Anderson LN, Cotterchio M, Cole DE, Knight JA. Vitamin D-related genetic variants, interactions with vitamin D exposure, and breast cancer risk among Caucasian women in Ontario. Cancer Epidemiology and Prevention Biomarkers. 2011;20(8):1708-17.
14. Hashemi SM, Arbabi N, Hashemi M, Mashhadi MA, Allahyari A, Sadeghi M. Association between VDR Gene Polymorphisms (rs 1544410, rs 7975232, rs 2228570, rs 731236 and rs 11568820) and Susceptibility to Breast Cancer in a Sample of Southeastern Iranian Population. International Journal of Cancer Management. 2017;10(3).
15. Buyru N, Tezol A, Yosunkaya-Fenerci E, Dalay N. Vitamin D receptor gene polymorphisms in breast cancer. Experimental & molecular medicine. 2003;35(6):550-5.
16. McCullough ML, Stevens VL, Diver WR, Feigelson HS, Rodriguez C, Bostick RM, et al. Vitamin D pathway gene polymorphisms, diet, and risk of postmenopausal breast cancer: a nested case-control study. Breast Cancer Research. 2007;9(1):1-7.