Original Article Sudan Medical Laboratory Journal

Vitamin D Receptors Gene Polymorphisms among Sudanese Patients with Prostate Cancer

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Abstract

Background: Prostate cancer has been most frequently diagnosed cancer in men in Sudan regarding to statistics data from the National Cancer Registry. Moreover, it was the second most prevalent cancer in men globally. BsmI, TaqI, FokI, and ApaI restriction enzymes are the most extensively investigated single nucleotide polymorphisms (SNP) in VDR, respectively, they are found in exon 2 (FokI), intron 8 (ApaI and BsmI) (TaqI).

Material and method: Study attempted to investigate the vitamin D receptor gene polymorphisms in Sudanese prostate cancer patients, this case control study included eight seven subjects they were split into two groups, cases group which included 42 prostate cancer patients who were identified by histology and control group which included 45 healthy individual as control. Data were collected by questionnaire and analyzed by statistical package for social sciences version (21) and SNP stat online web-based application program.

Results: Total of 87 individuals were included; 42 as case and 45 as control with age mean and median (71.78±8.04) and (70) respectively, rs731236, rs1544410 and *rs2228570* SNPs has significant association between prostate cancer risk when compared with control group, contrary to this finding *rs7975232* failed to find any associated with prostate cancer, furthermore all four SNP failed to find any associated with stage, Gleason grade, age, BMI, PSA level, and vitamin D levels.

Conclusion: The study concluded that polymorphisms of VDR gene have significant association with prostate cancer except ApaI SNP.

Key Words: VDR SNPs, prostate cancer, vitamin D receptor, Sudan.

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Introduction

Prostate Cancer is most frequently diagnosed cancer and is the second most prevalent cause of death among males (1). Its prevalence is highest in Africans, then Whites, then Mongolians, which may be attributed to genetic, social, and environmental variations (2). The active form of vitamin D (1,25-dihydroxyvitamin D3), which aids in controlling prostate development, is produced by prostate epithelial cells. A synthetic version of vitamin D3 called calcitriol has antiproliferative and prodifferentiation effects in prostate cancer. By attaching to the vitamin D receptor, 1,25-dihydroxyvitamin D3 is able to perform its intended function (VDR).To control the genes that produce vitamin D, the VDR forms a heterodimer, generally with the retinoid X receptor (3). The steroid hormones like testosterone and vitamin D which operate through the androgen receptor (AR) and VDR, respectively, on prostate cell division (4). Multiple nuclear receptors are expressed by prostate epithelial cells, which control cell division and proliferation in the prostate gland. Nuclear hormone receptor VDR controls gene transcription. The relevance of the VDR in prostate cancer is still debatable, despite evidence that its regulation is altered in prostate cancer (5). There was no link found between the frequency of any VDR genotype and prostate cancer in the Taiwanese (Asian) population (6). Other research, on the other hand, suggested that ApaI polymorphism confers vulnerability to sporadic disease (7, 8). A link between the VDR gene's in exon 2 and intron 8 SNPs (rs2228570 and rs1544410), and the risk of cancer and other diseases has been discovered (9 - 11). Only the rs2228570 variation in the VDR gene affects the structure of the protein (12). The identification of genetic variations connected to disease vulnerability, particularly the wide range of cancers, may be the key to advancing preventative medicine. In order to aid in early detection and therapy, polymorphisms in the VDR gene may be helpful in identifying those who are more likely to acquire disease. The relationship between vitamin D levels and VDR polymorphism and prostate cancer has not been previously investigated in Sudan. This observational case-control study aims to association the Vitamin D Receptors Gene Polymorphisms among Sudanese Patient with Prostate Cancer.

Material and Method

Study design

This case control based-hospital study conducted in the National Cancer Institute (NCI), Gezira University, Wad Medani, Sudan. Eight Seven subjects were included they were divided into two groups, cases group which included 42 prostate cancer patients who were identified by histology and control group which included 45 healthy individuals**.**

Sample collection by using venipuncture from a peripheral vein techniques, 5 mL of whole blood was collected into EDTA containing tubes (Becton Dickinson, USA) and stored at -20 °C until the DNA isolation procedure was performed. The G-spinTM Total DNA Extraction Mini Kit from iNtODEWORLD, Inc. USA was used for DNA extraction from blood samples**.**

CTPP Technique for mutation detection

In order to genotype a sizable number of samples quickly and affordably, PCR-CTPP (polymerase chain reaction using confronting two-pair primers) was developed. These techniques designed two-pair primers (four primers) into a standard PCR tube to amplify allele-specific DNA products, resulting in variable band sizes that allow differentiation between alleles.

 DOI: 10.52981/smlj.v10i2.2862

ACTPP primers sequence and product size:

SNP rs:	Primer sequence	Product size
Fok1 (rs2228570)	AP570T: 5- CTGGCCGCCATTGCCTtCA – 3 CP570T: 5- CCAGGCAGCTGATTCCAAG -3 AP570C: 5- GCTTGCTGTTCTTACAGGtAC–3 CP570C: 5- TCACCTGAAGAAGCCTTTGC -3	240 bp (T) 169 bp (C) Common 390 bp
Bsm1 (rs1544410)	AP410A: 5- GCCACAGACAGGCCTaCA – 3 CP410A: 5- GTCAAGGGTCACTGCACATT -3 AP410G:5- AGCCTGAGTATTGGGAAcGC–3 CP410G: 5- CTGGGCAACCTGAAGGGAG -3	180bp (A) 221 bp (G) Common 368 bp
Taq1 (rs731236)	CPXFw: 5 - AGGTGCGCCCATGGAAGGA -3 AP236C: 5- CAGGACGCCGCGCTGCTC -3 AP236T: 5- CAGGACGCCGCGCTGCTC -3 CPXRev: 5 – TGGATAGGGGAGGTGGCAG -3	382 bp (C) 271 bp (T) common 617 bp
Apa1 (rs7975232)	CPXFw: 5 - AGGTGCGCCCATGGAAGGA -3 AP232A: 5- CAGGAGCTCTCAGCTGGTCA -3 AP232C: 5- GTGGGATTGAGCAGTGATGG -3 CPXRev: 5 – TGGATAGGGGAGGTGGCAG -3	192 bp (A) 464 bp (C) common 617 bp

PCR mix

The MAGSYBR qPCR Kit was supplied as a 2X concentrated used to amplification of DNA fragments from (APSLABS India A-8 Surya Terrace, Pratik Nagar, Yerwada, Maharashtra, Pune-411006.Three sets of primers had been provided by Macrogen Inc. (Korea). The PCR carried out with each set of primers on all samples in a 15µl reaction magnitude. Each reaction for Fok1(rs2228570), Taq1 (rs731236) and Apa1 (rs7975232) SNP consisted of 7µl of 2X MAGSYBR qPCR Master Mix (APSLAB, India), 1µl of AP for both alleles, 0.5µl of CP (F and R) and DNA template was 5µl, 6µl and 6µl of for Fok1, Taq1 and Apa1 respectively. For Bsm1(rs1544410) it was 7µl of 2X MAGSYBR qPCR Master Mix (APSLAB, India), 0.8µl of AP for both alleles, 0.4µl of CP (F and R), 5µl of DNA template and 0.6µl of Distil water.

PCR Conditions

For all four SNPs, there were 40 thermal cycles, and the PCR conditions were performing as the following: Taq1 (rs731236) and Apa1 (rs7975232) Denaturation, Annealing and Extension program was ($94^{°C}$ / 40 sec, $60^{°C}$/ 40 sec and $72^{°C}$ / 40 sec) respectively. Fok1 (rs2228570) program was ($94^{°C}$/1 min, $55^{°C}$/45 sec and $72^{°C}$/45 sec) for Denaturation, Annealing and Extension respectively. Bsm1 (rs1544410) Denaturation, Annealing and Extension program was ($95^{°C}$/ 40 sec, $58^{°C}$/30 sec and $72^{°C}$/ 40 sec).

Data collection and analysis

Well-designed questionnaires with all the necessary biographical and diagnostic information were used to collect the data. SPSS software program was used to analyze the data (Chi squire was used to calculated association). Number and percentage of patients or means and standard deviation range of data were used to summarize patient clinic and demographic variablesr. SNP stat online web-based application program was used for analysis of single SNPs: multiple inheritance models (co-dominant, dominant, recessive, over-dominant and log-additive).

Ethical approval

The purpose of the study was explained to each participant, and they all gave their agreement to participate. The National Cancer Institute at the University of Gezira and the Gezira State Health Ministry's ethical committee both gave their approval to this study.

Results

Table (1): Comparison of Fok1 and Bsm1SNPs between case and control group

Fok1 (Total =87)					
Model	Genotype	Cases (42)	Controls (45)	OR (95% CI)	P-value
Co-dominant	C/C	12 (28.6%)	32 (71.1%)	1.00	0.000
	C/T	26 (61.9%)	12 (26.7%)	0.17 (0.07-0.45)	
	T/T	4 (9.5%)	1 (2.2%)	0.09 (0.01-0.93)	
Dominant	C/C	12 (28.6%)	32 (71.1%)	1.00	0.000
	C/T-T/T	30 (71.4%)	13 (28.9%)	0.16 (0.06-0.41)	
Recessive	C/C-C/T	38 (90.5%)	44 (97.8%)	1.00	0.130
	T/T	4 (9.5%)	1 (2.2%)	0.22 (0.02-2.02)	
Over dominant	C/C-T/T	16 (38.1%)	33 (73.3%)	1.00	0.000
	C/T	26 (61.9%)	12 (26.7%)	0.22 (0.09-0.55)	
Log-additive	-	-	-	0.21 (0.09-0.48)	0.000
Bsm1 (Total =87)					
Model	Genotype	Cases (42)	Controls (45)	OR (95% CI)	P-value
Co-dominant	A/A	8 (19.1%)	23 (51.1%)	1.00	0.004
	G/A	18 (42.9%)	9 (20%)	0.17 (0.06-0.54)	
	G/G	16 (38.1%)	13 (28.9%)	0.28 (0.10-0.84)	
Dominant	A/A	8 (19.1%)	23 (51.1%)	1.00	0.002
	G/A-G/G	34 (81%)	22 (48.9%)	0.23 (0.09-0.59)	
Recessive	A/A-G/A	26 (61.9%)	32 (71.1%)	1.00	0.360
	G/G	16 (38.1%)	13 (28.9%)	0.66 (0.27-1.62)	
Over dominant	A/A-G/G	24 (57.1%)	36 (80%)	1.00	0.020
	G/A	18 (42.9%)	9 (20%)	0.33 (0.13-0.86)	
Log-additive	---	---	---	0.54 (0.32-0.92)	0.020

Table (2): Comparison of Apa1 and Taq1 SNPs between case and control group

Apa1 (Total =87)					
Model	**Genotype**	**Cases (42)**	**Controls (45)**	**OR (95% CI)**	**P-value**
Co-dominant	A/A	18 (42.9%)	25 (55.6%)	1.00	0.180
	A/C	21 (50%)	14 (31.1%)	0.48 (0.19-1.19)	
	C/C	3 (7.1%)	6 (13.3%)	1.44 (0.32-6.54)	
Dominant	A/A	18 (42.9%)	25 (55.6%)	1.00	0.240
	A/C-C/C	24 (57.1%)	20 (44.4%)	0.60 (0.26-1.40)	
Recessive	A/A-A/C	39 (92.9%)	39 (86.7%)	1.00	0.340
	C/C	3 (7.1%)	6 (13.3%)	2.00 (0.47-8.57)	
Over dominant	A/A-C/C	21 (50%)	31 (68.9%)	1.00	0.072
	A/C	21 (50%)	14 (31.1%)	0.45 (0.19-1.08)	
Log-additive	---	---	---	0.86 (0.46-1.62)	0.650
Taq1 (Total =87)					
Model	**Genotype**	**Cases (42)**	**Controls (45)**	**OR (95% CI)**	**P-value**
Co-dominant	C/C	6 (14.3%)	20 (44.4%)	1.00	0.007
	T/C	21 (50%)	16 (35.6%)	0.23 (0.07-0.70)	
	T/T	15 (35.7%)	9 (20%)	0.18 (0.05-0.62)	
Dominant	C/C	6 (14.3%)	20 (44.4%)	1.00	0.002
	T/C-T/T	36 (85.7%)	25 (55.6%)	0.21 (0.07-0.59)	
Recessive	C/C-T/C	27 (64.3%)	36 (80%)	1.00	0.100
	T/T	15 (35.7%)	9 (20%)	0.45 (0.17-1.18)	
Over dominant	C/C-T/T	21 (50%)	29 (64.4%)	1.00	0.170
	T/C	21 (50%)	16 (35.6%)	0.55 (0.23-1.30)	
Log-additive	---	---	---	0.43 (0.23-0.79)	0.004

 DOI: 10.52981/smlj.v10i2.2862

Table (3): Association of Fok1, Bsm1, Apa1 and Taq1 polymorphism with vitamin D level status among prostate cancer patients

Model	Genotype	Abnormal VD	Normal VD	OR (95% CI)	P-value
Fok1 (Total = 42)					
Co-dominant	C/C	2 (14.3%)	10 (35.7%)	1.00	0.200
	C/T	11 (78.6%)	15 (53.6%)	0.23 (0.04-1.35)	
	T/T	1 (7.1%)	3 (10.7%)	0.39 (0.02-6.85)	
Dominant	C/C	2 (14.3%)	10 (35.7%)	1.00	0.084
	C/T-T/T	12 (85.7%)	18 (64.3%)	0.24 (0.04-1.40)	
Bsm1 (Total = 42)					
Co-dominant	G/G	5 (35.7%)	11 (39.3%)	1.00	0.008
	G/A	9 (64.3%)	9 (32.1%)	0.39 (0.09-1.71)	
	A/A	0 (0%)	8 (28.6%)	NA (0.00-NA)	
Dominant	A/A	0 (0%)	8 (28.6%)	NA (0.00-NA	0.004
	G/A-G/G	14 (100%)	20 (71.4%)	1.00	
Apa1 (Total = 42)					
Co-dominant	A/A	8 (57.1%)	10 (35.7%)	1.00	0.430
	A/C	5 (35.7%)	16 (57.1%)	2.47 (0.62-9.83)	
	C/C	1 (7.1%)	2 (7.1%)	1.38 (0.10-18.99)	
Dominant	A/A	8 (57.1%)	10 (35.7%)	1.00	0.220
	A/C-C/C	6 (42.9%)	18 (64.3%)	2.28 (0.61-8.60	
Taq1 (Total = 42)					
Co-dominant	T/T	5 (35.7%)	10 (35.7%)	1.00	0.690
	T/C	6 (42.9%)	15 (53.6%)	1.47 (0.33-6.55)	
	C/C	3 (21.4%)	3 (10.7%)	0.67 (0.09-5.15)	
Dominant	T/T	5 (35.7%)	10 (35.7%)	1.00	0.770
	T/C-C/C	9 (64.3%)	18 (64.3%)	1.24 (0.30-5.12)	

Table (4): Association of Fok1, Bsm1, Apa1 and Taq1 polymorphism with Total PSA levels among prostate cancer patients

Model	Genotype	TPSA < 100	TPSA >100	OR (95% CI)	P-value
Fok1 (Total = 42)					
Co-dominant	C/C	5 (25%)	7 (31.8%)	1.00	0.680
	C/T	12 (60%)	14 (63.6%)	0.95 (0.23-3.96)	
	T/T	3 (15%)	1 (4.5%)	0.35 (0.03-4.78)	
Dominant	C/C	5 (25%)	7 (31.8%)	1.00	0.820
	C/T-T/T	15 (75%)	15 (68.2%)	0.85 (0.21-3.47)	
Bsm1 (Total = 42)					
Co-dominant	G/G	9 (45%)	7 (31.8%)	1.00	0.600
	G/A	8 (40%)	10 (45.5%)	1.85 (0.45-7.65)	
	A/A	3 (15%)	5 (22.7%)	2.17 (0.36-13.24)	
Dominant	A/A	3 (15%)	5 (22.7%)	1.57 (0.31-8.08)	0.320
	G/A-G/G	17 (85%)	17 (77.3%)	1.00	
Apa1 (Total = 42)					
Co-dominant	A/A	12 (60%)	6 (27.3%)	1.00	0.046
	A/C	7 (35%)	14 (63.6%)	5.41 (1.23-23.89)	
	C/C	7 (35%)	14 (63.6%)	5.41 (1.23-23.89)	
Dominant	A/A	12 (60%)	6 (27.3%)	1.00	0.014
	A/C-C/C	8 (40%)	16 (72.7%)	5.57 (1.30-23.93)	
Taq1 (Total = 42)					
Co-dominant	T/T	11 (55%)	4 (18.2%)	1.00	0.092
	T/C	7 (35%)	14 (63.6%)	4.83 (1.09-21.41)	
	C/C	2 (10%)	4 (18.2%)	3.97 (0.47-33.47)	
Dominant	T/T	11 (55%)	4 (18.2%)	1.00	0.030
	T/C-C/C	9 (45%)	18 (81.8%)	4.64 (1.11-19.46)	

Table (5) Distribution of Fok1, Bsm1, Apa1 and Taq1 polymorphism According to body weight of prostate cancer patients

Fok1 (Total = 42)					
Model	Genotype	Abnormal Weight	Normal Weight	OR (95% CI)	P-value
Co-dominant	C/C	6 (26.1%)	6 (31.6%)	1.00	0.760
	C/T	14 (60.9%)	12 (63.2%)	0.91 (0.23-3.65)	
	T/T	3 (13%)	1 (5.3%)	0.40 (0.03-5.39)	
Dominant	C/C	6 (26.1%)	6 (31.6%)	1.00	0.800
	C/T-T/T	17 (73.9%)	13 (68.4%)	0.84 (0.21-3.29	
Bsm1 (Total = 42)					
Co-dominant	G/G	10 (43.5%)	6 (31.6%)	10 (43.5%)	0.520
	G/A	10 (43.5%)	8 (42.1%)	1.41 (0.35-5.66)	
	A/A	3 (13%)	5 (26.3%)	2.76 (0.47-16.18)	
Dominant	A/A	3 (13%)	5 (26.3%)	2.30 (0.46-11.40)	0.300
	G/A-G/G	20 (87%)	14 (73.7%)	1.00	
Apa1 (Total = 42)					
Co-dominant	A/A	8 (34.8%)	10 (52.6%)	1.00	0.280
	A/C	14 (60.9%)	7 (36.8%)	0.41 (0.11-1.53)	
	C/C	1 (4.3%)	2 (10.5%)	1.87 (0.14-25.92)	
Dominant	A/A	8 (34.8%)	10 (52.6%)	8 (34.8%)	0.280
	A/C-C/C	15 (65.2%)	9 (47.4%)	0.50 (0.14-1.76)	
Taq1 (Total = 42)					
Co-dominant	T/T	8 (34.8%)	7 (36.8%)	1.00	0.100
	T/C	14 (60.9%)	7 (36.8%)	0.51 (0.12-2.12)	
	C/C	1 (4.3%)	5 (26.3%)	4.72 (0.41-54.75)	
Dominant	T/T	8 (34.8%)	7 (36.8%)	1.00	0.690
	T/C-C/C	15 (65.2%)	12 (63.2%)	0.76 (0.20-2.91)	

 DOI: 10.52981/smlj.v10i2.2862

Table (6): Association of Fok1, Bsm1, Apa1 and Taq1 polymorphism with Gleason stage among prostate cancer patients

Fok1 (Total = 42)					
Model	**Genotype**	**G. Stage< 7**	**G. Stage< 7**	**OR (95% CI)**	**P-value**
Co-dominant	**C/C**	**5 (27.8%)**	**7 (29.2%)**	**1.00**	**0.970**
	C/T	**11 (61.1%)**	**15 (62.5%)**	**1.00 (0.25-4.06)**	
	T/T	**2 (11.1%)**	**2 (8.3%)**	**0.78 (0.07-8.09**	
Dominant	**C/C**	**5 (27.8%)**	**7 (29.2%)**	**1.00**	**0.970**
	C/T-T/T	**13 (72.2%)**	**17 (70.8%)**	**0.97 (0.25-3.85)**	
Bsm1 (Total = 42)					
Co-dominant	**G/G**	**4 (22.2%)**	**12 (50%)**	**1.00**	**0.026**
	G/A	**12 (66.7%)**	**6 (25%)**	**0.17 (0.04-0.75)**	
	A/A	**2 (11.1%)**	**6 (25%)**	**1.00 (0.14-7.09)**	
Dominant	**A/A**	**2 (11.1%)**	**6 (25%)**	**2.63 (0.46-14.97)**	**0.250**
	G/A-G/G	**16 (88.9%)**	**18 (75%)**	**1.00**	
Apa1 (Total = 42)					
Co-dominant	**A/A**	**10 (55.6%)**	**8 (33.3%)**	**1.00**	**0.330**
	A/C	**7 (38.9%)**	**14 (58.3%)**	**2.59 (0.70-9.63)**	
	C/C	**1 (5.6%)**	**2 (8.3%)**	**2.76 (0.20-37.62)**	
Dominant	**A/A**	**10 (55.6%)**	**8 (33.3%)**	**1.00**	**0.140**
	A/C-C/C	**8 (44.4%)**	**16 (66.7%)**	**2.61 (0.73-9.36)**	
Taq1 (Total = 42)					
Co-dominant	**T/T**	**7 (38.9%)**	**8 (33.3%)**	**1.00**	**0.300**
	T/C	**7 (38.9%)**	**14 (58.3%)**	**1.61 (0.40-6.51)**	
	C/C	**4 (22.2%)**	**2 (8.3%)**	**0.37 (0.05-2.97)**	
Dominant	**T/T**	**7 (38.9%)**	**8 (33.3%)**	**1.00**	**0.780**
	T/C-C/C	**11 (61.1%)**	**16 (66.7%)**	**1.21 (0.32-4.55)**	

Table (7): Distribution of Fok1, Bsm1, Apa1 and Taq1 polymorphism according to prostate cancer patient's age

Fok1 (Total = 42)					
Model	**Genotype**	**> 70 Years**	**< 70 Years**	**OR (95% CI)**	**P-value**
Co-dominant	**C/C**	**2 (14.3%)**	**10 (35.7%)**	**1.00**	**0.035**
	C/T	**12 (85.7%)**	**14 (50%)**	**0.22 (0.04-1.24)**	
	T/T	**0 (0%)**	**4 (14.3%)**	**NA (0.00-NA)**	
Dominant	**C/C**	**2 (14.3%)**	**10 (35.7%)**	**1.00**	**0.100**
	C/T-T/T	**12 (85.7%)**	**18 (64.3%)**	**0.27 (0.05-1.50)**	
Bsm1 (Total = 42)					
Co-dominant	**G/G**	**4 (28.6%)**	**12 (42.9%)**	**1.00**	**0.640**
	G/A	**7 (50%)**	**11 (39.3%)**	**0.50 (0.11-2.23**	
	A/A	**3 (21.4%)**	**5 (17.9%)**	**0.56 (0.09-3.52)**	
Dominant	**A/A**	**3 (21.4%)**	**5 (17.9%)**	**0.82 (0.16-4.12)**	**0.810**
	G/A-G/G	**11 (78.6%)**	**23 (82.1%)**	**1.00**	
Apa1 (Total = 42)					
Co-dominant	**A/A**	**7 (50%)**	**11 (39.3%)**	**1.00**	**0.810**
	A/C	**6 (42.9%)**	**15 (53.6%)**	**1.55 (0.40-5.97)**	
	C/C	**1 (7.1%)**	**2 (7.1%)**	**1.18 (0.09-15.99)**	
Dominant	**A/A**	**7 (50%)**	**11 (39.3%)**	**1.00**	**0.540**
	A/C-C/C	**7 (50%)**	**17 (60.7%)**	**1.50 (0.41-5.52)**	
Taq1 (Total = 42)					
Co-dominant	**T/T**	**5 (35.7%)**	**10 (35.7%)**	**1.00**	**0.990**
	T/C	**7 (50%)**	**14 (50%)**	**1.09 (0.26-4.66)**	
	C/C	**2 (14.3%)**	**4 (14.3%)**	**1.19 (0.14-9.87)**	
Dominant	**T/T**	**5 (35.7%)**	**10 (35.7%)**	**1.00**	**0.88**
	T/C-C/C	**9 (64.3%)**	**18 (64.3%)**	**1.11 (0.27-4.52)**	

Discussion:

This case control study examined 4 SNPs of the VDR gene included rs731236 (*TaqI*), rs7975232 (*ApaI*), rs1544410 (*BsmI*) and rs2228570 (*Fok1*) in prostate cancer. In (rs731236 (*TaqI*): Overall C and T allele frequencies were almost similar in proportions but there was mild increased in C, the majority of C allele was in control group. Regarding the genotype frequencies, the TC was common and extensive of this increased in prostate cancer group, this finding agreed with several previous studies reported for the same SNP polymorphisms among prostate cancer patients in Japanese population and Piedmont region USA (4, 5). In contrast Oakley-Girvan, working with African American and White populations, they did not find any association among TaqI with PCa risk in the family or case control data. Similar results were reported by Chen *et al* (2009) (10) when they investigated British individuals of European origin. These findings might strength our conclusion that TaqI has no link with different races in the study area. It was observed that the link between TaqI polymorphism and risk factors for prostate cancer was not fully investigated. This study did not record any association between these risk factors (stage, age, BMI, PSA level, and vitamin D level) and prostate cancer. Study showed a higher frequency of T allele gene and TC genotype increased in the Gleason >7 stage, PCa compared with Gleason < 7, suggesting that PCa patients carrying T allele and TC genotype are most probably more prone to entering an advanced stage. On the other hand, for rs7975232 (*ApaI*): The C allele frequencies was the most frequent overall study population, with mild proportion increased in control group, furthermore AA genotype frequency was the most frequent overall population and it has same proportion of mutant genotype frequencies (A/C-C/C). One of our standing finding is that *ApaI (*rs7975232) SNP failed to find any associated with prostate cancer under the additive, dominant, or recessive genetic models. Other studies conducted elsewhere showed the same findings (13, 14), on the other hand; (7, 12, 15) reported an association between *ApaI (rs7975232)* and prostate cancer risk. This could be attributed to heterogeneity of studies populations. The *ApaI (*rs7975232) SNP failed to find any associated with stage, Gleason grade, age, BMI, PSA level, and vitamin D level. This in line with Suzuki who didn't observe a relationship between clinic and pathologic parameters and genotype distributions of the ApaI (4). Whereas in rs1544410 (*BsmI*): A and G allele, they appear in similar proportions frequencies overall population, C allele was more common in control group while G alle common in prostate cancer, A/G – G/G mutant showed predominated genotype frequencies overall population. Moreover, it was common in prostate cancer. This finding is supported by several previous studies among Japanese population, Piedmont region USA, and Caucasians population (4, 5, 16). In our study, we found an association between *BsmI* genotypes and high Gleason score. The findings are directly in line with previous findings (12).

Contrary to this finding several studies done in Brazil by Sarah Braga Rodrigues Nunes, a meta-analysis of 27 published studies and Mahesh B. Keitheri Cheteri study in USA which find no association between *BsmI* genotypes and high Gleason score (17, 18). It seems that A/G – G/G genotype alleles have strong tendency for expression regarding to development of prostate cancer among population under current study. The *BsmI(rs1544410)* SNP failed to find any associated with other risk factor such as stage, age, BMI, PSA level, and vitamin D level. Finally in rs2228570 (Fok1): C allele frequencies was the most frequent among study population majority of them were in control group, C/C genotype frequencies was the major and vast of them were appear in control group. Furthermore, (C/T-T/T) the mutant genotype was major in prostate cancer group .This is an important finding in the *Fok1* (*rs2228570*) SNP which has strong association with prostate cancer and was agree with some previous studies (19 - 22).

Conclusion

The study was concluded to the (Fok1, Bsm1 and TaqI) SNPS showed significant association with risk to prostate cancer, whereas ApaI SNP was not crrelated with risk to prostate cancer.

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