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Vitamin D Receptors Gene Polymorphisms and their Association with Serum Vitamin D level among Sudanese Patients with Benign Prostatic Hyperplasia

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a non-malignant enlargement of the prostate and it is a not unusual reason of lower urinary tract symptoms (LUTS) among older men. BPH have a massive effect on health of older men and their health-care. Human VDR is a nuclear receptor gene located in the long arm of chromosome 12, and consists of 11exons and 11 introns. The most frequently studied single nucleotide polymorphisms are BsmI (rs1544410), TaqI (rs731236), FokI (rs2228570), and ApaI (rs7975232) as defined by using the endonucleases BsmI, TaqI, FokI, and ApaI. There was no previous study in Sudan regarding to association between vitamin D level and VDR polymorphism with Benign Prostatic Hyperplasia (BPH).This is case control study aimed to Assess Vitamin D Receptors Gene Polymorphisms and Their Association with Serum Vitamin D level among Sudanese Benign Prostatic Hyperplasia attending Gezira Hospital for Renal Disease and Surgery (GHRDS). Fifty patients with BPH were included in the study based on their histopathology result with age means (65.84±9.51) years and 40 healthy control group, vitamin D level was measured by immunoassay using cobase 411 ROCHE instrument and VDR was detected by using polymerase chain reaction with confronting two-pair primers technique, ApaI and TaqI SNP failed to find any associated with Benign Prostatic Hyperplasia but FokI and BsmI find association, all study single nucleated polymorphisms of VDR gene showed significant association with prostate cancer risk except ApaI SNP, vitamin D Levels were decreased among BPH patients (29.50±11.52) when compeer with control group (30.45±10.35) without significant differences p. value (0.207).Oral supplementation is recommended for individuals with low level of vitamin D, also Screening BsmI (A/G) allel in Benign Prostatic Hyperplasia.

Keywords: Benign prostatic hyperplasia, Vitamin D Receptor, Polymorphism, Sudanese.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common disease that reported to affects in 19-30% of men older than 50 years. Long-term outcomes of the disease might include acute urinary retention (AUR), the need for surgical operation, urinary tract infection, bladder characteristic deterioration, and infrequently renal failure [**1**]. So far, no molecular markers for the progression of BPH had been absolutely established. Several molecular biomarkers, consisting of PSA, P25/26, TGFBRII, and uPSA, had been reported to have excessive potential to fill this void [**2-3**]. Human VDR gene is placed on the chromosomal locus 12q13-14, and it is considered the main gene that determines VDR concentration in cells. The gene is a member of the nuclear hormone receptor which influences the function of genes and involve in cell regulation, growth, and immunity [**4**]. The VDR gene variations are associated with a variety of biological diseases together with prostate enlargement. It is expression has been observed in ordinary and malignant prostate cell [**5**], it has been hypothesized that distinctive SNPs within the VDR might also have an effect on BPH risk and many polymorphisms within the VDR gene were identified via PCR-RFLP, among which Fok1, Bsm1, Apa1, Taq1, and Poly(A) were studied [**6**]. Xu *et al* meta-analysis and Hamasaki *et al* and Bai *et al* reported that polymorphisms of the VDR gene have an impact on the development of various prostate diseases [**7, 8, 9**]. Up to now, few epidemiological researches have investigated the VDR gene polymorphisms with regards to BPH risk [**10**]. This study aimed to Assess Vitamin D Receptors Gene Polymorphisms and Their Association with Serum Vitamin D level among Sudanese Benign Prostatic Hyperplasia attending Gezira Hospital for Renal Disease and Surgery (GHRDS).

MATERIALS AND METHODS

Study design, setting and sample size: This is case control study, conducted in Gezira Hospital for Renal Disease and Surgery (GHRDS). 50 Sudanese males with Benign Prostatic Hyperplasia attending Gezira Hospital for Renal Disease and Surgery (GHRDS), they were histology; ically proven from TURP or TRUS biopsy and PSA, and 40 healthy controls.

Sample collection: Blood Samples: all blood samples (total 5 mL) were collected by venipuncture from a peripheral vein by using 5 mL plastic disposable syringes, 2.5 mL was dispended into lithium heparin tubes for PSA and Vitamin D, 2.5 mL in K EDTA tubes for DNA extraction.

Vitamin D measurement: Serum PSA and Vitamin D were measured by Immunoassay technique using (Cobase411, serial No:0868-16, manufactured by Hitachi high technologies corporation, Tokyo-Japan) the Elecsys reagents kit (Roche-Germany) was used.

DNA extraction method: DNA extraction was done by using G-spin™ Total DNA Extraction Mini Kit from iNtODEWORLD, Inc USA.

CTPP Technique for mutation detection: PCR-CTPP (polymerase chain reaction with confronting two-pair primers) was invented to genotype a relatively large number of samples in a cost-effective and time-saving manner. In this method, allele-specific DNA products are amplified by means of applying appropriately designed two-pair primers (four primers) into an ordinary PCR tube, producing different bands size to allow differentiation between alleles.

PCR mix: The MAGSYBR qPCR Kit was supplied as a 2X concentrated ready-to-use mixture for amplification of specific 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.

ACTPP primers sequence and product size

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

PCR Conditions: The number of thermal cycles was 40 for all four SNPs, with initial denaturation temperature of 95°C for 3 min and final extension at 72°C for 2 min.

The rest temperatures as follow:

SNP rs:	Denaturation/ time	Annealing/ time	Extension/ time
Fok1 (rs2228570)	94°C / 1 min	55°C / 45 sec	72°C / 45 sec
Bsm1 (rs1544410)	95°C / 40 sec	58°C / 30 sec	72°C /40 sec
Taq1 (rs731236)	94°C / 40 sec	60°C / 40 sec	72°C / 40 sec
Apa1 (rs7975232)	94°C / 40 sec	60°C / 40 sec	72°C / 40 sec

Data collection and analysis: Data were collected through well-constructed questionnaire containing all personal and diagnostic information. Data was analyzed by SPSS software (22) was used to analyze the data. Patient clinical and demographic characteristics were summarized as number and percentage of patients or means and standard deviation range of values, codominant, dominant, over dominant and recessive models were used to define significance of different combinations of genotype frequencies in cases compared to controls using SNP Stats (https://www.snpstats.net/start.htm). These models were classified based on the presence of specific allele in each genotype. Association between groups for genotypes and alleles were determined by calculating Odd's ratio. The p-value (<0.05) was considered as statistically significant.

Ethical approval: Each participant was informed about the goal of the study and each one was consent to be involved in the study. The ethical committee at the Gezira State Health Ministry and the National Cancer Institute, University of Gezira, approved this study.

RESULTS AND DISCUSSION

SNP rs2228570(*Fok1*) was found to be insignificantly associated with Benign Prostatic Hyperplasia risk under the codominant (OR=0.62, 95% CI: 0.25-1.51; p=0.28 and OR=**NA**; p=0.28) for C/T and T/T Genotype respectively, dominant (OR=0.67, 95% CI: 0.28-1.62; p=0.37) for C/T-T/T Genotype, recessive (OR=**NA**; p=0.24) for T/T Genotype and the log additive models (OR=0.76, 95% CI: 0.33-1.76; *p*=0.00001)**.**SNP rs1544410 (BsmI)was found to be significantly associated with Benign

Prostatic Hyperplasia risk under the codominant (OR=0.73, 95% CI: 0.25-2.10; *p*=0.00004 and OR=5.90, 95% CI: 1.89-18.40; *p*=0.00004) for G/A and G/G Genotype respectively, dominant (OR=1.97, 95% CI: 0.82-4.71; *p*=0.12) for G/A-G/G Genotype, recessive (OR=6.80, 95% CI: 2.40-19.21; p=0.00004) G/G Genotype and the log additive models (OR=2.27, 95% CI: 1.32-3.90; *p*=0.002) table 1.

Table 1. SNPs association with Benign Prostatic Hyperplasia risk

G570 association with response STATUS (n=90, crude analysis)					
Model	**Genotype**	**STATUS=BPH**	**STATUS=Co**	**OR (95% CI)**	**P-value**
Codominant	C/C	28 (62.2%)	32 (71.1%)	1.00	0.28
	C/T	17 (37.8%)	12 (26.7%)	0.62 (0.25-1.51)	
	T/T	0 (0%)	1 (2.2%)	NA (0.00-NA)	
Dominant	C/C	28 (62.2%)	32 (71.1%)	1.00	0.37
	C/T-T/T	17 (37.8%)	13 (28.9%)	0.67 (0.28-1.62)	
Recessive	C/C-C/T	45 (100%)	44 (97.8%)	1.00	0.24
	T/T	0 (0%)	1 (2.2%)	NA (0.00-NA)	
Over dominant	C/C-T/T	28 (62.2%)	33 (73.3%)	1.00	0.26
	C/T	17 (37.8%)	12 (26.7%)	0.60 (0.24-1.46)	
Log-additive	---	---	---	0.76 (0.33-1.76)	0.53
G410 association with response STATUS (n=90, crude analysis)					
Model	**Genotype**	**STATUS=BPH**	**STATUS=Co**	**OR (95% CI)**	**P-value**
Codominant	G/G	20 (44.4%)	13 (28.9%)	1.00	4e-04
	G/A	19 (42.2%)	9 (20%)	0.73 (0.25-2.10)	
	A/A	6 (13.3%)	23 (51.1%)	5.90 (1.89-18.40)	
Dominant	G/G	20 (44.4%)	13 (28.9%)	1.00	0.12
	G/A-A/A	25 (55.6%)	32 (71.1%)	1.97 (0.82-4.71)	
Recessive	G/G-G/A	39 (86.7%)	22 (48.9%)	1.00	1e-04
	A/A	6 (13.3%)	23 (51.1%)	6.80 (2.40-19.21)	
Over dominant	G/G-A/A	26 (57.8%)	36 (80%)	1.00	0.022
	G/A	19 (42.2%)	9 (20%)	0.34 (0.13-0.88)	
Log-additive	---	---	---	2.27 (1.32-3.90)	0.002

Table 2. SNPs association with Benign Prostatic Hyperplasia risk

G232 association with response STATUS (n=8790, crude analysis)					
Model	**Genotype**	**STATUS=BPH**	**STATUS=Co**	**OR (95% CI)**	**P-value**
Codominant	A/A	17 (37.8%)	25 (55.6%)	1.00	0.19
	A/C	22 (48.9%)	14 (31.1%)	0.43 (0.17-1.08)	
	C/C	6 (13.3%)	6 (13.3%)	0.68 (0.19-2.47)	
Dominant	A/A	17 (37.8%)	25 (55.6%)	1.00	0.09
	A/C-C/C	28 (62.2%)	20 (44.4%)	0.49 (0.21-1.13)	
Recessive	A/A-A/C	39 (86.7%)	39 (86.7%)	1.00	NA
	C/C	6 (13.3%)	6 (13.3%)	1.00 (0.30-3.37)	
Over dominant	A/A-C/C	23 (51.1%)	31 (68.9%)	1.00	0.084
	A/C	22 (48.9%)	14 (31.1%)	0.47 (0.20-1.12)	
Log-additive	---	---	---	0.69 (0.38-1.26)	0.23
G.236 association with response STATUS (n=90, crude analysis)					
Model	**Genotype**	**STATUS=BPH**	**STATUS=Co**	**OR (95% CI)**	**P-value**
Codominant	T/T	20 (44.4%)	9 (20%)	1.00	0.0042
	T/C	18 (40%)	16 (35.6%)	1.98 (0.70-5.56)	
	C/C	7 (15.6%)	20 (44.4%)	6.35 (1.98-20.38)	
Dominant	T/T	20 (44.4%)	9 (20%)	1.00	0.012
	T/C-C/C	25 (55.6%)	36 (80%)	3.20 (1.25-8.17)	
Recessive	T/T-T/C	38 (84.4%)	25 (55.6%)	1.00	0.0024
	C/C	7 (15.6%)	20 (44.4%)	4.34 (1.60-11.78)	
Over dominant	T/T-C/C	27 (60%)	29 (64.4%)	1.00	0.66
	T/C	18 (40%)	16 (35.6%)	0.83 (0.35-1.94)	
Log-additive	---	---	---	2.50 (1.40-4.47)	0.0011

The *ApaI(*rs7975232) SNP failed to find any associated with Benign Prostatic Hyperplasia risk under the codominant (OR=0.43, 95% CI: 0.17-1.08; *p*=0.19 and OR=0.68, 95% CI: 0.19-2.47; *p*=0.19) for A/C and C/C Genotype respectively, dominant (OR=0.49, 95% CI: 0.21-1.13; *p*=0.09) for A/C-C/C Genotype, recessive (OR=1.00, 95% CI: 0.30-3.37; p=NA) C/C Genotype and the log additive models (OR=0.47, 95% CI: 0.20-1.12; *p*=0.23)**.** SNP (rs731236 (Taq I) was found to be significantly associated with Benign Prostatic Hyperplasia risk under the codominant (OR=1.98, 95% CI: 0.70-5.56; *p*=0.0042 and OR=6.45, 95% CI: 1.98-20.38; *p*=0.0042) for T/C and T/T Genotype respectively, dominant (OR=3.20, 95% CI: 1.25-8.17; *p*=0.012) for T/C-T/T Genotype, recessive (OR=4.34, 95% CI: 1.60-11.78; p=0.0024) T/T Genotype and the log additive models (OR=3.50, 95% CI: 1.40-4.47; *p*=0.00) table 2.

Association between Vitamin D levels in different participants: Lower levels of vitamin D in Benign Prostatic Hyperplasia patients, they didn't reach the significant difference compared with the control group (P= 0.207) (Table 3).

Table 3. Compared vitamin D between Prostate Cancer, Benign Prostatic Hyperplasia and control

Groups	N	Mean	STD	P. Value
Benign Prostatic Hyperplasia	50	29.50	11.52	0.207
Control	40	30.45	10.35	

In the present case control study, we examined four well-characterized SNPs of the VDR gene (rs731236 (*TaqI*), rs7975232 (*ApaI*), rs1544410 (*BsmI*) and rs2228570 (*Fok1*)) for their associations with Benign Prostatic Hyperplasia. For rs731236, the ratios of CC/CT genotypes are lower than TT in the subjects with BPH, the difference is statistically significant. The results were a match with old study done in Japan [**11**] and recent study done in Iraq by RawaaAl Chalabi found association between SNP and BPH patients [**12**], and Conversely to this finding different previous studies Combined analysis of 4 studies suggested nosignificant correlation between Taq1 VDR polymorphism and BPH risk[1**3**]. Additionally Bangkok, Taiwan, Netherland and another Japanese studies also find no association with the VDR **[8-14, 16**]. BsmI (A/G) polymorphism (rs544410) of the VDR gene is an important genetic variation that altered the level and metabolism of vitamin D which play a vital role in the various biological processes (36), regarding to this the statistical finding of the results exposed a significant difference in allelic frequencies of the rs1544410 genotype. 6 Patients had homozygote AA while G/A (G>A was found in 19 patients and 20 persons had homozygous allele (GG), this in agreement with and recent study done in Iraq by RawaaAlChalabi found association between SNP and BPH patients [**12**], and disagree with previous studies done in Bangkok, Taiwan, Netherland and another Japanese studies also find no association with the VDR [**8-14, 16**].

*ApaI(*rs7975232) SNP was unassociated with BPH .The same results have been reported by [**14-17**]. An association between this SNP and BPH has been document in old study from Japan [**11**] and recent one from Iraq [**12**].

Consistent with the previous studies done in Taiwan and North Indian [**15-18**] , this study did not find association between SNP (rs2228570(*Fok1*)and BPH, on the other hand study done in Iraq by RawaaAlChalabi found association between SNP and BPH patients [**12**].

The study did not find significant differences between vitamin D level in BPH and control group, this finding in line with study done in Iraq by RawaaAlChalabi [**12**] and study done in Wuhan, China by Zhang *et al* [**19**]

CONCLUSION

This is the first study that attempts to detect and describes VDR polymorphisms and their association with Benign Prostatic Hyperplasia in Sudan.

- A/G-G/G genotype alleles have strong tendency for expression regarding to development of prostate cancer.
- There was significant association between VDR-polymorphisms rs544410 and rs731236 with risk of Benign Prostatic Hyperplasia.
- There was insignificant association between VDR-polymorphisms rs7975232, and rs2228570 with risk of Benign Prostatic Hyperplasia.

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