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RESEARCH ARTICLE

Gene Polymorphism of Vitamin D Receptor (VDR) in Iraqi Population

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Abstract

Vitamin D receptor (*VDR*) gene has several variants.The distribution of these polymorphisms may vary among different ethnic groups therefore in this paper; we seek to determine population frequencies of selected *VDR* polymorphisms (*FokI* rs2228570 and *TaqI* rs731236) in the healthy Iraqi population. Genotyping for two *VDR* single nucleotide polymorphism were performed for a total of sixty healthy individuals using Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP). For *FokI* polymorphism, genotypes frequencies were 70%, 26.6% and 3.3% for FF, Ff and ff respectively and 50%, 45 % and 5% for TT, Tt and tt genotype for *TaqI* polymorphism respectively. As for the Allelic frequencies, the readings were 83.3 % opposed to 16.6 % for *F* and *f* alleles and 72.5 % opposed to 27.5 % for *T* and *t* alleles. In addition, the pattern regarding distribution of *FokI* and *TaqI* genotypes and alleles in the Iraqi Arab population was different from other ethnic groups. *VDR* polymorphisms data distribution among the healthy population in this work can be capitalized to investigate possible associations of *VDR* gene with different diseases. Further, anthropological comparisons can be attained using the same data set.

Keywords: *Vitamin D receptor, VDR polymorphism, VDR SNPs.*

Introduction

Vitamin D is believed to contribute to the body's immune system, endocrine system cardiovascular system, neuropsychological functioning, neuromuscular performance as well as its major role in bone health and calcium homeostasis. It is also being investigated in the research circles as a potent antioxidant that fights against free radical damage. On top of that, it induces cellular differentiation, where it protects against carcinogenesis [1].

The biological actions of vitamin D are exerted by binding to its intracellular receptor known as Vitamin D receptor (VDR) [2].Vitamin D-Vitamin D receptor complex attaches to retinoid X receptor and forms aheterodimer which is dependable for expression/ suppression of genes complicated in cell cycle arrest, apoptosis and differentiation [3].VDR (also known as calcitriol receptor or NR1I1) [4] which is a member of the steroid/ thyroid hormone nuclear receptor family, is expressed in mass of nucleated human cells at alternating concentrations[5].The *VDR* gene is well thought-out a candidate for vitamin D controlling responses [6].Mapping of the *VDR* gene at the chromosomal locus 12q12-14, consists of two promoter regions, eight coding exons (namely, 2-9), and six untranslated exons (1A-1F) [7].*VDR* gene is larger than 100 kb in size [3] and contains more than 470 single-nucleotide polymorphisms (SNPs) that thought of being capable to affect the structure and function of VDR [8].

Classically, there have been four typed single-nucleotide polymorphisms (SNPs), *FokI*, *TaqI*, *BsmI* and *ApaI*, all of which were researched intensively for association with various human traits and were acknowledged to affect risk of a variety of diseases [9].The *Fok1* polymorphism (rs2228570) of the *VDR* gene, which is positioned in the translation initiation start site, produces two copies of the VDR protein with dissimilar lengths (3 amino acids), longer one which is encoded by the '*f*' allele, is less active than the short

protein encoded by '*F*' allele [10]. On a different aspect the remaining variants (*TaqI*, *BsmI* and *ApaI*) are situated in the 3´-UTR region and are shown to impose no direct effect on the protein sequence. Nevertheless, the mRNA stability may alter by 3´-UTR region of the *VDR* gene [3]. T→C (ATT to ATC codon substitution) of *TaqI* polymorphism variation leads to a silent change in codon 352 in exon nine. The previous polymorphism is far from being able to alter VDR function as it pushes for isoleucine. Investigations had shown that there are no allele particular differences in *VDR* mRNA or even an association between the '*t*' allele and the reduced levels of *VDR* mRNA. However, alternative research indicated that the *TaqI* may direct the T helper cells type 1 (TH1) toward T helper cells type 2 (TH2) where Homozygotes tt genotype has a tendency to produce a TH1 immune response and TT homozygotes genotype produces a TH2 response [11].

Differences in *VDR* gene polymorphisms in the term of genotype and allelic frequencies between populations of different ethnicities have been reported [12].So the specific aim of this study is to investigate and characterize the distribution of *VDR* (*FokI* and *TaqI*) gene polymorphism in unrelated healthy Iraqi Arabs individuals.

Subjects and Methods

Study Population

Sixty unrelated healthy Iraqi Arab individuals were enrolled in the stud) mean age ± S.E. = 42.5 ±1.7) years. An informed consent was obtained from each participant.

DNA Extraction

Three milliliters of venous blood were collected in tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant and kept frozen until use. Genomic DNA was extracted from frozen whole blood using Blood g DNA Mini prep kit (Promega, USA). Extracted DNA was quantified by Nanodrop Analyzer spectrometer, checked for purity and stored at -20ºC until further analyses.

Genotyping of *VDR Gene*

VDR gene (*FokI* and *Taq1*) polymorphism was determined by using restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) technique.

The sequences of primer used for amplification *VDR* gene polymorphism *FokI* (rs2228570) (C/T) is ′5′AGCTGGCCCTGGCACTGACTCTGCTCT-3 (forward) and 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′ (reverse) and for *TaqI* polymorphism (rs731236) (T/C) 5′-CAGAGCATGGACAGGGAGCAAG-3′ (forward) and 5′GCAACTCCTCATGGCTGAGGTCTCA-3′reverse [13].The PCR profile was performed as follows: initial denaturation at 94◦C for 5min then 35 cycles of denaturation at 94◦C for 30s followed by annealing (*FokI* polymorphism at 60◦C and *TaqI* polymorphism at 64◦C) for 30s , extension at 72◦C for 30s and final extension at 72◦C for 5min. The PCR product was digested with FokI and TaqI restriction enzyme respectively. Digested PCR products were separated by electrophoresis on a 2.5 % agarose gel containing ethidium bromide and finally visualized under U.V light.

According to generated fragment patterns the genotypes were recorded. The homozygote genotype (FF) of *Fok1* polymorphism yielded only one band of 265bp. The genotype (ff) generated two fragments of 196 and 69 bp whereas heterogygote genotype (Ff) displayed three fragments of 265, 196, and 69 bp. *TaqI* polymorphism assigned as follows: TT genotype generated only two bands (495 bp and 245 bp), tt genotype presented with three bands (290 bp, 245 bp and 205 bp) and Tt genotype exhibited four bands (495 bp, 290 bp, 245 bp and 205 bp).

Statistical Analysis

Genotype and allelic frequencies for *VDR* gene polymorphisms (*FokI* and *TaqI*) were calculated by direct gene counting .The Pearson's chi-squared test was used for conformity distribution of genotypes with Hardy–Weinberg equilibrium (HWE) and for comparing genotype and Allelic frequencies obtained in our study to those from other studies. These estimations were calculated by using the WINPEPI package version 11.36.

Results and Discussion

For the two investigated SNPs (*Fok*I and *TaqI*) of *VDR* gene the observed genotype and allelic frequencies are summarized in Table 1. The genetic polymorphism of *VDR* was introduced with three genotypes (FF, Ff,

ff for *FokI* and TT, Tt, tt for *TaqI*) and two alleles (*F*, *f* for *FokI* and *T,t* for *TaqI*). The FF genotype frequency for *FokI* SNP was found to be 70% opposed to 26.6 % and 3.3% for Ff and ff genotypes respectively while analyzing the other SNP (*TaqI*) in this study resulted in the finding the distribution of homozygous TT and tt genotype to be 50% and 5% Meanwhile the heterozygous Tt genotype frequency was found to be 45%. The current study showed that allelic frequency of *F* allele opposed to *f* allele and *T* allele opposed to *t* allele in Iraqi population was 83.3 % opposed to 6.6 % and 72.5 % opposed to 7.5 % respectively. The genotype frequencies of *VDR* gene polymorphisms (*FokI* and *TaqI*) were in agreement with Hardy-Weinberg equilibrium (i.e, no significant differences between the observed and expected genotype frequencies).

Table1: Observed numbers and percentage frequencies of *VDR* gene polymorphisms (*FokI* and *TaqI*) in Iraqi population

Variables	Total No. = 60	
	Observed No.	Observed %
***FokI* Genotypes**		
FF	42	70
Ff	16	26.6
ff	2	3.3
Alleles		
F	100	83.3
f	20	16.6
***TaqI* Genotypes**		
TT	30	
Tt	27	50
tt	3	45
Alleles		5
T	87	72.5
t	33	27.5

The importance of the results regarding genotype and allelic frequencies in Iraqi population comes from the fact that Iraq has an inheritance dating back to 6000 years BC Moreover, it is a mixture of different ethnicities, Arabs are more proportion among ethnicities (75-80%), then Kurds (15-20%) followed by Turkmens, Assyrians and others (5%).Consequently, Iraqis represent a vital population for genetic studies due to their earliest history and natural genetic variation [14].As shown in Table 2, Analysis of Genotype frequencies of the *VDR* gene (*FokI* SNP) in Iraqi population with the genotype frequencies of other population from different ethnic groups found significant difference between Iraqi Arabs with Poland [18], South Korea [19] and Turkey [16] populations. Despite of that, none of genotypes showed a significant variation with Morocco [15], South Africa [17], India [20], Iran [10] and Paraguay populations [21] in their frequencies. Results of allelic frequencies for previous SNP illustrated that Iraqi individuals marginally differ from Turkey [16], South Africa [17], Poland [18], South Korea [19] and Morocco [15] populations. At the same time, it consistent with India [20], Iran [10], and Paraguay populations [21].

Table 2: Distribution of genotypes and allele frequency of *VDR* gene polymorphism (*FokI*) in Iraqi and different populations

Study	Country	Ethnicity	Total no.	Genotype			P	Alleles (%)		P
				FF	Ff	ff		*F*	*f*	
Ref.	Iraq	Arab	60	42 (70)	16 (26.6)	2 (3.3)	Ref.	100 (83.3)	20 (16.6)	Ref.
Arji et al.[15]	Morocco	Arab and Berber	203	109 (53.6)	82 (40.3)	12 (5.9)	NS	300 (73.8)	106 (26.1)	*
Ates et al.[16]	Turkey	Anatolian	80	35 (43.7)	37 (46.2)	8 (10)	**	107 (66.8)	53 (33.1)	**
Babb et al.[17]	South Africa	African	352	203 (57.6)	129 (36.6)	20 (5.6)	NS	406 (70.6)	169 (29.3)	**
Cieślińska et al.[18]	Poland	Polish	196	68 (34.6)	92 (46.9)	36 (18.3)	***	228 (58.1)	164 (41.8)	**
Kang et al.[19]	South Korean	Korean	105	41 (39)	43 (32.3)	21 (20)	***	125 (59.5)	85 (40.4)	**
	India	Indian	205	118	80	7	NS	316	94	NS

Rathored et al.[20]				(57.5)	(39)	(3.4)		(77)	(22.9)	
Salimi et al.[10]	Iran	Iranian	131	93 (71)	31 (24)	7 (5)	NS	217 (83)	45 (17)	NS
Wilbur et al.[21]	Paraguay	Ache, Chiripa, GuaranÍ	124	81 (65.3)	42 (33.8)	1 (0.3)	NS	204 (82.2)	44 (17.7)	NS

Ref. = Current study, NS = Not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Neither genotype nor allelic frequencies in the other polymorphism (*Taq1*) reported a significant difference between our study population and other ethnic groups with the exception of Morocco [15] and South Korea [19] populations in genotype frequencies nor South Korea [19] populations in allelic frequencies as demonstrate in Table 3. The distribution of the two VDR gene polymorphisms in this report revealed variation among different ethnic groups, such common variation occurs in DNA sequences has an extensive biological impact on the progress of certain diseases [22].

Studies have revealed that VDR polymorphisms across ethnics were correlated with different incidences of many diseases, For instance, Case–control study on ovarian cancer considered *FokI* polymorphism a risk factor for ovarian cancer (rs2228570) in the Polish population [23], A strong association was reported between *FokI* polymorphism and Type2 Diabetic mellitus **(**T2DM) in Kashmir Indian population [1]. In Chinese population, *TaqI* of *VDR* polymorphisms was linked with obesity where the genotype TT and allele *T* were established as potential predictors [13]. *TaqI* TC heterozygotes and *C* allele of *FokI* seemed to have some protective effect on Prostate Cancer risk in the Pakistani Population [3].In an Egyptian population Vitamin D receptor gene polymorphisms (*ApaI* and *TaqI*) confer high breast cancer susceptibility [8].While many hypotheses have been proposed to reveal the cause-effect relation regarding the geographic variation.

It seems possible that evolutionary selection pressures have given increase to frequent polymorphisms in genes and so contributed to noticeable differences in allelic frequency at the same loci. When geographic difference in certain polymorphism is superimposed on host genetic heterogeneity, considerable variation may occur in allelic associations and ethnic populations showed dissimilar frequencies of alleles, Moreover gene-environment interactions are likely to set up a further layer of complexity [24].

Table 3: Distribution of genotypes and allele frequency of *VDR* gene polymorphism (*TaqI*) in Iraqi and different populations

Study	Country	Ethnicity	Total NO.	Genotype			P	Alleles (%)		P
				TT	Tt	tt		*T*	*t*	
Ref.	Iraq	Arab	60	30 (55)	27 (45)	3 (5)	Ref.	87 (72.5)	33 (27.5)	Ref.
Arji et al.[15]	Morocco	Arab and Berber	203	109 (53.6)	48 (23.6)	46 (22.6)	***	266 (65.5)	140 (24.4)	NS
Ates et al.[16]	Turkey	Anatolian	80	30 (37.5)	39 (48.7)	11 (13.7)	NS	99 (61.8)	61 (38.1)	NS
Babb et al.[17]	South Africa	African	352	190 (53.9)	140 (39)	22 (6.2)	NS	520 (73.8)	184 (26.1)	NS
Cieślińska et al.[18]	Poland	Polish	196	92 (46.9)	85 (43.3)	19 (9.6)	NS	269 (68.6)	123 (31.3)	NS
Kang et al. [19]	South Korean	Korean	94	85 (90.4)	8 (8.5)	1 (1)	***	178 (94.6)	10 (5.3)	**
Rathored et al.[20]	India	Indian	205	97 (47.3)	79 (38.5)	29 (14.1)	NS	273 (66.5)	137 (33.4)	NS
Salimi et al.[10]	Iran	Iranian	131	67 (51)	50 (83)	14 (11)	NS	184 (70)	78 (30)	NS
Wilbur et al.[21]	Paraguay	Ache, Chiripa, GuaranÍ	122	59 (48.3)	58 (47.5)	5 (4)	NS	176 (72.1)	68 (27.2)	NS

Ref. = Current study, NS = Not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$

Conclusion

The offered data can be used as a useful mean for anthropological analysis and for the prediction of genetic susceptibility to diseases linked to vitamin D deficiency in Iraqi Arab population. Such approach of identifying *VDR* gene variants in populations of different ethnicities may recognize rare and prevailing variants that can be employed to form the starting point for the understanding of inconsistency among different populations in the development and outcome of diseases. However, the study is limited by low sample size and further studies with a larger number of individuals will give a more informative genetic profile of *VDR* gene polymorphisms.

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