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Frequency Of Vitamin D3 Receptor Gene Polymorphism Among Iraqi Individuals

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Abstract:

VitaminD(VD) has been various functions in different biological actions like homeostasis of calcium, also its play critical role in cell differentiation additional to proliferation in many target organs. All these functions were culminated through the effective style of VD(1,25(OH)2D3), that attached with specific proteins that found in the cell cytoplasm which ererepresentedthe receptorsof vitamin D.Those receptors termed vitamin D receptor (VDR). Different single nucleotide polymorphisms (SNPs) of VDR have been determined by specific enzymatic restriction positions such as rs1544410 BsmI(B/b).There is a closed correlation between VDR gene polymorphism and several human diseases have been registered. Many projects about VDR genotyping wereperformed by single amplification refectory mutation system-polymerase chain reaction (ARMS-PCR) technique. In the present project for the first time, 100 DNA samples of Iraqi individuals were analyzed for VDR gene polymorphism atposition rs1544410 BsmI(B/b) by using of ARMS-PCR system to identify and thento compare the results of current study with other similar studies. Our findings demonstrated that the genotypic frequencies of BB, Bb, bb were 52, 36, 12 % respectively. On theotherhandweobtainedallelic frequencies of B/b were 70 versus30 % respectively. In comparison with present data as a reference, the genotypic and allelic frequency of VDR gene polymorphism with data gained from previous studies, it was found non-significant difference between BsmI(B/b) gene polymorphismof Iraqi population and other nationalities and races such as Japanese and South Africa. Whereas the Caucasian Polish and Iranian population displayed statistically significant difference in VDR BsmI(B/b) gene allele and genotypes frequencies (P< 0.0001& 0.0009respectively).

Introduction

VitaminD,isan important dietary factor that play role in different biological functions, the most important of them are calcium homeostasis, and severalnon-classical functions. Action of VD can be classified in critical important roles; suchashormone secretion regulation, immune system action,additional to celldifferentiation and proliferation (1). These functions

areculminatedthroughtheactivemodeofvitaminD,(1,25(OH)2D3), that attached to cell cytoplasm proteins termed vitamin Dreceptors(VDR).VitaminD3 receptor is located on chromosome 12q13.11(2). The active form of VDR which is 125(OH)2D3 regulating the secretion of several hormone that play remarkable role in preservation the normalbone mineral homeostasis and regulation of glucose levels in blood stream. As well as the active form of VD3 reduce the synthesis and secretion of other hormone like para-thyroid hormone (PTH) (3). Other activities of vitamin D3were reported. It was found that the active form of vitamin D,125(OH)2D3 playrole in adaptiveimmunity through reductionof immunoglobulinproduction and delay the proliferation and differentiation of B cell Tlymphocyte into plasma cells(4). Concerning cell mediated immunity reported that the active form of vitamin D3also itsinhibits T cell proliferation (5). Other expertsshowed that the active form 125(OH)2D3 of vitamin D3 act as anti-cancer action(6). Vitamin D deficiency also result in impairs bone mineralization, leading to bone softening disorders such as rickets inchildren.

Usually single-nucleotide polymorphisms (SNPs) may de occurred in coding or in non-coding regions of genes, or within the intergenicregions (regions between genes). SNPs in the coding region of gene are of two types: synonymous and non-synonymous. Synonymous SNPs don't affect the protein's function, on the other hand the changing in amino acid sequence of protein because the non-synonymous SNPs was reported then resulting in change of protein activities (7).

Several studies reported that gene polymorphism of vitamin D receptor was varied in many different ethnic groups. Single nucleotide polymorphisms in vitamin D receptor were investigated extensively and included many locations one of them is rs1544410 BsmI(B/b). Actually BsmI (B/b)polymorphism in intron eight characterized that T converted to C, since allele T is engineered torepresenting B allelewhile the allele C its styledto representballele. The correlation between vitamin D receptor genepolymorphism with different human diseaseshad been searched. ItwasreportedthatVDRgene polymorphism correlated to hyper-parathyroidism, infectious diseases, inflammatory bowel disease (IBD), and prostatic tumor (8,9,10,11).

Usually most of researcherswho conducted studies aboutvitaminDreceptor gene genotyping utilized PCR and RFLP techniques, which is characterized by consuming of time, cumbersome,additional to the difficult interpreting of results. On the other hand there is advanced method it has been used for DNA amplification called allele specific polymerase chain reaction (PCR) or the amplification refractory mutation system ARMS, which has been described by Lombaredin 2006 (12). So the present project was conducted tostudy the genotype frequencies for sample of Iraqis individuals then compare it with previous studies by using of ARMS-PCRtechnique.

Material & Methods

In the present study the total number of samples was 100 subjects were conducted in our project which is consisted of 50 women and 50 men. Age mean of all samples was 41 ± 30 years. All of samples were of Iraqi ancestry. More or less 3 ml of whole venous blood was collected in ethylene diaminetetra acetic acid (EDTA) tubes from each person then deoxyribonucleic acid (DNA)wasisolatedbyusingofasaltingoutmethod(13).Then we genotyped vitamin D receptor gene polymorphism of BsmI(B/b) for all samples by usingofsingleamplificationrefractorymutationsystemPCR (ARMS-PCR) technique.

The principle of single ARMS-PCR technique, is the using of two complementary reactionsforeach one of polymorphisms; one involved specific primer to the mutant allele andthe 2nd involved to the Wild type allele. Additional to common primer was used for both reactions. Genotypic principle were depended on if there is allele specific amplification in one reaction or both. Amplification of pair of internal control result in formation of a 796-bp. By using of size identification and PCR sequencing products, the legitimacy of amplification reactions was conformed. Sequences forspecificandinternal control primers as well as their mixes additional to specificity of ARMS-PCR assay were showed in Table(1).

Table 1: Showed the used primers and their specificities in ARMS-PCR technique in present study (14).

Gene	Primersequences	Annealing Temperature (C^0)	Amplicon size (bp)
BsmI rs1544410*	BsmI/B 5′AGCCTGAGTACTGGGAATGT 3′	62	534
	BsmI/b 5′AGCCTGAGTACTGGGAATGC 3′	60	
	BsmI/C 5′GGGAGGGAGTTAGGC ACC 3′	62	
Internal Control	DRB1 5′TGCCAAGTGGAGCACCCAA 3′ (F)		796
	DRB1 5′GCATCTTGCTCTGTGCAGAT 3′ (R)		

The PCR optimum reaction mixture volume consisted of (75 ng) from extracted DNA added to 15 µl involved specific &internalcontrolthen1xofammoniumsulfate-based PCR buffer, and 200 µM of each deoxynucleotidetriphosphate(dNTP,) 0.6 unit TaqDNA polymerase, and 1.5 -2.5 mMMgCl2 were added according to guide line (Cinnagencompany, Tehran, Iran). Then the mixture reactions were amplified by PCR gradient master-cycler PCR machine (BIO-RAD USA),The beginning of reaction started with 94^{0}C for two minutes, then 10 cycles for10 seconds at 94 ^{0}C, and 60 seconds at 65^{0}Cfollowed by 20 cycles of 10 seconds at 94 ^{0}C, 50 seconds at 61 ^{0}C, and 30 seconds at 72 ^{0}C. PCR products identification performed by gel electrophoresis by using of 2% agarosestained with ethidiumbromide.

Statistical analysis

Statistical analysis was performed using IBM SPSS 23.0 (NY, USA). Genotype and allele frequency were analyzed by PopGen32, version 1.31 (15). In this study, the Chi-square or Fisher's exact tests were used to determine whether there were significant differences in frequencies between this sample of the Iraqi population and other populations of other studies. P value ≤ 0.05 it wasrelied as statistically significant. Genotypic frequency andits distribution was consistent with the chi-squared test (x^2) Hardy-Weinberg equilibrium(16).

Results

The PCR products that representing vitamin D gene receptor genotypes BsmI(B/b)in present project is illustratedin Figure1. Outlines the distribution of VDRgenotypes and alleles BsmI (B/b). Allelic frequencies of B and b alleleswere 70% and 30% respectively. The genotypic distribution wasconsistentwithHardy-Weinbergequilibrium.With comparison to our study as a reference, genotypic and allelic frequencies of vitamin D gene receptor polymorphismwith data gained from other projects is shown in (Table 2) (17,18,19,20,21,22). The comparison of the present study population versusIranian populations (19) and the Caucasian Polish populations (22) displayed statistically significant differences (P< 0.0009 and 0.0001 respectively) in VDR BsmI (B/b)allele and genotypefrequencies.

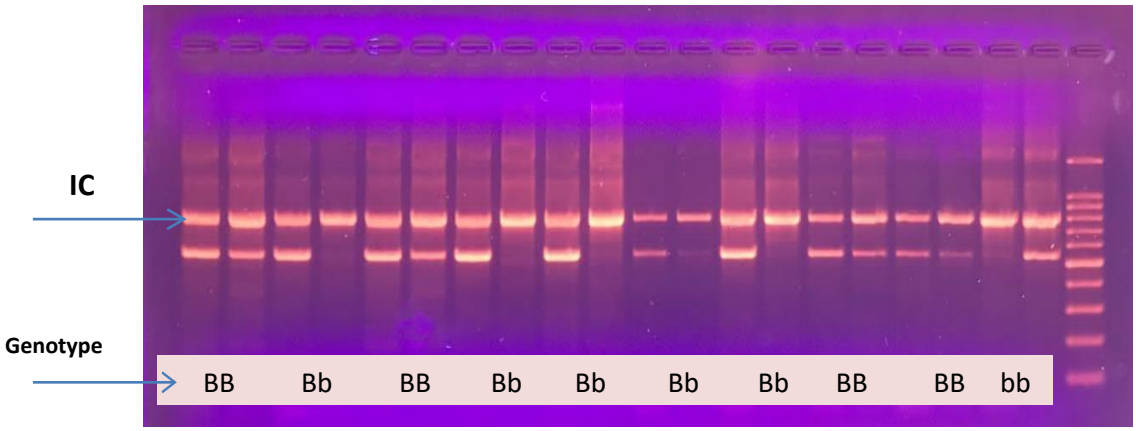

Figure. 1 Ten DNA samples have been analysed for ARMS-PCR reactions. Electrophoresis of PCR products were **A):** only one band for wild types reaction that represented a homozygous to wild type alleles (BsmIB/B genotype). **B):** Only one band in mutant reaction well that indicated a homozygous to the wild mutant alleles (.BsmI B/b genotype). **C):** One band in both reactions demonstrated a heterozygous (BsmI, b/b genotype) state. Each reaction internal control result in amplification of796-bp. The Genotypes of vitamin D gene receptor polymorphism of these ten samples were: BB, Bb, BB, Bb, Bb ,Bb, Bb, BB, Bb, bb, And IC = internalcontrol.

Table 2 . Distribution of VDR gene polymorphism in Iraqi population compare with previouslydata.

Project	Nationality	Total No.	Genotypes %			Alleles %		P value
			B/B	B/b	b/b	BsmI/B	BsmI/b	
Our Project	Iraq	100	52	36	12	70	30	Ref.
Niimi. et al. (17)	Japan	105	1.0	20	79	11	89	N.S
Lombard. et al. (18)	South Africa	117	8.0	27.33	64.70	24.10	75.90	N.S
Nadri. et al. (19)	Iran	150	25.30	42	32.70	46.32	53.70	**0.0009**
Mohammadnejad. et al. (20)	Iran	100	9.0	45	46	31.5	68.50	N.S
Jarari. et al.(21)	Iran	218	12	43	45	34	66	N.S
Maciejewski. et al. (22)	Caucasian polish	130	20.83	43.33	35.38	42.50	57.50	**0.0001**

NS= non-significant, **VDR=** Vitamin gene receptor

Discussion

VitaminDisdistinguishedbyitstwo characteristics,which is considered one of the most important our nutrientsand its actingas a hormone that synthesized in our bodies. It was found that there is essential role of vitamin D in metabolism of calcium and bone homeostasis. Actuallyvitamin D is a fat-soluble vitamin (22). It is found that play roleinhelpingour bodiesto absorb and retain calcium, both of which are important forconstructing the body. Other researchersshowed that vitamin D actively contribute in decrease the multiplication of cancer cells, as well as to control on infections and reducing severity of inflammation (23). Many organs and body'stissues have beenreceptors for vitamin D, this confirms that vitamin D roles beyond bone health, and some of experts and researchers believe that there is other possible functions it needs to be investigated (24). Abnormal signaling of vitamin D receptors if occurred due to SNPswould beinvolvedinseveral of pathophysiological mechanisms of many human diseases, including viral hepatitis (25, 26, 27). Single gene polymorphisms (SNPs) can occur in any region of the vitamin D3 receptor gene. Most of polymorphismsare silent, that mean they don't alter the function or gene expression. Some

polymorphisms are visible and they can altergene expression(28). Axiomatic scientific facts indicate that genetic variation (SNPs)are spread in the genome. The prevalence of these variations makes them important and had impactful role in causing a number of diseases(29).

Previous studies demonstrated that theindividuals with B/B genotype of BsmIhave been significantly lower levels of active from of vitamin D125(OH)2D3 compared to those with othergenotypes. Individuals with BB genotype were associated with higher risk of vitaminD deficiency and insulin resistance (30). In 2018 several studies reported that VDR BsmI polymorphismwas significantly associatedwith adolescent idiopathic scoliosis (AIS) susceptibility in the overall of Asian populations (31). Presently ourstudy demonstrated that there are varies between genotypes and allelic frequencies among the population in the same country;this is what has been seen in the Iranian ethnic races. The logical explanation for such results may agree with the opinion of Cheng and Thomas; they reported that substantial factors of environmental relevance on patterns of polymorphism among genes. In addition, the association between environmental relevance and gene variations is positive, consistentwith the expectation thatbalancing selection among heterogeneous environments maintains genetic variation at ecologically important genes (32). These study suggest an important role for environmental effects in shaping genome wide patterns of polymorphism and indicate another direction of genomicstudy.

In present conducted study PCR technique was adapted to performance by PCR technique with amplification refectory mutant system (ARMS) to investigating ofsingle nucleotide polymorphism in the vitamin D gene receptor polymorphism under optimum detection conditions. We are chosen ARMS-PCR technique due to it is economic, rapid, and it's a user-friendly method for genotyping. On the other hand we used internal control for each reaction to ensures the false negative results if itsoccurred.

Present findings demonstrated that the distribution of VDR gene polymorphism BsmI(B/b) genotype and allelicfrequenciesamong Iraqiindividuals then compared them with the genotypes frequency that gained from other race worldwide. It was found there is no significant correlation betweenBsmI (B/b)genotypesdistributioninIraqi population versusJapaneseand South African. Whereasfoundthatthereis statisticallysignificant difference betweenBsmI(B/b)genotypes frequencyIraqiindividuals compared with Iranianand Caucasian Polishpopulation.

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