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Biochemistry

Association between Vitamin (D) Receptor Gene Polymorphisms and Cardiovascular disease in Saudi Population

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ABSTRACT

Objective : To investigate three polymorphic sites ApaI,BsmI and TaqI of vitamin D receptor (VDR) gene on chromosome 12q (12–12q14) as candidate Cardiovasculrr disease (CVD) in Saudi Population.

Method : A sample of 100 Saudi classified as healthy (control,N= 50 (without CVD) or with CVD (N= 50) was investigated the relationship of VDR gene polymorphisms at three restriction sites ApaI, BsmI and TaqI to the risk of CVD .Blood samples were taken and DNA was extracted from whole blood. The target part of VDR gene was isolated and amplified by the polymerase chain reaction (PCR). PCR products were digested by restriction enzymes: ApaI, TaqI, and BsmI and electrophoresed on agarose gel.

Result: The difference in Vitamin D Total (Vit.D) values between each two shows non-significante difference p-value =0.1627. However,we observed that was distribution of genotypes frequency of the ApaI VDR gene polymorphisms differed highiy significantly in CVD patients (p-value =0.0085), but was no significant difference in control or between CVD patients and control groups. On the other hand, there was no significant difference in genotypes and alleles frequencies of the VDR gene polymorphisms at position BsmI and TaqI between CVD patients and control groups in Saudi population.

Conclusion: The VDR gene polymorphisms at one restriction sites ApaI to the risk of CVD in Saudi population.

KEYWORDS : Cardiovasculra disease (CVD);Vitamin (D) receptor (VDR) ;Gene polymorphisms ;Saudi population .

Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the world (1). Although the role of traditional risk factors is already consolidated, it is known that they cannot fully explain the development of CVD, which has caused continuous search for new risk factors. Growing evidence, obtained in recent years, has suggested that vitamin D deficiency may be associated with an increased risk of CVD (2). Vitamin D is classically known for its role in bone metabolism, being important for the maintenance of calcium homeostasis by ensuring physiologic calcium absorption by the gut (3,4,5). The discovery that the vitamin D receptor (VDR) is ubiquitously expressed in almost all body cells, such as immune, vascular or myocardial cells, suggests an involvement of vitamin D-mediated effects in several other systems apart from muscul oskeletal tissues(5). This has led to extensive research on vitamin D as a potential influencing factor in the pathogenesis of several chronic non-skeletal diseases, such as infectious or autoimmune diseases, cancer or cardiovascular diseases (CVD) (6,7,8).

The VDR gene is located on chromosome 12q (12–12q14) and is highly polymorphic (9).This gene includes eight protein-coding exons (exons 2–9) and six untranslated exons (exons 1a–1f) which are alternatively spliced. Four common single nucleotide poly morphisms (SNPs) including: FokI, BsmI, *ApaI* and *TaqI* which are located at the 3′ end of the VDR gene have been investigated extensively (10,11).

Association of vitamin D and different CV risk factors and diseases has been extensively evaluated during the last few years. Numerous observational studies, prospective meta-analyses, as well as some interventional studies have addressed the possible linkage of vitamin D deficiency and the development of CVD and its risk factors (12,13,14,15). However, BsmI (rs1544410), ApaI (rs7975232), and TaqI (rs731236) SNPs, located near the 3′ end of the VDR gene, are in strong linkage disequilibrium (LD) with each other. These three SNPs don't change the amino acid sequence of the encoded protein but have been shown to affect gene expression through regulation of mRNA stability (16). Therefore, we have investigated three polymorphic sites *ApaI*,BsmI and *TaqI* of VDR gene on chromosome 12q (12–12q14) as candidate for CVD susceptibility locus for the first time in Saudi population.

Material and Methods

Subjects

The VDR gene polymorphisms analysis study included a total of 100 Saudi volunteers. The diagnosis criteria of CVD were according to the World Health Organization (WHO).Subjects were divided in two groups CVD and non-CVD. Total number of 50 Saudi patients (25men and 25 women) with CVD. Fifty healthy control subjects were studied (25 men and 25women). They consisted of individuals with no history of diabetes or other autoimmune disease.

This criterion depends upon one test: Vitamin D Total Test **(Vit.D)** . We recorded the clinical parameters for each CVD volunteer upon diagnosis : Vit.D test .These medical details were obtained from patient hospital files after obtaining the consent from the administration .The previous history of CVD and family history of cardiovascular disease were taken from patient.The same parameters were recorded for the control volunteer. All subjects were selected from those who routinely attended diabetic clinic, Association of Diabetic Patient Friends Taif, King Abdul Aziz University Hospital (KAUH), Jeddah, Saudi Arabia. The study was approved by the ethical committee.All participants in the study filled a questionnaire and also signed a consent form.

Study Design

Genomic DNA was extracted from whole blood samples, in bio safety cabinet, using Thermo Scientific DNA extraction kit Blood (Thermo

Scientific, USA, Cat.no. K0781). The extracted DNA was stored at -20˚C for PCR amplification. On the other hand, concentration and purity of the extracted DNA was calculated automatically by Nanodrop2000c instrument from Thermo Scientific (USA).

For Polymerase Chain Reaction (PCR), the reactions were prepared using Maxima Hot Start Green PCR Master Mix (2x). The primers were from Biolegio, Nijmegen, Netherlands. The forward primer was (5'-CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA-3') and the reverse one was (5'-GCA ACT CCT CAT GGC TGA GGT CTC-3'). For polymerase Chain Reaction (PCR), the master mix from Thermo Scientificwas used. The mixture (50µl) contained 2X reaction buffer, 4mM Mg^{+2}, 4µM deoxyribonucleoside triphosphates, 0.2µM of each primer, 0.45 U Taq DNA polymerase and 10-30 ng of DNA template. The total reaction volume was made up to 50µl with nuclease free water.

The PCR tubes were transferred to thermal cycle. The amplification conditions were ; an initial denaturation for 4 min at 95˚C , 30 cycles each of which consisted of (denaturation at 95 ˚C for 30 S, annealing at 60 ˚C for 1 min and an extension at 68 ˚C for 2 min), and final extension for 5 min at 72˚C and ended at hold at 4˚ C. The PCR products were verified by 1% agrose gel electrophoresis at 100 V for 30 min .Purification of Purification was done for the PCR product using Gene JET PCR Purification Kit from Thermo scientific.

VDR genotyping

Amplified PCR products (5 µl) were digested with 3000 U of each restriction enzyme from Thermo Scientific. These enzymes are Acetobacter pasteurianus ApaI , Bacillus stearothermophilus BsmI and Thermus aquaticus YTI TaqI .These enzymes were used to the supplier-recommended protocols by Thermo Scientific. By using the thermal cycler, the reaction was incubated in 37˚C for 1 hour which is the activation temperature of BsmI and ApaI, then the enzymes were inactivated by incubation at 65˚C for 20 min. After that, the reaction was incubated 65˚C for 1 hour which is activation temperature of TaqI, and then the enzymes were inactivated by incubation at 80˚C for 20 min.

Statistical analysis

All statistical analyses were performed using the MegStat® version 9.0 computer program. Descriptive data were given as mean ± standard deviation (SD).Differences among groups were tested using analysis of variance (ANOVA) in distribution of the genotypes between the females and males according to CVD and Non-CVD were examined with the chi-square analysis. Differences in genotype frequencies were considered statistically significant for p-value ARI0.05.

Results

The Main Characteristic of the study group

The volunteers in this study were classified to two groups according to healthy subject and cardic patient totally 50 subjects in each groups. The clinical and biochemical parameters for the two groups cardic patient and control samples are shown in Table 1. The vitamin D Total (Vit.D) values between each two groups females and males are no significant difference p-value =0. 1627 >0.05 between CVD and control.

Vitamin D Total (Vit.D)

Table 1 Clinical and biochemical characteristics of non-CVD and CVD

Parameters	CVD Mean ± SD	Non- CVD Mean ± SD	P-value
18.2±5.81	20.2±5.33	0.1627	
(nmol/L)			

No significant difference.
Data is represented as mean ± **SD**
P-Value for control groups and CVD

Figure 1 Digestion results of the 3 polymorphism sites. Lane 1 :1kb DNA ladder .Lane 2 :negative control, lane 3 :PCR Products (2229 bp).*ApaI*

digestion:control(2229bp,1900bp,500bp)patient(2229bp,1900 bp,500bp).*BsmI* digestion:

control(2229bp),patient(2229bp,1900bp,700bp).*TaqI* digestion :control(2229bp) ,patient (2229bp,250bp).

The PCR products in our samples from Saudi volunteers, ~ 2229 bp as shown in Figure 1, were digested with the BsmI ,TaqI and ApaI restriction enzymes.

Statistical analysis of Genotypes Frequencies of BsmI,TaqI and ApaI Polymorphisms in study Groups at CVD and Control samples

Frequencies of VDR alleles in the two groups, irrespective of whether it was at females and males, shown in Table 2 . Accordingly, the distribution of alleles frequency of the BsmI , AapI and TaqI VDR polymorphisms differed significantly between Cardiovascular patients and controls. There was no significant difference in alleles frequencies of the VDR gene polymorphisms at positions BsmI ,TaqI and ApaI between CVD patients and control groups (p=0.4237 >0.05) for BsmI (p=0.9768>0.05) for ApaI. (p=0.3898>0.05) for TaqI. The frequency of B allele was more frequent in control group compared with Cardiovascular patients and b more in Cardiovascular patients. The frequency of A allele was more frequent in Cardiovascular patients compared with control group and a more in control group. Furthermore ,the frequency of T allele was more frequent in control group compared with Cardiovascular patients and t more in Cardiovascular patients. But There was highly significant difference in genotype frequencies of the VDR gene polymorphisms at positions ApaI in Cardiovascular patients (p=0.0085<0.05) . The frequency of AA genotype was more frequent in Cardiovascular patients compared with control group and aa more in control group compared with Cardiovascular patients.

Table2 Distribution of VDR gene polymorphisms in patients with CVD and non-CVD

VDR polymorphisms	Case (n=50)		Controls (n=50)		P-value
BsmI Genotypes	**NO.**	**%**	**NO.**	**%**	
BB	6	12	24	48	0.6075
Bb	11	22	21	42	
bb	33	66	5	10	
ApaI Genotypes					0.0861
AA	25	50	14	28	
Aa	8	16	17	34	
aa	17	34	19	38	
TaqI Genotypes					0.7270
TT	20	40	18	36	
Tt	21	42	15	30	
tt	9	18	17	34	
Bsm I Alleles					0.4237
B	23	23	69	69	
b	77	77	31	31	
ApaI Alleles					0.9768

A	58	58	45	45	
a	42	42	55	55	
TaqI Alleles					0.3898
T	61	61	51	51	
t	39	39	49	49	

Discussion

Vitamin D has increasingly been recognized to play a role in a broad range of bodily functions beyond bone health, including CV health ((3,17,18)).

Vitamin D is initially metabolized to the intermediate compound 25-hydroxyvitamin D in the liver which subsequently binds to the intracellular receptors to regulate gene expression. Results from cross-sectional studies examining the relation between vitamin D and CVD in the general population are conflicting (19). Furthermore, a low vitamin D level is associated with increased cardiovascular morbidity and mortality in the general population (20).

VDR is an important regulator of vitamin D pathway, which involves the conversion of serum 25-hydroxyvitamin D into the active hormone, 1,25-dihydroxyvitamin D. VDR is required for the functions of vitamin D (21). VDR is an intracellular hormone receptor that specifically binds the biologically active form of calcitriol or vitamin D, 1,25-dihydroxyvitamin D and interacts with specific nucleotide sequences of target genes to produce a variety of biologic effects (22).

VDR gene plays an important role in the vitamin D pathway. VDR protein is known to display polymorphic variation and belongs to the steroid hormone family of nuclear receptors which are responsible for the transcriptional regulation of a number of hormone responsive genes. As VDR is expressed in a large number of tissues, it is not surprising that ligand-activated VDR modulates the expression of multiple targeted genes (23), which is consistent with the fact that vitamin D deficiency has been associated with risk factors for cardiovascular disease, metabolic syndrome and even with overall mortality (24) .VDR harbors several known functional polymorphisms and several of these polymorphisms have been commonly investigated (10). The human VDR gene is mapped tp chromosome 12q12-q14, and five common polymorphisms have been typically associated with VDR activity ((25, 26)).

Due to these reasons, this study has been chosens to investigate the association of CVD in a sample of Saudi population with VDR BsmI,ApaI and TaqI polymorphisms and measured biochemical total vitamin D as a target for this study.In the present study, samples were divided into two groups CVD and non-CVD according females and males that measure Vit.D. Results showed no significant difference in the mean value of vitamin D between the CVD and controls was found (p=0.1627 >0.05) and there was no significant difference between females and males of each two groups (p=0.3489>0.05, p=0.3489>0.05).

Therefore, vitamin D deficiency can result from inadequate exposure to sun ,in adequate alimentary intake, decreased absorption ,abnormal metabolism, or vitamin D resistance (3).Recently, many chronic diseases such as cancer ((27,28)).Osteoporosis ((29,30)) and several autoimmune diseases ((31,32)) have been linked to vitamin D deficiency. Furthermore, vitamin D deficiency was highly prevalent in Saudi population .They cover their body completely except their faces. Wearing concealing clothing and restriction of outdoor activities has been reported previously as a risk factor for vitamin D deficiency in Female Saudi Arabia adolescents (33).

Data analysis showed that the association between the biochemical parameter and results we obtained from the RFLP for each two groups .No significant differences was found (p > 0.05) according to their ApaI,BsmI and TaqI genotypes. In addition, we examined the association of VDR gene polymorphisms at three position (ApaI, BsmI and TaqI) with CVD. Our results demonstrated alleles of BsmI gene polymorphisms was more frequent in CVD patients compared with controls and alleles of ApaI more in CVD patients compared with controls and alleles of TaqI more in CVD patients compared with controls and there was no significant difference in alleles frequency between cases and controls (p= 0.4327>0.05) for BsmI (p=0.9768>0.05) for AapI and (p= 0.3898>0.05) for TaqI .But there was highily significant difference in genotype frequencies of the VDR gene polymorphisms at positions ApaI in Cardiovascular patients (p=0.0085<0.05) . The frequency of AA genotype was more frequent in Cardiovascular patients compared with control group and aa more in control group compared with Cardiovascular patients. Therefore, genotype AA in ApaI region , al is risk genotype in CVD.

This outcome matches the outcome of the largest meta-analysis which found in Similar in nature was the study conducted by (34), who studied the link between the polymorphisms BsmI and FokI of the VDR with coronary artery disease incidence in a Chinese population. No significant associations were found between the SNPs and the incidence of the disease.

Studied the association of five SNPs in the VDR, none of which were TaqI ApaI, BsmI, FokI and Tru9I with both the incidence of CAD and 25(OH)D levels in an Indian population. The resulting genotypes were not associated with either coronary artery disease incidence or 25(OH)D levels (35).

Vitamin D's relationship with CVD has been a highly investigated theme in the past decade. Whether on an epidemiological or a molecular level, vitamin D has been repeatedly demonstrated to positively influence cardiovascular health ((36, 37)).

A prospective study found a significant association between the t allele of TaqI VDR and baseline cases of coronary artery disease in a French population(38).

In our present study population, the genotypes at Fok I and Apa I SNPs of VDR were associated with vitamin D deficiency. Also noted were significant relationships between 25OHD levels and allelic variants of Fok I and Bsm I SNPs. Carrying the f allele of the Fok I SNP or the aa genotype of the Apa I SNP might be protective against vitamin D deficiency: an earlier study found that they can affect circulating levels of vitamin D and may also influence cardiovascular risk (39).

Support of the involvement of VDR polymorphisms with the cardiovascular system was provided by (40), who identified both the FF genotype and the F allele of the FokI polymorphism as being associated with the development of essential hypertension in their study cohort comprising Indian subjects.

In United Kingdom the clinical studies showing associations between vitamin D status and cardiovascular disease and the experimental studies that explore the mechanistic basis for these associations (41).

Conclusion

our case control study indicated that the VDR polymorphism in BsmI ,TaqI and ApaI regions are associated with Cardiovascular in the Saudi population .We recommend using the same methodology with larger sample size in the same population. In addition, future studies on the correlation between environmental factor such as UV, immune response, VDR SNPs and CVD may also be considered.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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