Research article

The association of the Fok1(rs2228570) vitamin D receptor (VDR) polymorphism with cardiovascular diseases predisposition in Sudanese patients

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Abstract

Cardiovascular diseases (CVDs) are public health concerns globally. The role of genetics in CVDs predisposition was evidenced in previous studies. The nuclear vitamin D receptor (VDR) regulates the transcription of a number of different genes implicated in a variety of diseases including CVDs. In this study we aimed to investigate “for the first time” the association of vitamin D receptor (VDR) FokI(rs2228570) gene polymorphism with the risk of heart diseases (CVDs) in Sudanese patients. A cross sectional case-control study was conducted, including 60 of proven heart disease (CVD) patients and 77 controls. The demographic information was obtained using well designed questionnaire. The genotypes of the VDR FokI polymorphism (rs2228570) were determined by polymerase chain reaction-restriction fragment length polymorphism method using FokI restriction enzyme. The results of this study showed that atherosclerosis represents 57.1% of the CVD cases. No gender difference was observed when compare cases to the controls ($P=0.13$). Previous attack of CVDs was reported in 16.7% of the CVD cases. 50% of the patients have family history of CVDs with high significant difference when compared to the controls ($P=0.0001$). 52% and 48% of the CVD patients are hypertensive and diabetic respectively. The genotypes of the FokI polymorphism of the VDR gene did not differ between CVD patients and control subjects ($P=0.72$). This indicates that this mutation is unlikely to play a major role in CVDs predisposition in our sample. However, the frequencies of the mutant CC and TC genotypes among CVD patients who encountered previous attack was 33.3% and 55.6% respectively. The frequency of the mutant allele among CVD cases and controls is 71% and 74% respectively and among who patients have family history of CVD was 72%, indicating a potential presence of the mutant allele in the general population. This result suggests that the FokI polymorphism of the VDR gene is unlikely to contribute to CVDs predisposition in this samples, however, a considerable frequency of the mutant allele among CVD cases and controls and among those who have family history of CVDs, indicating a latent presence of the mutant allele in the general population and might possibly contribute to disease susceptibility in Sudanese CVD patients.

Keywords: Cardiovascular diseases; VDR polymorphisms, rs2228570 (*FokI*)

Introduction

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide and the burden of the disease is increasing rapidly because of the changes of the lifestyle; consumption of junk foods, smoking, stress, being overweight and sedentary life. All these factors raise the possibility of acquiring hypertension, diabetes, high cholesterol; all are Cardiovascular diseases risk factors. In Sudan the (CVDs) is a public health concern (Suliman 2011, Yassin et al., 2015, Ali et al., 2017), as higher prevalence of many of the risk factors CVDs, including diabetes, hypertension and hyperlipidemia were reported. According to the WHO; the CVDs represent 31% of all global deaths (Roth et al., 2017). CVDs are multifactorial diseases. Strong evidence of the genetic contribution to disease predisposition was reported in previous studies however the results are inconsistent (Pan et al., 2009, Sygitowicz, et al., 2014, Tarighiet al., 2017). Vitamin D is a steroid hormone which has pleiotropic functions. Its regulation in the transcription of a number of different genes implicated in a variety of diseases including heart diseases (Holick 2004, Wang et al., 2005, Wu-Wong et al., 2006, Wang et al., 2017), has been mediated through its binding to the nuclear vitamin D receptor (VDR) (Zmuda et al., 2000), which is present in many cells such as endothelial cell, smooth muscle and myocardium (Dusso et al., 2005). The VDR gene located on chromosome 12q13.1. Several polymorphisms of the VDR gene have been identified and intensively studied as a possible candidate for cardiovascular disease predisposition in different ethnic groups but the results of these studies are controversial (Pan et al., 2009, Laczmanski et al., 2013, Dorsch et al., 2014, He and Wang 2015, Abu el Maaty et al., 2016, Alizadeh et al., 2017). In this study we

aimed to investigate “for the first time” the association of vitamin D Receptor (VDR) FokI(rs2228570) gene polymorphism with the risk of cardiovascular diseases (CVDs) in Sudanese patients.

Patients and methods:

A cross sectional case-control study including 60 of proven cardiovascular disease (CVD) patients and 77 controls. Verbal consent was obtained from patients and controls. Three ml of blood were collected in EDTA tube from each participant. The demographic information was obtained using well designed questionnaire. DNA was extracted using conventional guanidine method and the quality of the genomic DNA was checked on Nanodrop Spectrophotometer. PCR amplification targeting the FokI polymorphism of the VDR gene (rs 2228570) was performed using iNtRON Biotechnology, Inc., mixture containing 6.5 -10.5 µL of ddH₂O, 1 µL of 10 pmol/µL of the primer mix (Forward 5'AGCTGGCCCTGGCACTGACTCTGGC TC T`3, Reverse 5'ATG GAA ACA CCT TGC TTC TTC TCC CTC 3`) and 1-5 µL of 50 ng/µL of template DNA. The PCR program was as follows: 95°C for 5 min for initial denaturing, 35 cycles of denaturing at 95°C for 30s, annealing at 63°C for 1 min, followed by extension at 72°C for 30s. Final extension at 72°C for 10 min to ensure complete amplification of the sequence. The amplicon of the FokI polymorphism was digested with a FokI restriction enzyme (New England Biolab). 2-3 µL of the amplicon was added to 0.25 µL of the FokI enzyme, 1 µL of 10x buffer and the mix completed to 8 µL. The digested product was incubated at 37° C for 4 hours and the digested reaction mixture was then electrophoresed for 45 mins. using 2% agarose gel stained with ethidium bromide and visualized on gel documentation system. The recognition site of the FokI enzyme 5'-

GGATG(N)₉₋₃'. The digested product indicates the presence of the TT genotype with band size of 206 and 61bp. The undigested product reflects the presence of the CC genotype with 267bp and the heterozygous indicated by three bands of 267 bp, 206 and 61 bp.

All the statistical analysis was done using IBM SPSS Statistics for Windows, Version 20.0. The results were considered statistically significant at P-value <0.05.

Results

The results of this study showed that 90% of the cardiovascular disease (CVD) patients are at age more than 40 years. No gender difference was observed when compared to the controls (P= 0.13). Atherosclerosis represents 57.1% of the cases. The frequency of Dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) was 7.1% each (Table 1). Previous attack of CVD was reported in 16.7% of the

cases. 50% of the patients have family history of CVD with high significant difference when compared to the controls (P=0.0001). High frequencies of hypertension (52%), Diabetes (48%) were observed among the patients.

An insignificant association between the genotypes of the FokI polymorphism of the VDR gene and CVD predisposition was observed (P=0.72) (Table 2).

However, 50% and 45.5% of the patients who have family history of CVD carry the CC and TC genotypes respectively, in addition, high frequencies of the C allele in both CVD cases and controls was observed (71% and 74% respectively) (Table 2). The frequencies of the CC and TC genotype among CVD patients who encountered previous attack was 33.3% and 55.6% of respectively and 91.6% of atherosclerotic patients have the CT and CC genotypes, indicating a non-negligible role of the mutant genotypes in CVDs susceptibility.

Table 1: Shows the demographic data of the CVD cases and controls

Parameter		Case N=60	Control N=77	P-value
Age	<40 years	6 (10%)	26 (33.8%)	P=0.001
	>40 years	54 (90%)	51 (66.2%)	
Sex	Male	30 (50%)	30 (39%)	P=0.13
	Female	30 (50%)	47 (61%)	
Type of CVD	Atherosclerosis	57.1%	0%	
	DCM	7.1%		
	HCM	7.1%		
	Other	28.7%		
Previous attack	Yes	16.7%	0%	
	No	83.3%		
Family history of CVD	Yes	50%	3.7%	P=0.0001
	No	50%	96.3%	
Hypertension	Yes	52%	0%	
	No	48%		
Diabetes mellitus	Yes	48%	6.5%	P=0.001
	No	52%	93.5%	

Table 2: Shows the distribution of the FokI genotypes of VDR gene and allele frequency among cardiovascular patients (CVD) and controls.

Genotypes of FokI (rs 2228570)	Cases (CVDs) N=60	Controls N=77	P value
CC	28 (46.7%)	39 (50.6%)	P=0.72
TC	29 (48.3%)	36 (46.8%)	
TT	3 (5.0%)	2 (2.6%)	
Allele frequency			P=0.75
C	0.71	0.74	
T	0.29	0.26

Figure 1: Shows the genotype of FokI polymorphism of the VDR gene among CVD cases and controls

Figure 2: Shows the genotypes of the FokI(rs 2228570) vitamin D receptor (VDR) polymorphism among cardiovascular diseases patients and the controls: Lane 1,2,6,7,8,9,12,13,14 represent the homozygous genotype CC (267bp). Lane 3,4,11 represent the heterozygous CT (267,206,61bp). Lane 5 is homozygous genotype TT (206,61bp)

Discussion

Cardiovascular disease (CVD) is a serious public health problem associated with high morbidity and mortality worldwide. In Sudan, CVD is a challenging health problem, as a high burden of many of the risk factors of CVD, including diabetes, hypertension and hyperlipidemia was reported (Suliman 2011). Strong evidence of the genetic contribution to disease predisposition was reported in previous studies (Zmuda et al., 2000, Holick 2004). The expression of vitamin D receptor (VDR) in a wide variety of cells including vascular endothelial cells, smooth muscle and cardiomyocytes, makes it a target for thorough investigation to demonstrate its association with cardiovascular diseases [Dusso et al., 2005, Laczmanski et al., 2013, Dorsch et al., 2014, Alizadeh et al., 2017].

To the best of our knowledge this is the first genetic study aimed to investigate the association of vitamin D Receptor (VDR) FokI (rs2228570) gene polymorphism with the risk of cardiovascular diseases (CVDs) in Sudanese patients.

The statistical genotype analysis did not provide evidence for the association of the FokI (rs2228570) polymorphism of vitamin D receptor (VDR) with the risk of cardiovascular diseases (CVDs) in Sudanese patients ($P=0.72$). This result in contrast with those reported in other populations (Laczmanski et al., 2013, Dorsch et al., 2014, He and Wang 2015). However, there are some light in the tunnel to think further for a possible association of the FokI polymorphism with CVD in our population. First; we observed a high frequency of the mutant allele among CVD cases and controls (71% and 74% respectively) and among patients who have family history of CVD, indicating a potential presence of the mutant allele in the general population and an expected significant role in cardiovascular disease specially that high

frequency of the disease risks of CVD such as hypertension and diabetes (52% and 48% respectively) was also observed. Second the frequencies of the mutant CC and TC genotypes among CVD patients who encountered previous attack was 33.3% and 55.6% respectively. In addition, 91.6% of atherosclerotic patients have the CT and CC genotypes, indicating a non-negligible role of the mutant genotypes in CVDs susceptibility.

Conclusions:

This study did not provide evidence for the association of the FokI (rs2228570) polymorphism of vitamin D receptor (VDR) with the risk of cardiovascular diseases (CVDs) in Sudanese patients. This might be related to the complex nature of CVDs that is unlikely that a single polymorphism to contribute to disease susceptibility. Not only the small size of the studied subjects, but also the subjects are not homogeneous, belonging to multiethnic tribes, all these do not enable us to validate the association of the FokI polymorphism of vitamin D and the VDR gene with cardiovascular risks. However, the potential role of the FokI polymorphism in CVD predisposition is probable, as a considerable presence of the mutant allele and genotypes in both cases and controls and in atherosclerotic patients and among those who have family history of the CVDs was observed.

Recommendations:

Cardiovascular diseases are a public health concern. They donot only associate with severe morbidity but also harvest live of many affected patients, therefore a thorough knowledge of disease risk factors; both genetic and nongeneticsis recommended.

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