The Impact of Vitamin D Receptor Gene Polymorphisms and Haplotypes on the susceptibility to Toxoplasmosis

Qasim Sharhan Al-Mayah (PhD) [1]

Abstract

Background: Toxoplasmosis is a cosmopolitan infectious disease caused by *Toxoplasma gondii*. Vitamin D is an immune modulator exerting its effect through a nuclear receptor called vitamin D receptor. Genetic polymorphisms in the vitamin D receptor gene could affect the activity of vitamin D and hence the individual's susceptibility to toxoplasmosis.

Objective: To evaluate the impact of four single nucleotide polymorphisms (FokI, BsmI, TaqI and ApaI) and different haplotypes of vitamin D receptor gene on the susceptibility of Iraqi women to toxoplasmosis.

Patients and Methods: This case-control study involved 72 women with confirmed toxoplasmosis and 50 women as controls, DNA was extracted from blood samples and allele specific polymerase chain reaction technique was used for genotyping of the four polymorphisms using specific primers. Haplotypes and linkage disequilibrium were calculated using single nucleotide polymorphism analyzer 2.0 software.

Results: Only the FokIpolymorphism had significant reverse association with toxoplasmosis in homozygote form (OR=0.140, 95%CI= 0.027-0.717, P=0.018). At allelic level, FokI F allele had significantly higher frequency in patients than controls (OR= 0.552, 95%CI=0.314-0.972, P=0.043).The frequency of two haplotypes differed significantly between patients and controls where FBAT haplotype was more frequent in patients while fta B was more frequent in controls. Moderate linkage disequilibrium correlations were found between FokI and TaqI in patients and controls.

Conclusion: Allele f of FokI polymorphism and fBat haplotype in vitamin receptor gene is associated with a protective role against toxoplasmosis.

Key words: Toxoplasmosis, Vitamin D receptor gene polymorphisms, Haplotypes, Linkage disequilibrium

Corresponding Author: Kasim19672003@yahoo.com.

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[1]Medical Research Unit - College of Medicine - Al-Nahrain University - Baghdad - Iraq.

Introduction

Toxoplasma gondii is an obligate intracellularapicomplexan parasite which infects about one-third of the world's human population [1]. The prevalence of toxoplasmosis varies according to many factors with certain geographical regions having seroprevalence rates as high as 84% [2].

Because *Toxoplasma* is an intracellular microorganism, the principle immune response against its infection is cell-mediated immunity (CMI) where IFN-γ and IL-2 are the main effectors. Vitamin D acts as immune modulator through its active form (1, 25(OH)2D3) [3]. It was found that high serum levels of vitamin D causes downregulation of CMI response by

blocking IFN-γ secretion which causes impairment in macrophage activation. However, in vivo and in vitro growth of Toxoplasma was found to be inhibited by the treatment with vitamin D may be through restriction of tachyzoite proliferation within the parasitophorous vacuole [4].

Single nucleotide polymorphism (SNP) in vitamin D receptor (VDR) can affect the binding activity of this receptor with the active form of the vitamin and eventually the immuneresponse against Toxoplasma. Four importantpolymorphisms in VDR gene which are FokI (rs 10735810), BsmI [rs 154410], ApaI (rs 7975232) and TaqI [rs 731236]. These SNPs were subjected to intensive investigation regarding their role in different diseases. Significant associations were found between these SNPs and different infectious diseases such astuberculosis [5]. Malaria [6]. Chagas disease [7]. Human immunodeficiency virus (HIV)[8]. Pertussis [9]. And hepatitis C virus [10]. However, according to available literature there is no previous study concerning the association of VDR gene polymorphism with toxoplasmosis. Thus, the present study aimed to investigate the role of VDR gene polymorphisms and haplotypes in women's susceptibility of toxoplasmosis.

Patients and Methods

Subjects and Samples

A total of 243 women (age range 19-49 years, mean= 33.16 years) attending the Obstetrics and Gynecology Department at Al-Yarmouk Teaching Hospital and Al-Iamamain Al-Kadhumain Teaching Hospital in Baghdad suffering from different gynecological diseases during the period from November, 2014 to June, 2016 were recruited for this study. Five milliliters of venous blood were obtained from each woman and distributed into two aliquots. The first aliquot (3ml) was dispensed into aplain tube from which serum was separated. The second aliquot (2ml) was transferred to EDTA tube. Both aliquots were kept at -20˚C until be used.

Serum samples were tested for anti-*T. gondii* antibodies by two laboratorymethods; Rapid Test Cassette(CTK Biotech Inc., USA), and enzyme linked immunosorbent assay for detection of anti-Toxoplasma IgG and IgM antibodies (Human anti-toxoplasma(tox) antibody (IgG and IgM) ELISA Kit/ Cusabio/China). Onlypatients with positive results in the two testswere involved in the study. Woman was considered positive for ELISA if the score was 1.4-fold higher than the cut-off. Out of 243 investigated women, 72 were found to be positive for toxoplasmosis. From the rest toxoplasma-negative, 50 women were randomly selected to represent the control group.

DNA was extracted from whole blood using ready kit (Favor prep DNA extraction mini kit/ Favor Gene Biotechnologies/ Taiwan) according to the manufacturer's manual. Allele specific polymerase chain reaction (AS-PCR) was used for genotyping. The primer sets and used for amplification and PCR conditions were according to Jafari *et al* [11]. With some modifications.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences Version 16.0 (SPSS Inc., Chicago, IL). Chi-square test was used to find out genotype deviation from Hardy-Weinberg Equilibrium (HWE), and to compare the distributions of allele frequencies in the disease and control groups. The risk associated with individual alleles or genotypes was calculated as the odds ratio (OR) with 95% confidence intervals (95% CI) using binary logistic regression test.For this analysis, subjects who were homozygous

for the wild type allele were considered as reference, and polymorphisms as dependent variables, SNP analyzer 2.0 software was used for Haplotype analysis and Pairwise linkage disequilibrium between VDR gene with construction of LD plots. A two-sided significant level of 0.05 was considered to indicate a statistically significant difference.

Results

The results of ARMS-PCR for the four FokI, BsmI, TaqI and ApaI are shown in figures 1, 2, 3 and 4 respectively.

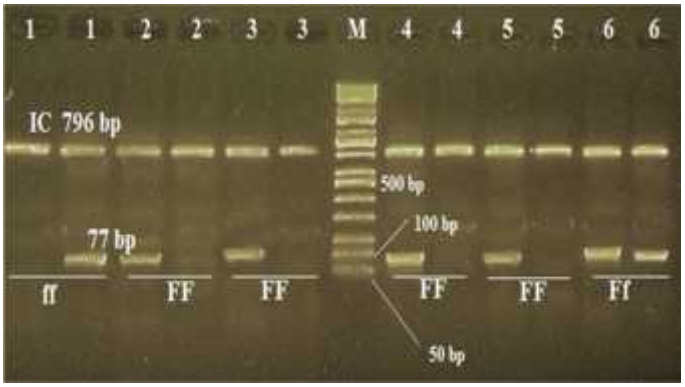


Figure (1): FokI polymorphism genotyping using two tubes AS-PCR visualized under U. V light after staining with ethidium bromide. M: 100 bp DNA marker. The 796bp represents the amplification of internal control (DRB1 gene) while the 77bp represents the amplification FokI polymorphism. IC: internal control.

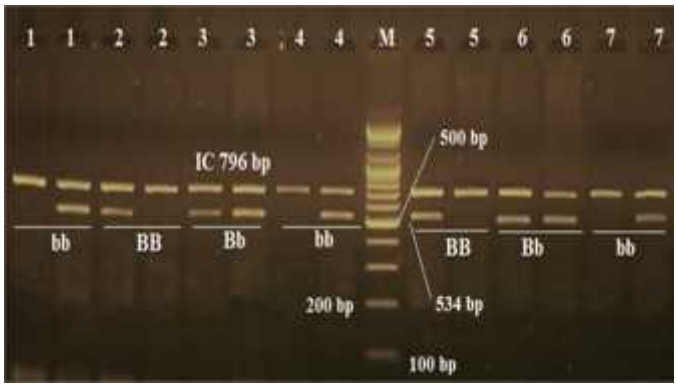


Figure (2): BsmI polymorphism genotyping using two tubes AS-PCR visualized under U. V light after staining with ethidium bromide. M: 100 bp DNA marker. The 796bp represents the amplification of internal control (DRB1 gene) while the 534bp represents the amplification BsmI polymorphism. IC: internal control.

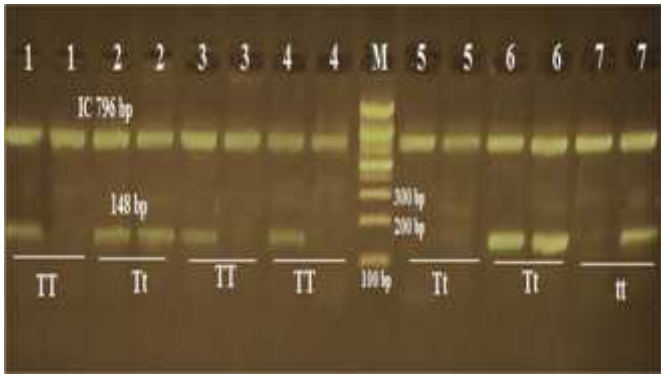


Figure (3): TaqI polymorphism genotyping using two tubes AS-PCR visualized under U. V light after staining with ethidium bromide. M: 100 bp DNA marker. The 796bp represents the amplification of internal control (DRB1 gene) while the 148bp represents the amplification TaqI polymorphism. IC: internal control.

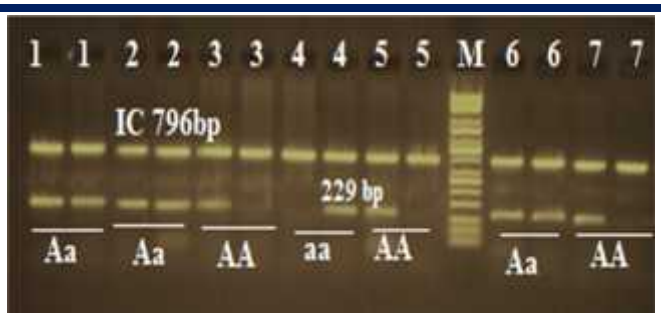


Figure (4): ApaI polymorphism genotyping using two tubes AS-PCR visualized under U. V light after staining with ethidium bromide. M: 100 bp DNA marker. The 796bp represents the amplification of internal control (DRB1 gene) while the 229bp represents the amplification ApaI polymorphism. IC: internal control.

Genotype and Allele Frequencies

The genotype distributions of the four polymorphisms were in Hardy-Weinberg equilibrium in patients and controls. Genotype and allele frequencies of these SNPs in patients and controls are represented in table 1.

The only VDR polymorphism which appeared to have significant inverse effect on toxoplasmosis is FokI. The frequency of homozygote mutant genotypeff was higher in controls (16%) than in patients (2.77%) with significant difference (OR=0.140 95%CI=0.027-0.717, P=0.018). For the other polymorphisms,the frequencies of different genotypes did not significantly differ between patients and controls.

Table (1): Genotypes and allele frequencies of VDR gene polymorphisms in studied groups**.**

Variables	Patients N=72	Control N=50	*P*-value	OR(95%CI)
FokI-Genotypes				
FF	41(56.94%)	23 (46%)	0.061	1.0
Ff	29(40.28%)	19(38%)	0.693	0.856(0.396-1.852)
ff	2(2.77%)	8 (16%)	0.018	0.140(0.027-0.717)
Alleles			0.043	
F	111 (77.08%)	65(65%)		1.0
f	33(22.91%)	35(35%)		0.552 (0.314-0.972)
BsmI-Genotype				
BB	57(79.67%)	31 (62%)	0.119	1.0
Bb	13(18.06%)	16(32%)	0.280	0.363(0.057-2.287)
bb	2(2.78%)	3(6 %)	0.841	0.821(0.119-5.670)
Alleles			0.072	
B	127(88.19%)	78(78%)		1.0
b	17(11.81%)	22(22%)		0.504 (0.250-1.012)
TaqI-Genotypes				
TT	44 (61.11%)	30 (60%)	0.670	1.0
Tt	25 (34.72%)	16(32%)	0.874	1.065(0.488-2.325)
tt	3 (4.17%)	4(8%)	0.402	0.511 (0.107-2.251)
Alleles			0.650	
T	113(78.47%)	76 (76%)		1.0
t	31 (21.53%)	19 (24%)		0.869(0.473-1.594)
ApaI-Genotypes				
AA				
Aa	28 (38.89%)	15 (30%)	0.348	1.0
aa	39 (54.17%)	28 (56%)	0.150	0.383(0.103-1.4150
	5 (6.94%)	7 (14%)	0.294	0.513(0.147-1.783)
Alleles			0.227	
A	95 (65.97%)	58 (58%)		1.0
a	49 (34.03%)	42 (42%)		0.712 (0.421-1.205)

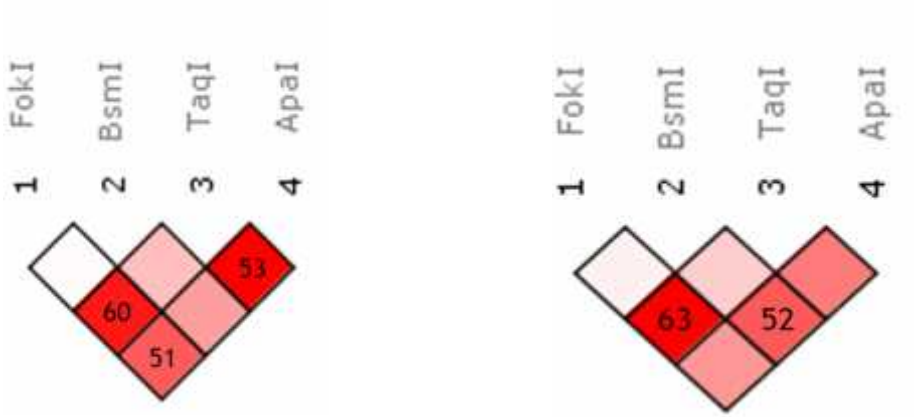
Haplotype Frequencies

Table 2 shows the frequency of different haplotypes in Toxoplasma patients and controls. The frequency of FBAThaplotype was higher in patients (0.51) than controls (0.32) with significant difference (P=0.038). On the other hand fBat haplotype had higher frequency in controls (0.24) than patients (0.061) with significant difference (P=0.012).

Table (2): Haplotype blocks of VDR polymrophisms in Toxoplasma patients and healthy controls.

Haplotype Blocks	Sequence	Frequency in Cases	Frequency in Controls	*P*-value
Block 1	FBAT	0.51	0.32	0.038
Block 2	fBat	0.089	0.15	0.378
Block 3	fBat	0.061	0.24	0.012
Block 4	fBAT	0.059	0.045	0.696
Block 5	FbAT	0.034	0.1	0.236

Figure 5 shows the result of pairwise LD calculation. In Toxoplasma patients, there were a moderate correlations between FokI and each of TaqI and ApaI, and between TaqI and ApaI. Similar correlations were also seen in controls between FokI and TaqI, and between BsmI and ApaI.



(a) (b)

Figure (5): Linkage disequilibrium between the four most common SNPs (FokI, BsmI, TaqI and ApaI) of VDR gene in (a) patients and (b) controls. Numbers in boxes represent the correlation coefficient value of LD (r^2) multiplied by 100.

Discussion

The current study showed the significant protective role of both hetezygotegenotype (Ff) and homozygote mutant genotype (ff) of FokI polymorphism against toxoplasmosis (OR=0.140, 95%CI=0.027-0.717, P=0.018 and OR= 0.164, 95%CI=0.031-0.856, P=0.032 respectively). This association was further confirmed at allelic level where carriers of "f" allele have approximately half opportunity to get toxoplasmosis compared to "F" allele carriers under the same circumstances (OR= 0.552, 95%CI=0.314-0.972, P=0.043). These results are in accordance with that obtained by Gelderet al. [12] who recorded a decreases prevalence of FokI f in British patients with pulmonary diseases caused by Mycobacterium malmoense. However, several other studies support the reverse idea that FokI f is a risk factor for infection intracellular

microorganism especially tuberculosis [13-15].

In the liver cells, vitamin D is converted into the active form (1, 25(OH)2VD3) which binds to VDR that present in almost all body [16]. The 1, 25(OH) 2VD3-liganded VDR can directly induce the transcription of different genes. However, the most prominent effect of this ligand is the downregulation of signaling transcription factors among which the nuclear factor NF-κB [17]. Moreover, the active form of vitamin D could affect the pattern recognition receptor such as toll like receptor 2 (TLR2) and thus inhibits the maturation and differentiation of antigen presenting cells (APCs) [18]. Finally, there is some evidences which indicate that 1, 25(OH)2VD3 can directly skew T cell differentiation toward Th2 and Treg at the expense of Th1 and Th17 [19][20]. For toxoplasmosis, allthese activities can increase the individual's susceptibility and exaggerate the infection.

FokI polymorphism (C to T transition) indirectly affects the activity of vitamin D. It is located in the translation initiation site. Two versions of protein with different lengths, The presence of wild type C allele (F) at this site produces VDR with 424 amino acids, while a receptor with 427 amino acids is produced by the variant T allele (f)[21]. Biologically, the longer protein was found to be less active than the shorter one [22]. In the same regard, Li et al. [23] demonstrated that individuals carrying f/f genotype had significantly higher vitamin D concentration compared to those carrying F/F genotype; however such result was not always obtained [24]. Nevertheless it can be state that the activity of vitamin D is reduced somewhat with the longer protein VDR (encoded by f allele), which may explain the protective role of this allele against toxoplasmosis.

While FokI is located in coding region, BsmI and ApaI (in intron 8) and TaqI (in exon 9) are located at the 3'UTR of the gene. Accordingly, the expected influence of this polymorphism on VDR will be less than that of FokI despite their minor role in VDR mRNA stability [21]. And the rate of transcription [25]. Although there were inverse association of these polymorphisms with toxoplasmosis, the differences were not significant. Similar results were obtained by Gaoet al [26]. Who did not find significant association of these SNPs with tuberculosis in African and South American population.

Certain haplotypes in VDR gene seems to form a genetic combination that influence he susceptibility or resistance against different infections.Alagarasuet al. [27] .Showed that the b-A-T haplotype had inverse association with HIV infection while B-A-t haplotype links with increase the susceptibility to this infection. In the current study the haplotype involving the wild type alleles of the SNPs (FBAT haplotype) predisposes to toxoplasmosis while the protective haplotype (FBat) involves almost the mutant alleles. These results partially support the result of genotyping which indicated a protective role of f allele of FokI against the disease.

Linkage disequilibrium describes the association of alleles of adjacent polymorphisms into each other [28]. This implies than one polymorphism can predict another which is significantly linked to it. Analysis of LD (with haplotypes) is a very effective tool to understand the contribution of different variants in a gene as a risk for certain diseases [21]. In this study, FokI is linked to TaqI in both patients and controls. As the genotyping results indicate a significant association of FokI f with the resistance to toxoplasmosis, it is reasonable to assume that FokI f acts in a synergistic effect with TaqI t in this resistance.

Collectively these data indicate the protective role against toxoplasmosis ofFokI f allele which is in LD with TaqI t allele. In addition, what can be called wild

typehaplotype (FBAT haplotype) could predispose to the disease while FBat haplotype is a protective genetic constituent.

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