Vitamin-D Receptor (VDR) Gene Polymorphisms (FokI and TaqI) in Patients with Hashimoto's Thyroiditis of Iraqi Population

Wajdy Jabbar Majid[1], Abdul Hussein Alwan Algenabi[2], Alaa Abood Alwadees[3]

Wajdy Jabbar Majid[1], Abdul Hussein Alwan Algenabi[2], Alaa Abood Alwadees[3]
[1]Department of Biochemistry, College of Medicine, University of Thi-Qar, Iraq.
[2]Department of Biochemistry, College of Medicine, Kufa University, Iraq.
[3]College of Medicine, Department of Surgery, Kufa University, Iraq.

Correspondence:
Wajdy Jabbar Majid
Department of Biochemistry, College of Medicine, University of Thi – Qar, Iraq

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ABSTRACT

Background: Hashimoto's thyroiditis is a multifactorial disease in which different environmental factors may trigger the already carried genetic susceptibility in affected individuals. One of the involved theories in the progression of autoimmune disorders is the impairment of immunomodulatory activities of vitamin D or its receptors. The gene polymorphism of vitamin D receptor was reported as an associate of numerous autoimmune diseases including Addison's disease or type I diabetes mellitus. The association between vitamin D receptor gene (VDR) polymorphisms and risk of Hashimoto's thyroiditis was not analyzed in our community yet.

Methods: A case-control study was conducted on 182 Hashimoto's thyroiditis patients visiting the Al-Hussein teaching hospital in Al-Nasiriah city and Al-Sadder medical city in Al-Najaf in addition to 200 healthy individuals as a control group. Serum vitamin D3, FT3, FT4, TSH, anti-TG and anti TPO concentrations were determined by Electrochemiluminescence methods using Cobas e411 from Roche Company. The genotyping of VDR gene (TaqI and FokI) was achieved by PCR-RFLP method in all participants.

Results: Dominant homozygous genotype (FF) were significantly higher in the Hashimoto's thyroiditis patients compared with those of the control group (OR= 2.22; P=0.0002), while the OR for the heterozygous Ff genotype (0.63; p=0.029) and recessive homozygous ff genotype were (0.40; p=0.017) suggesting that the individuals carrying homozygous dominant FF genotype were two times more susceptible for development of Hashimoto's thyroiditis than individuals carrying the ff or Ff genotypes which were more protective from disease. In addition, results regarding VDR-TaqI polymorphism showed that individuals carrying dominant homozygous TT genotype or T allele have higher risks to develop Hashimoto's thyroiditis (OR= 2.64 and OR=1.78 respectively).

Conclusion: The current study data of VDR gene polymorphisms suggesting that Iraqi individuals carrying dominant homozygous FF of rs2228570 SNP and dominant homozygous TT of rs731236 SNP were more susceptible for development of Hashimoto's thyroiditis while individuals carrying the ff homozygous or Ff heterozygous of rs2228570 SNP and heterozygous Tt of rs731236 SNP were more protective from disease. However, such associations were not related to vitamin D status of the studied Hashimoto's thyroiditis population.

Keywords: Vitamin D Receptor gene polymorphism, Vitamin D levels, Hashimoto's Thyroiditis.

INTRODUCTION

Hashimoto's thyroiditis (HT) is an autoimmune thyroid disorder in which a pathologic attack of lymphocytes to the thyroid follicles occurs. The thyroid hormones are produced by thyroid follicles on a large protein referred to as thyroglobulin, which also regarded as storage location of thyroid hormones (Zaletel, 2007). One of the specific biochemical features of HT disease is the presence of anti-thyroid antibodies, which are anti-thyroglobulin (anti-TG) and anti-thyroid peroxidase (anti-TPO). Small amounts of thyroglobulin are released into the blood where the average half-life is about three days (Frohlich and Wahl, 2017; Indrasena, 2017). So that, both antibodies play pivotal role in the pathogenicity of HT although in various degrees, for example, in contrast to Anti-TG antibodies, the Anti-TPO antibodies capable to activate complement components and cause thyroid cell toxicity (McLachlan and Rapoport, 2004). Nevertheless, both thyroid antibodies are useful as markers for the diagnosis of diseases of autoimmune thyroiditis.

In HT disease, autoantibodies against TPO are present in more than 90 percent of patients, while Tg antibodies can be distinguished in nearly 80 percent (Zaletel, 2007, 4). The prevalence is approximately 2 percent in all age groups, with incidence of (0.3-1.5)/1000 persons every year (Hiromatsu et al., 2013). HT is more common in female than in male. Among females, disease incidence is at least eight times higher than in males (Caturegli et al., 2014). Nevertheless, according to laboratory results for females the occurrence of anti-TPO antibodies occur in approximately 10% of the population (Hiromatsu et al., 2013). During the early beginning of HT when the immune responses is passively attacking and breaking down the thyroid follicules, elevated the levels of triiodothyronine (T3) and thyroxine (T4) develops produced by destroyed thyroid glands cells into the peripheral blood simulating a transient hyperthyroid state (Unnikrishnan et al., 2013).

HT occurs more frequently in patients with other autoimmune diseases, such as primary adrenal hypofunction, Gravis disease, rheumatoid arthritis, pernicious anemia, gluten enteropathy, lupus disease and type 1 diabetic disease (Wiebolt et al., 2011). In addition, these disorders are associated to a involvement of genetic and environmental factors (Weetman, 2011). No data on the relationship between FokI and TaqI polymorphisms and HT was performed in Iraq. Therefore, the aim of present study was to

evaluate FokI and TaqI gene polymorphisms in HT patients and healthy control groups as possible risk factors for HT in the Iraqi population.

MATERIALS AND METHODS

This study consists of 182 adult patients (mean age: 39.06±12.2 years; range: 18 – 68 years), all of them are previously or newly diagnosed with HT according to criteria of American Association of Clinical Endocrinology those who referred to the Al-Hussein Teaching hospital in Nasiriyah city and Al-Najaf medical city, were selected to participate in this case-control study. For control group, we carefully selected 200 apparently healthy individuals (21 males and 179 females) aged 18 and 68 years (mean age: 36.03±10.66 years), with no history of any chronic autoimmune disease like diabetes mellitus, SLE, rheumatoid arthritis and celiac disease and also those with normal thyroid function test , anti-TPO and anti-Tg antibody at the time of samples collection. For both groups we measure the levels of freeT3, freeT4, TSH, anti-TPO and anti-Tg antibody (Table 1).

Table 1: characteristics of autoimmune hypothyroidism patients and healthy control.

Variable	Patient (n=182) No. and %	Control (n=200) No. and %	P value
Sex			
Male	21(11.53)	21(10.5)	0.746
female	161(88.46)	179(89.5)	
Age (mean±SD)	39.06±12.2	36.03±10.66	0.01
18-27	38(20.87)	51(25.5)	
28-37	41(22.52)	55(27.5)	
38-47	55(30.21)	63(31.5)	0.023
48-57	34(18.68)	28(14)	
58-68	14(7.69)	3(1.5)	
Biochemical parameters			
Anti-TPO	300.36±173.97	13.61±7.67	<0.0001
Anti-TG	1052.22±1207.66	32.93±17.65	<0.0001
Free T3	3.73±0.96	5.17±0.61	<0.0001
Free T4	10.70±3.32	16.06±5.44	<0.0001
TSH	32.69±34.67	2.31±1.07	<0.0001

Variables are shown as mean±SD; Statistical significance at p value < 0.05

Genotype analysis

Two milliliters of whole blood samples were collected from all participants in EDTA tubes and stored at - 20 0C according to the manufacturer's recommendations. Human DNA was extracted using a ReliaPrepTM Blood gDNA Miniprep System Kit (Promega, USA) to be used for gene study.

Table 2: Primers sequences and PCR conditions used in this study

Sequences of primers		PCR protocols
TaqI rs731236	F:5'CAGAGCATGGACAGGGAGCAA-3' R:5'GCAACTCCTCATGGCTGAGGTCTC-3'	94° 10 min, 35X(94°C 15 s,55°C30 s, 72°C 30 s), 72° 10 min
FokI rs2228570	F:5'AGCTGGCCCTGGCACTGACTCTGCTCT-3' R:5'ATGGAAACACCTTGCTTCTTCTCCCTC-3'	95° 5 min, 30X(94°C 60 s,60°C60 s, 72°C 60 s), 72° 5 min

TaqI (rs731236) and FokI (rs2228570) polymorphisms in VDR gene were amplified with polymerase chain reactions (PCR). For these reactions, 12.5 µl of GoTaq®G2 green master mix (ready to use) (Promega, USA), 2.0 µl of forward primer, 2.0 µl of reverse primer, 5 µl of extracted DNA sample, and 3.5 µl of nuclease free water. The PCR conditions and forward, reverse primers sequences are showed in Table 2. PCR products obtained were 740 bp and 273 bp for TaqI and FokI on 2.0% agarose gel, respectively. The PCR products were digested (RFLP or Restriction fragment length polymorphism) by TaqI and FokI restriction enzymes (Promega, USA; BioLabs, UK; respectively). TaqI PCR product was digested for 2 hours in 65° C and then inactivated by water bath at 850 C for 10 minutes, FokI PCR product was digested for 60 minutes in 37° C then were inactivated in water bath at 650 C for 20 minutes, and electrophoresis of digested PCR products on 2.0% agarose gel was used to evaluate of TaqI and FokI polymorphisms. For TaqI, wild genotype (TT) detected with two fragments at 495 and 245 bp, heterozygous genotype (Tt) presented with fragments at 495, 290, 245 and 205 bp, whereas for homozygous genotype (tt) display by bands at 290, 245 and 205 (Fig. 1). For FokI, wild genotype (FF) presented with one band at 273 bp, heterozygous genotype (Ff) detected with three fragments at 273, 198 and 75 bp, while for homozygous genotype (ff) revealed by two fragments at 198 and 75 bp (Fig. 2).

Figure 1: **Agarose gel electrophoresis for the analysis of TaqI (rs731236) genotypes in fourteen Hashimot'o thyroiditis patients. (2% agarose, 90v for 45 min).**

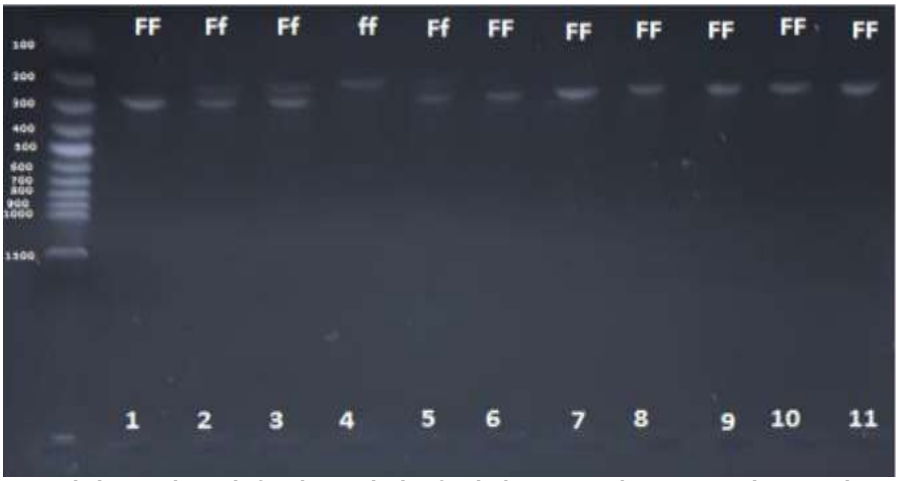

Figure 2: **Agarose gel electrophoresis for the analysis of FokI (rs2228570) genotypes in 11 patients. (2% agarose, 90v for 45 min).**

RESULTS

To analysis the distribution of genotypes and alleles, Hardy-Weinberg equilibrium (HWE) test was done and the findings showed that both FokI and TaqI polymorphisms were in HWE in patients and control groups (p> 0.05). According to our study, dominant homozygous genotype (FF) were significantly higher in the Hashimoto's thyroiditis patients compared with those of the control group (OR= 2.22, CI95%= 1.45 – 3.36, P: 0.0002), while the OR for the heterozygous Ff genotype (0.63; p=0.029) and recessive homozygous ff genotype were (0.40; p=0.017) suggesting that the individuals carrying homozygous dominant FF genotype were two times more susceptible for development of Hashimoto's thyroiditis than individuals carrying the ff or Ff genotypes which were more protective from disease. On the other hand, results regarding VDR-TaqI polymorphism showed that individuals carrying dominant homozygous TT genotype or T allele have higher risks to develop Hashimoto's thyroiditis (OR= 2.64 and OR=1.78 respectively) (Table 3).

Table 3: Distribution of the Vitamin D receptor FokI (rs2228570), TaqI (rs731236) genotypes and alleles in patients and controls

Genotype / allele	Patients (N=182)		Control (N=200)		P value	OR	95% CI
	No.	%	No.	%			
FokI genotypes :							
FF	93	51.10	64	32	0.0002	2.22	1.45 – 3.36
Ff	78	42.86	108	54	0.029	0.63	0.43 – 0.96
Ff	11	6.04	28	14	0.017	0.40	0.19 – 0.82
Alleles :							
F	264	72.52	236	59	0.0001	1.83	1.35 – 2.48
F	100	27.47	164	41	0.0001	0.54	0.40 – 0.73
TaqI genotypes :							
TT	90	49.45	54	27	<0.0001	2.64	1.73 – 4.05

Tt	75	41.21	119	59.5	0.0005	0.47	0.32 – 0.72
Tt	17	9.34	27	13.5	0.265	0.66	0.35 – 1.26
Alleles							
T	255	70.05	227	56.75	0.0002	1.78	1.32 – 2.40
T	109	29.95	173	43.25	0.0002	0.56	0.42 – 0.76

DISCUSSION

Hashimoto's thyroiditis is a common chronic autoimmune disorder. Its pathogenesis is mainly explained by an immune system disorder. Studies have also confirmed the presence of immune-linked genes associated with this disorder (Stefanić et al., 2008). Also the HT is a multifactorial disease in which different environmental factors (Ajjan and Weetman., 2015; Trimarchi,2015) may trigger the already carried genetic susceptibility in affected individuals. Several genes that been to be associated with occurrence of disease, disease progression and disease severity. HLA and CTLA-4 genes are mostly involved (Zaletel and Gaberscek, 2011; Pastuszak-Lewandoska, et al., 2009). VDR is the main nuclear vitamin D receptor and plays an significant role in the regulation of vitamin D (Kato, 2000). The VDR gene on Chromosome 12 has a few single nucleotide polymorphisms (SNP) such as FokI and TaqI, both of which are involved in vitamin D (Li et al., 2012).

This report was the first analysis of potential effects of the VDR FokI and TaqI on genetic susceptibility in the Iraqi population to Hashimoto's thyroiditis. Our findings showed that genotypes FokI "FF" and "TT" have a major impact on HT susceptibility. In addition, the genotype of FokI "FF" and TaqI "TT" has been considered a risk factor for patients with Hashimoto's thyroiditis.

The current study results for the distribution of dominant homozygous FF genotype for FokI (rs 2228570) SNP showed OR=2.22 with p value=0.0002 while the OR for the heterozygous Ff genotype (0.63; p=0.029) and recessive homozygous ff genotype were (0.40; p=0.017) suggesting that the individuals carrying dominant homozygous FF genotype were two times more susceptible for development of Hashimoto's thyroiditis than individuals carrying the ff or Ff genotypes which were more protective from disease.

It has been shown that the expression of VD receptor could be affected by genetic polymorphism of VD receptor gene (Uitterlinden et al., 2004; 19). For example, Ogunkolade *et al*. reported that the VD receptor SNP (rs2228570) in coding region is associated with elevated mRNA quantity of VD receptor. Some studies were consistent with the present study results and confirm such assumption as they showed significant correlation between FokI polymorphisms and occurrence risk of thyroid autoimmune diseases. For instance, studies conducted by Djurovic et al. Yazici et al., and Lin WY and their colleagues in their studies showed that rs2228570 of VD receptor gene were associated with Hashimoto's disease in Serbian(Djurovic et al., 2015),Turkish (Yazici et al., 2013) and Taiwan (Lin et al., 2006) societies respectively. However, other studies including genome-wide association study showed that this polymorphism did not influence individual susceptibility to Hashimoto's thyroiditis (ElRawi et al., 2019).

This discrepancy in results among the published literature as well as the current findings in VDR gene polymorphisms distributions in different population could be due to the distinct ethnicities and different geographical regions (Semino et al., 2000). That is to say, based on these conflicting results, the involvement of rs2228570 SNP in Hashimoto's thyroiditis pathogenesis and development required to be interpreted carefully (Gao and Yu, 2018).

In addition, the present study was recorded significant difference (p=0.029) in VDR Fok1 genotype Ff between patients and control groups. Such findings are in agreement with the results of Yazici et al. who found that *the heterozygous genotype of VDR* rs2228570 SNP were significantly associated with lower risk of HT in their study population (Yazici et al., 2013).

In contrast to the current results, Guleryuzi B. et al. found no significant differences (p = 0.28) in the distribution of Ff genotype between patients (24.2%) and control groups (32%) (25). Based on the current results regarding the genotype frequency of rs731236 SNP between Hashimoto's thyroiditis and control groups, individuals carrying dominant homozygous TT genotype or T allele have higher tendency for occurrence of Hashimoto's thyroiditis (OR= 2.64 and OR=1.78 respectively).

Previous studies evaluated the association of VDR gene SNPs with Hashimoto's thyroiditis patients and found that Turkish persons carrying homozygous dominant genotype (TT) of rs731236 SNP were more prone to develop Hashimoto's disease (Yazici et al., 2013), and also with Meng Feng et al., in their meta-analysis study (Giovinazzo et al., 2017).

However, several other researches from Polish, Italian, Chinese and Japanese population did not revealed significant differences in the distribution of VDR-TaqI genotypes between HT patients and healthy controls (Maciejewski et al., 2019) and also J. Djurovic et al. Observed a higher allele frequency, but not significant from control, of rs731236 SNP in Serbian population with Hashimoto's thyroiditis, (P > 0.05) (Wang et al., 2017).

CONFLICT OF INTEREST: Nil

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