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RESEARCH ARTICLE

HUMAN LEUKOCYTES ANTIGENS HLADQB1 DETERMINE SUSCEPTIBILITY TO THYROID DISEASE IN SAMPLE OF PATIENTS

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ABSTRACT

Background: Thyroid disease is a common prevalent disease in the women of reproductive age. This disease arises due to complex interactions between environmental and genetic factors. On the other hand, the relations between genes and environment are yet to be identified. The most important susceptibility genes that have been identified is the HLA-DQB1 gene locus on chromosome number six. The major environmental factors include iodine, medications, infection, smoking, and possibly stress.

Aim of study: the association between HLA-DQB1 alleles and goitrous thyroid disease in a group of Iraqi Arab Muslims.

Patients and methods: A case-control comparative research was carried out in Al-Kindy Teaching Hospital, Baghdad-Iraq. Patients with thyroid diseases attended this hospital in the period September-2013 to June-2014 for thyoidectomy. HLA –DQB1genotyping was done using a group of sequence-specific oligonucleotide probes (SSOP) method by means of HLA-DQB1amplification kit and other hybridization kit (SSO HLA type DQB1 plus and Mastermix for HLA type DQB1 Amp plus kits - Innogenetics- from Belgium) by using AutoLipa – 48Innogenetics-Belgum.

Results: There was an increased frequency of HLADQB1*03:01and 0601 in control group compared with patients group (P=0.005, Odds ratio=0.0164, 95% Confidence Interval: 0.0009-0.2926) and (P=0.01, Odd ratio=0.1667, 95% Confidence Interval: 0.0412 to 0.6750) respectively. Other alleles like HLA-DQB1* 0202, 03:02, 0501 and 06:02 were detected in patients group and not in control group.

Conclusions: HLA alleles have an effect on development thyroid disease. HLADQB1* 0301 and 0601 is a protective in Iraqi Arab Muslims individuals.

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INTRODUCTION

Thyroid enlargement whether diffuse or nodular is common problem in the general population named as goiter. Goiter is defined as a thyroid gland enlargement exceeds the upper maximum value of normal for the individual's age and sex: 18 mL for females, 25 mL for males.

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This goiter may be linked with a euthyroid, hyperthyroid, or hypothyroid metabolic condition (Fuhrer *et al.,* 2012).Goiter is a multifactorial in its origin which is due to iodine deficiency in genetically susceptible individual with other factors like cigarette smoking, pregnancy, selenium or zinc deficiency, goitrogens and emotional stress (Krohn *et al.,* 2005). Pathogenesis of nodular goiter is iodine deficiency with mutagenic environmental factors in genetically predispose person that leads to cellular proliferation and formation of free radicals that promotes the appearance of somatic mutations in

thyrocytes (Führer *et al.,* 2010). Consequently, the cause of this disease seems to involve complex interactions between different factors which they are genetic and environmental factors. Genetic factor is an important aspect that leads to thyroid disease. Four genetic loci were associated with thyroid volume which two of them are free loci that situated upstream of and within CAPZB. These two genes encodes the beta subunit of the barbed-end F-actin binding protein and its function is modulating actin polymerization. Other locus blots FGF7, which its gene encodes fibroblast growth factor 7. The last fourth locus corresponds to a ‘‘gene desert’’ located on chromosome 16q23 downstream of the coding sequence LOC440389 (Teumer *et al.,* 2011). Other hereditary studies have shown the association between human leukocyte antigen (HLA) region that located on chromosome 6p21.31 is a significant factor in development thyroid disease. HLA region contains many immune response genes and it is highly polymorphic region that everybody had HLA differs from others. This HLA region encodes genes that are classified into three classes (class I,II and III), first class is class I genes that contains HLA-A, HLA-B and HLA-C, second class is class II genes that involves HLA-DR, HLA-DP and HLA-DQ(5). Numerous studies have been performed about thyroid disease and HLA association among diverse ethnic and racial populations. The main function of HLA class II molecules is presentation of antigenic peptide to T helper cells in the thymus and in the blood. It had been reported that HLA class II region DRB1 and DQB1 have been related with thyroid disease. For example, predisposing or positive effect (increased risk of disease) for HLA-DRB1*03, HLA-DQB1*02, HLA-DQA1*05 (DR3) and a protective or negative result (decreased risk of disease) for HLA-DRB1*07, HLA-DQB1*02, HLA-DQA1*02 (DR7) have been always demonstrated (Yanagawa *et al*., 1994; Zamani *et al*., 2000). We try to demonstrate the association between HLA-DQB1 alleles and goitrous thyroid disease in a sample of Iraqi Arab Muslims.

Patients and Methods

A case-control study was carried out in Al-Kindy Teaching Hospital, Baghdad-Iraq. Patients with thyroid diseases attended this governmental hospital in the period September-2013 to June-2014 and thyoidectomy was done according to physion and surgeon openion. An informed consent procedure from the patients was approved by the Al-Kindy College of Medicine - Scientific and ethical committee. Population under study consisted of 30 Iraqi Arab Muslims patients who had the following inclusion criteria : clinically diagnosed as goiter (nodular, multinodular and diffuse) and by laboratory tests diagnosed as hypothyroidism (symptoms & low levels of T4 and T3 with high level of TSH), hyperthyroidism (symptoms & high levels of T4 and T3 with low level of TSH), Euothyroid (symptoms due to pressure effects & normal levels of T4, T3 and TSH)**.** The exclusion criteria were any patient with other autoimmune disease like diabetes mellitus and any other diseases. Twenty four of the patients were females, 6 were males, with ratio 12:3 females to males. The age mean of the patients is 35.5 years with range 20-61 years. The control group consisted from 30 healthy individuals ethnically matched with patients group. They do not have any past history of thyroid disease. 15 of them were males and the rest was females, with ratio 3:1 males to females. The age mean of the control group is 36.5 years with range 23-60 years. Ten mL of venous blood were collected by venipuncture from study population, thyroid

Table 1. Distribution of human leukocytes antigens (HLA-DQB1) allele's frequencies in patients with thyroid disease and healthy control groups

HLA-DQB1* alleles	Thyroid patients group No.=30		Healthy control group No.=30		Odd ratio (95% confidence interval)	P- value
	No.	%	No.	%		
02:01	15	25	12	20	1.5 0.5395 to 4.1707	0.43
02:02	9	15	0	0	na	na
03:01	15	25	30	50	0.0164 0.0009 to 0.2926	0.005
03:02	3	5	0	0	na	na
05:01	6	10	0	0	na	na
06:01	3	5	12	20	0.1667 0.0412 to 0.6750	0.01
06:02	6	10	0	0	na	na
06:04	0	0	6	10	na	na

na=not applicable

Table 2. Gene frequencies of patients with thyroid disease and control group

HLA-DQB1* alleles	Thyroid patients group No.=30	Healthy control group No.=30
02:01	0.29	0.22
02:02	0.16	0
03:01	0.29	1
03:02	0.05	0
05:01	0,10	0
06:01	0.05	0.22
06:02	0.10	0
06:04	0	0.10

disease patients and normal Iraqi Arab Muslims individuals with no family history of thyroid disease, ethnicity, age and sex were matched. Five mL in plane container, which used for thyroid function test (TSH, T4, T3). The other five mL in EDTA containers for DNA extraction by blood kit (QIAmp DNA blood Mini Kit, QIAGEN INC- Germany). The presence of DNA was assessed by using 2% agarose gel containing ethidium bromide and was visualized under UV illumination source light. DNA amplification was done for DQB1 by SSOP method using HLA-DQB1amplification and hybridization kits (SSO HLA type DQB1 plus and Mastermix for HLA type DQB1 Amp plus kits –Innogenetics from Belgium using AutoLipa – 48Innogenetics-Belgum in HLA research unit. LiRas version-5.0 software- Innogenetics from Belgium was used to interpret the results. Statistical analysis was done using using MiniTab version. 3.0 software to analyze the results. Chi-square was used to analyze the distribution of HLA alleles in patients with thyroid disease and control groups. Fisher's exact test, the Odds ratio (OR) along with the 95% confidence interval (95% CI) were used. P-value less than 0.05 were considered statistically significant.

RESULTS

This study included thirty patients with thyroid diseases. 24 (80%) of the patients were females, 6 (20%) were males, with ratio 12:3 females to males. The age mean of the patients is 35.5 years with range 20-61 years. 4 diagnosed as hypothyroidism, 1 hyperthyroidism, and 25 goiters. Tissue typing for both control and thyroid patients groups were done for DQB1* alleles using PCR-SSOP method. Table-1- showed allele's frequencies of HLA-DQB1 for thyroid patients and control group. There was an increased frequency of HLADQB1*03:01and 0601 in control group compared with patients group (P=0.005, Odds ratio=0.0164, 95% CI: 0.0009-0.2926) and (P=0.01, Odd ratio=0.1667, 95% CI: 0.0412 to 0.6750) respectively. Other alleles like HLA-DQB1* 0202, 03:02, 0501 and 06:02 were detected in patients group and not in control group. Gene frequencies of both patients and control groups were shown in Table 2.

DISCUSSION

Thyroid disease is a common public health problem in women in reproductive age. Genetic factor is one of the causative agents of this disease. HLA alleles are one of the predisposition or protective against this disease. Many studies were done in this field regarding the association of thyroid disease and HLA typing. Grumet *et al*. first showed this association with alleles of MHC class I – HLA-B8 (8). The first team who demonstrated the association between HLA-DR3 in Canadian Caucasians and strong linkage disequilibrium with HLA-B8 was Farid *et al*. in 1979 (9). Our study found that an increased frequency of HLADQB1*03:01and 0601 in control group compared with patients group (P=0.005, Odds ratio=0.0164, 95% CI: 0.0009-0.2926) and (P=0.01, Odd ratio=0.1667, 95% CI: 0.0412 to 0.6750) respectively. This means that DQB1*03:01 and 06:01 is protective against development of thyroid disease. Other study demonstrated HLADRB1*0301 and HLA-DQB1*0201 considered to be a possibility for developing disease and a protective allele in Sudanese population is DQB1*0601(Elmugadam *et al.,* 2014). In Romanian population, HLA-DRB1*03 and DRB1*11 may be the primary susceptibility to thyroid disease whereas HLA-DRB1*01 and DRB1*15 seem to be protective (Martin *et al.,* 2014). In other races like nonwhite South African blacks populations, has been found the associated of HLA-DR1 and DR3 (Omar *et al.,* 1990). MHC haplotype association with thyroid disease was reported in many studies. Heward *et al.,* have a relation of HLA DRB1*0304-DQB1*02- DQA1*0501 haplotype (Smith *et al.,* 1998). This is in agreement with our study, HLA-DQB1*02 was detected in patients group and not in control group. The other linkage disequlibrium is DRB3*020/DQA1*0501 haplotype in African Americans population (14). Other haplotypes in Hong Kong Chinese individuals are associated with thyroid disease are B46, DR9, DRB1*303, and DQB1*0303 (15). This discrepancy in the results are due to many factors like, race of the population, religion, criteria of sample selection, size of sample under study, method used in testing either serology or molecular.

Conclusion

HLA alleles has an effect on development thyroid disease . HLADQB1* 0301 and 0601 is a protective in Iraqi Arab Muslims individuals.

Acknowledgment

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Competing Interests

The authors have declared that no competing interest exists. There is no conflict of interest.

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