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Association of HLA-DRB1, DQB1 with Thyroid Disorders in Sample of Iraqi Patients

Mithal A. Abdoun[1] **Amina N. AL-Thwani [2]** **Laith A. Kamel [3]**

[1]Iraqi Ministry of Science and Technology (IMST), Baghdad, Iraq
[2]Genetic Engineering and Biotechnology Institute for Post Graduate studies, University of Baghdad
[3]Al-Karama hospital, Ministry of Health Baghdad, Iraq

Abstract: This study was carried out to evaluate some immunogenetic parameters of some Iraqi patients with thyroid disorders. Forty patients suffered from thyroid disorders who were admitted to the Department of Radiation, Nuclear Medicine Hospital in Baghdad and 30 healthy control during the period from June to October- 2011. The age of patients and healthy individuals ranged between 13-71 years. The HLA-class II typing was conducted for three groups the first group consisted of patients with hyperthyroidism, hypothyroidism and thyroid non toxic goiter patients, the second group consisted of thyroid cancer patients and the third group consist of healthy individuals. By using molecular methods polymerase chain reaction- sequence specific oligonucleotide (PCR- SSO) for HLA-Typing , the results showed that high frequencies of HLA DRB1*13and HLA-DRB1*10 ($P<0.01$ and $P<0.05$, respectively) in thyroid cancer patients when compared with control group. Whereas the frequency of HLA-DQB1*06 significantly ($P<0.05$) increased in healthy control group compared to thyroid disorders patient groups. The results suggested that the immunogenetic markers such as HLA-DQB1*06 allele may have a protective effects against the development of thyroid disorders.

Key words: HLA-DRB1, DQB1, Thyroid disorders, PCR.

ارتباط المؤشرات المناعية HLA-DRB1,DQB باختلالات الغده الدرقية في عينة من المرضى العراقيين

مثال عبد الكريم عبد عون[1] **امنة نعمة الثويني[2]** **ليث عبد الاله كامل[3]**

[1]وزارة العلوم والتكنولوجيا، بغداد، العراق
[2]معهد الهندسة الوراثية والتقانات الاحيائية– جامعة بغداد
[3]وزارة الصحة – مستشفى الكرامة، بغداد، العراق

الخلاصة: تعود اختلالات الغدة الدرقية لعدد من الاسباب وقد تبين ان اقتران العوامل الوراثية والبيئية والذاتية ضروري لحدوث هذه الحالة المرضية. تضمنت الدراسة تقييم بعض مؤشرات المناعة الوراثية لدى عينة من المرضى العراقيين المصابين باختلالات الغدة الدرقية. اجريت الدراسة لـ 40 شخصا يعانون من اختلالات الغدة الدرقية من الذين تم فحصهم في قسم الطب النووي لمستشفى الإشعاع والطب الذري في بغداد و 30 شخصا سليماً تم اتخاذهم كمجموعة سيطرة وذلك للفترة من حزيران الى تشرين الاول 2011. تراوحت أعمار المرضى والأصحاء من 13 – 71 سنة. لتنميط مستضد الكرية البيضاء البشري للصنف الثاني (- , Class-II- HLA-DRB1 DQB1) لدى مرضى اختلالات الغدة الدرقية تم تحليل توزيع هذه الاليلات لثلاثة مجاميع تضمنت المجموعة الاولى المرضى المصابين بقصور الغدة الدرقية، الدراق ومرضى النشاط المفرط للغدة الدرقية ،اما المجموعة الثانية فشملت مرضى سرطان الغدة الدرقية والمجموعة

الثالثة تضمنت 30 شخصا سليماً (مجموعة سيطرة) وقد اشتملت الدراسة الجزيئية استخدام تقنية PCR–SSO، أظهرت النتائج بأن هنالك زيادة معنوية في تكرار مستضدات الكرية البشرية البيضاء HLADRB1*10,13 لدى مرضى سرطان الغدة الدرقية مقارنة بمجموعة السيطرة (P<0.01 , P<0.05 ، على التوالي)، في حين كان هناك زيادة معنوية (P <0.05) في تكرار HLA-DQB1*06 عند مجموعة السيطرة (الاصحاء) مقارنة بمرضى اختلالات الغدة الدرقية. تشير النتائج الى ان المعلم المناعي الجيني كالاليل HLA -06 *DQB1ممكن ان يساهم في التقليل من خطورة الاصابة باختلالات الغدة الدرقية لدى المواطنين العراقيين.

Introduction

Thyroid disorders increase in an ubiquitous global phenomenon suspected to further rise in the upcoming decades (1,2). According to the American Thyroid Association (ATA), More than 12 percent of the U.S. population will develop a thyroid condition during their life time (ATA, 2013) . Thyroid disorders are also health problems in Iraq,(3) reported that disorders rate was(50.82%), the prevalence in female (55.24%)was higher, than in male (34.57%), these percentages reflect severe endemicity. In a recent study of convenience sample of students from AL- Russafa 2 sector, found that thyroid disorders prevalence was estimated to be 14.35% (4). These disorders have a multifactorial etiology, and the right combination of genetic, environmental, and endogenous factors are required for the initiation of the disease process.
The Human Leukocyte Antigen (HLA)is a vital genetic factor that initiates or regulates immune response by presenting foreign or self antigens to T-lymphocyte, therefore lower or higher representation of some HLA alleles may contribute to susceptibility and severity of thyroid disorders.

Materials and methods

Subjects

Thyroid Disorders Groups

Forty patients of thyroid disorders who attend the endocrinologist in Nuclear Medicine Hospital in Baghdad, were selected. Clinical, ultrasonication and serum thyroid hormones were used for diagnosis. Patients' ages ranged from 13-71 years. All patients were suffering from thyroid disorders (hyperthyroidism, thyroid non-toxic goiter, hypothyroidism and thyroid cancer) who have been referred to the Department of Radiation, Nuclear Medicine Hospital in Baghdad during aperiod from June 2011 to October 2011.

Healthy control group

Apparently healthy control group consistsed of thirty healthy individuals with ages ranged (13-71). Free from obvious abnormalities were selected after informed consent was obtained.

HLA Typing

For this, a peripheral blood sample was taken in a tube containing EDTA. The steps for this technique include the QIAamp DNA Mini Kits method genomic DNA purification (QIAGE 2010). Subsequently, the HLA-DRB1and HLA-DQB1 alleles were genotyped using polymerase chain reaction and sequence specific oligonucleotide (manufactured by innogenetics N.V Technologiepark 6, 9052 gentm Belgium).

Ethical use of data

Informed consent was obtained from all the study participants, and the

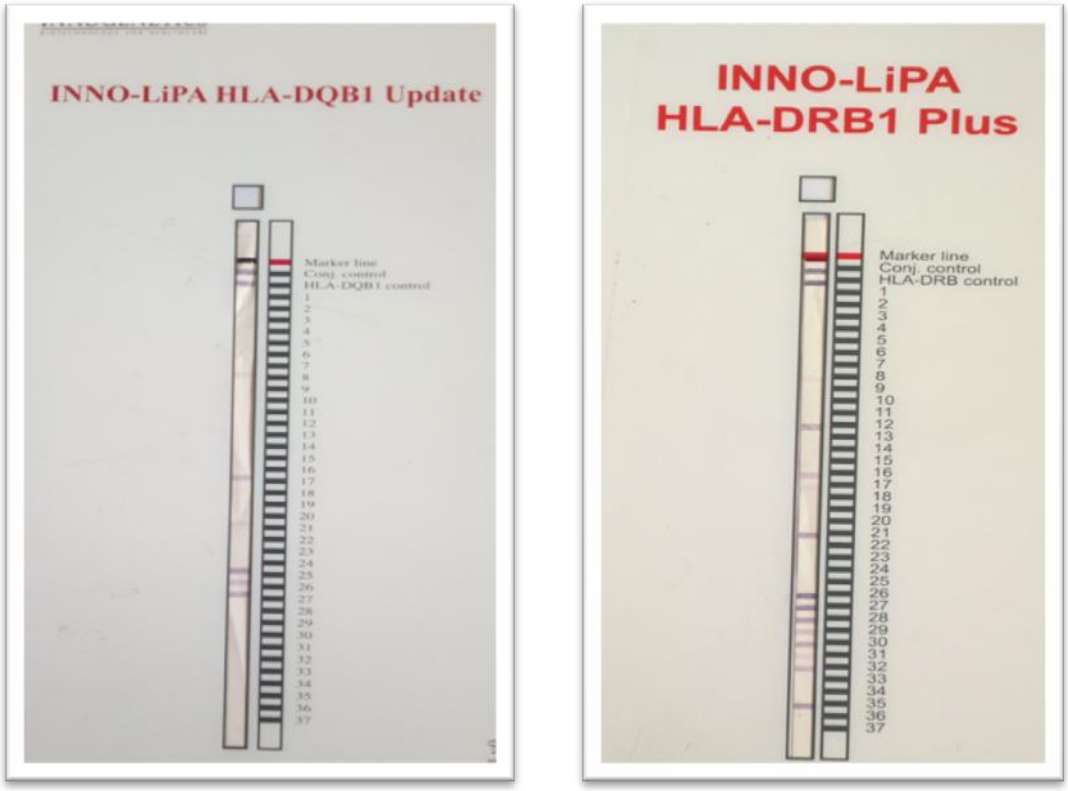
guidelines set by the ethics committee of our institute and hospitals were applied.

Statistical Analyses

The HLA-DRB1and HLA-DQB1allele frequencies were estimated by direct counts, and they represent the percentage of individuals who were positive for a particular allele. To compare the differences between the frequencies in the control andclean-up worker groups, a 2×2contingency table analysis was done using the pearson X 2 when the expected value for an HLA marker was less than 5. All of the analyses were performed using the SPSS version 15-software package). The relative risk (RR) was calculated according to the Woolf method. The P values were corrected by multiplying them by the number of alleles tested (Bonferroni correction). The association between the clinical variables and HLA-D alleles was made using the Pearson X 2 test, with the Fisher exact test when the expected value for an HLA marker was less than 5. Only P values of less than 0.05 were considered statistically significant.

Results and Discussion

Many studies have identified genetic human leukocyte antigen (HLA) markers as the determinants of the relative risk for pathological processes and the development of immune disorders, for the susceptibility to the pathogenic microorganisms, etc. (5).
Therefore lower or higher representation of some HLA alleles may contribute to the developing of Thyroid Disorders. The HLA typing conducted in this study for 40 Iraqi thyroid Disorders patients included hypothyroidism, hyperthyroidism, thyroid non toxic goiter , thyroid cancer,and 30 control subjects were successfully genotyped at the DRB1 and DQB1 loci , using PCR-SSO method with 16 HLA-DR and 6HLA-DQ specific primers mixture.
The comparison was made between frequencies of HLA alleles in patients and controls, to verify some association between these alleles and thyroid disorders. The frequency distribution was constructed to give an insight on which of the HLA- DR and DQ alleles was more frequent or infrequent in 70 subjects for each of the two loci, which were used in this study (Figure 1).



(A) **(B)**

Figure 1:A:HLA-DQB1 and,B: HLA-DRB1 Alleles Amplification by PCR-SSO of Thyroid Disorders Patients, Separated DNA Strandwere Hybridized with Specific Oligonucleotide Probes Immobilized as Parallel LinesonMembrane-Based Strips

Distribution of HLA-DQB1and HLA-DRB1 in Patients Group with Thyroid Cancer and Apparently Healthy Controls

We discuss thyroid cancer patient in aseparate group from other thyroid disorders because of the difference in its molecular basics .The frequency distribution of various HLA class II-DR antigens for Thyroid cancer (TC) group and healthy controls are presented in table (1). Highly significant $P \leq 0.01$ frequencies of HLA-DRBI*13 antigens were observed.

Hence these frequencies have elevated odds ratio (OR) (7.50) and 95% CI=1.532-36.714, as well as the following Antigen DRB1*10 was presented with higher frequencies in T C patients with $P \leq 0.05$. On the other hand, there were some antigens that showed higher frequency in healthy controls as compared with patients ,but statistically not significant like HLA-DQB1*06.

Positive association of specific HLA class II alleles in any malignant-tumor type reflects the specific role of these molecules in the promotion of chronic inflammation HLA expression suggests

that immune-system evasion of certain cellular populations could be responsible for promoting survival of the neoplasm (6).

The study of this disease and the factors that predispose its presentation is of prime importance for identification of at-risk groups, which translates into a more precise evaluation for each patient (6).The origin of malignant neoplasms is multifactorial nevertheless, there are certain factors that can increase not only the risk for appearance of the disease, but even more so that the tumor would continue to grow and would produce distal disease or metastasis (7).

The incidence of thyroid cancer has been rising at a high pace in the United States and in many other countries. On the basis of the data from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) dataset, the incidence has nearly doubled in the United States during the last decade and almost tripled since the early 1970s (8).

Chaudhuri *et al* .(9) stated that if immunological surveillance is an important mechanism in the tumorgenesis process, certain individuals who inherit specific HLAclass II alleles can be resistant or more susceptible to tumor presentation.

To date, few studies have been conducted as attempt to determine the association and impact that these represent in the risk of presenting thyroid cancer and the different HLA, especially HLA class II, and some studies lack sufficient power due to a reduced number of studied cases (Baccar *et al* .,2006). In 1994 Rigopoulouet al. , compared 161 Spanish patient with thyroid cancer, They performed HLA- classI (B35) and- classII (DR11) typification . The most important differences were found in the HLA-B locus, where the HLA-B35 allele was present with greater frequency in the group of sick patients than in the control group ($p \leq 0.05$) (10). In another study by (11) found that HLA-DRB1*04 frequency was significantly higher in Iranian patients compared to the controls [P=0.02, OR; 1.9, 95% CI (1.04-3.57)].

Table 1:- Distribution of HLA-DQB1and HLA-DRB1 in Patient with Thyroid Cancer, and Controls

Allele	Control Group NO.=30		Thyroid Cancer NO.=10		OR	X^2	P value	CI
HLA-DRB1	N	PF(%)	N	PF(%)				
*01	3	10	1	10	1.000	0.000	>05	0.092-10.865
*02	0	0	1	10	-	3.077	>05	--
*03	5	16.7	3	30	2.143	0.833	>05	0.408-11.255
*04	11	36.7	0	0	-	-	>05	-
*05	1	3.3	0	0	-	0.342	>05	-
*06	1	3.3	0	0	-	0.342	>05	-
*07	6	20.0	1	10	0.444	0.519	>05	0.047-4.222
*08	1	3.3	1	10	3.222	0.702	>05	0.183-56.883
*10	0	0	2	20	-	6.316	0.058	-
*11	6	20.0	4	40	2.667	1.600	>05	0.566-12.557
*13	5	16.7	6	60	7.500	7.064	0.014	1.532-36.714
*14	4	13.3	1	10	0.722	0.076	>05	0.071-7.340
*15	9	30.0	1	10	0.259	1.60	>05	0.028-2.360
*16	3	10.0	0	0	-	1.081	>05	--
HLA-DQB1								
*02	11	36.7	7	70	4.030	3.367	>05	0.861-18.855
*03	18	60.0	5	50	0.667	0.307	>05	0.158-2.810
*04	3	10.0	0	0	-	1.081	>05	-
*05	10	33.3	1	10	0.222	2.048	>05	0.025-2.008
***06**	**15**	**50.0**	**3**	**30**	-	**1.081**	**>05**	-

Distribution of HLA -DQB1and HLA-DRB1 in Patients with Thyroid Disorders and Controls

Data results of the frequency distribution of various HLA class II-DRB1 antigens for all thyroid disorders group and healthy controls are presented in table (2).No statistically significant differences between Thyroid disorder patients and healthy control group in HLA -DQB1and HLA-DRB1 alleles frequency. except Protective effects against the development of thyroid disorders were seen at DQB1*06, P=0.044 ,OR=0.333 and, 95% CI=0.121-0.917.

In addition the comparison with healthy control : some alleles DRB1*13andDRB1*11 were increased in individuals with thyroid disorders more than of control group but statistically not significant while DRB1*02,*10 and*12 was foundonly in thyroid disorders group as well as the following alleles : DQB1*03 which were presented with higher frequencies in thyroid disorders patients than with healthy control OR (1.758), but it did not shown any significance.

The results of different works show few reproducible results because there are important differences in the expression of the different HLAs, depending on the geographical area to which reference is made (12). This is due to that the frequency of presentation of the different HLA alleles which determined by the dominant pathogens of each geographic region in particular, and because these genes are highly polymorphic.

Thus rendering it necessary to continue evaluating these markers in different populations and to include greater numbers of patients to confirm the different associations and risks between alleles and haplotypes and to determine whether there are others that could be catalogued as risk factors for development of thyroid disorders , and at a determined moment whether the fact that some allele, alleles, or haplotypes are found expressed consistently in some group of Individuals affords the power to utilize HLA typifications as prognostic factors, (6) , at the present time few authors had performed characterization of HLA, and up to our knowledge this study one of the first attempt to find an association between HLA and thyroid disorders in Iraqi population , this field needs more attemps and larger sample to support our knowledge in this vital aspect.

Table 2: Distribution of HLA -DQB1and HLA-DRB1 in Patients with Thyroid Disorders and Controls

Allele	Control Group No.=30		Total No.=30		OR	X^2	P value	95%CI
HLA-DRB1	N	PF(%)	N	PF(%)				
*01	3	10	7	17.5	1.909	0.788	05>	0.450-8.098
*02	0	0	1	2.5	-	0.761	05>	-
*03	5	16.7	11	27.5	1.897	0.391	05>	0.580-6.201
*04	10	36.7	14	35	1.077	0.021	05>	0.397-2.925
*05	1	3.3	0	0	-	1.353	05>	0.968-1.106
*06	1	3.3	0	0	-	1.353	05>	0.968-1.106
*07	6	20.0	5	12.5	0.571	0.302	05>	0.156-2.087
*08	1	3.3	1	2.5	0.744	0.043	05>	0.045-12.390
*10	0	0	5	12.5	-	4.038	05>	-
*11	6	20.0	16	40	2.667	2.500	05>	0.892-7.976
*12	0	0	2	5	-	1.544	05>	-
*13	5	16.7	14	35	2.692	2.914	05>	0.845-8.583
*14	4	13.3	1	2.5	0.167	3.033	05>	0.018-1.576
*15	9	30.0	8	20	0.583	0.932	05>	0.194-1.752
*16	3	10.0	2	5	0.474	0.646	05>	0.074-3.031
HLA-DQB1								
*02	11	36.7	19	47.5	1.563	0.822	05>	0.594-4.113
*03	18	60.0	29	72.5	1.758	1.214	05>	0.642-4.814
*04	3	10.0	2	5	0.474	0.646	05>	0.074-3.031
*05	10	33.3	10	25	0.667	0.583	05>	0.235-1.892
*06	15	50.0	10	25	0.333	4.667	0.044	0.121-0.917

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