THE ROLE OF HLA-DRB1 ALLELE IN HYPOTHYROID PATIENTS WITH AND WITHOUT PERIODONTITIS

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ABSTRACT

Hypothyroidism is a frequent disorder in the general population, especially among women, is defined as a deficiency of thyroid activity that results from insufficient production or action of thyroid hormones leading to a total decrease of metabolic. Human leukocyte antigen is the most polymorphic genetic system in man. Genes of this region influence susceptibility to certain diseases.

Objectives: The purpose of the present study is to investigate the role of HLA-DRB1 genotyping in hypothyroid patients with and without periodontitis. Sixty hypothyroid patients 30 of patients were with periodontitis and 30 without periodontitis compare with 30 healthy subjects as control enrolled in this study. DNA was extracted from blood samples, then HLA- genotyping performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSO). The results showed the frequencies of HLA-DRB1*03 and *04 alleles are significantly increased in hypothyroid patients than control (P<0.001; P<0.05) respectively; whereas, the frequencies of HLA-DRB1*08 allele is significantly higher in control group as compared to patients group (P<0.05). Surprisingly comparison between groups of hypothyroid patients with and without periodontitis revealed significant higher frequency (P<0.05) of DRB1*03 allele among group of hypothyroid patients with periodontitis. The present findings suggested that the presence of HLA-DRB1*03 and HLA-DRB1*04 alleles may increase the susceptibility to hypothyroidism, while HLA-DRB1*08 allele could confer protective effects against this disease. Moreover, patients with HLA-DRB1*03 allele are more likely to develop periodontitis.

KEYWORDS: HLA-DR, hypothyroidism, periodontitis.

INTRODUCTION:

Hypothyroid is the decrease in thyroid hormones production and thyroid gland function (Pinto and Glick, 2002). It most frequently reflects a disease of the gland itself as primary hypothyroidism, and it affects hundreds of millions around the world (Jonklaas et al., 2014; Rowe et al., 2016). Autoimmune thyroid disease may occur when genetically susceptible individuals are exposed to environmental modulating triggers such as infection, stress, and iodine. Tissue infiltration of the thyroid gland by thyroid autoantibodies that mediate humoral immune reactions causes hyperthyroidism in Graves’ Disease (GD) and hypothyroidism in Hashimoto’s Thyroiditis (HT) (Ahn et al., 2011, Soomro, et al., 2016). Generally thyroid dysfunction is the second most common glandular disorder of the endocrine system which may rear its head in any system in the body including the mouth. The oral cavity is adversely affected by either an excess or deficiency of thyroid hormones (Chandra and Bathla, 2011; Babu and Patel, 2016; Al-Sabary, et al. 2017).

Periodontitis is considered as one of the most common and severe types of oral infections. It is an inflammatory disease of the supporting structures of the teeth caused by specific microorganisms resulting in progressive destruction of periodontal ligaments and alveolar bone with pocket formation, recession or both (Pushparani, 2015; Gomes et al., 2016). Patients are not equally susceptible to periodontal disease (Kornman, 2001), and individual susceptibility to the microbial challenge in periodontitis determined in part by a genetic predisposition (Hart, 1994). Studies have reported genetic risk factors as important for developing periodontitis (Boughman et al., 1986 and Boughman et al., 1990).

Over several decades, various forms of genomic analysis of the major histocompatibility complex (MHC) have been extremely successful in picking up many disease associations. This is to be expected, as the MHC region is one of the most polymorphic regions of the human genome. It also encodes proteins critical to immunity (Trowsdale and Knight, 2013; Chatterjee et al., 2014). The human leukocyte antigen (HLA) loci are part of the genetic region known as the MHC (Hugh et al., 1984). HLA allelic information is also useful in predicting immune responses to various infectious diseases, genetic disorders, autoimmune conditions and cancer (Gowda et al., 2016). The MHC genomic region is associated with more than 100 diseases (mainly autoimmune and infectious) than any other region of the genome (Price et al., 1999; Shiina et
Some HLA alleles occur at a much higher frequency in people suffering from specific diseases. The association between HLA allele and a given disease may be quantified by determining the frequency of that HLA allele expressed by individuals with the disease, then comparing with the frequency of the same allele in the general population (Kindt et al., 2007).

Sawai and DeGroot (2000) and Jacobson et al., (2008) have examined the binding and presentation of thyroidal auto-antigens to T-cells by different HLA-DR subtypes; one study has shown a higher affinity of HLA-DR3 to TSH- Receptor (TSHR) immune-dominant peptides than to TSHR non-immune-dominant peptides, suggesting that certain DR sequences influence the binding and presentation of TSHR peptides. However, HLA genes play a major role in susceptibility to autoimmune thyroid disease. For thyroid autoantigens to be presented by HLA molecules to T-cells, a mechanism of autoantigen presentation must exist within the thyroid gland or the draining lymph nodes of the gland. One potential intrathyroidal mechanism not utilizing professional antigen presenting cells \ may be through the aberrant expression of HLA class II molecules on thyrocytes (Bottazzo et al., 1983).

The aim of the present study is to investigate the role of HLA-DRB1 genotyping in hypothyroid patients with and without periodontitis as compared to control as well as to estimate and study the effect of thyroid hormones on periodontitis.

MATERIALS AND METHODS

This study was carried out in 60 Iraqi hypothyroid patients (total patients) their ages ranged between (20-64) years, were rounded up from Nuclear Medicine and Radiation Therapy Department, Educational Oncology Hospital. 30 of patients were diagnosed with periodontitis. The diagnosis was made through specialized dentists in the Department of Periodontics, College of Dentistry–Baghdad University. Beside 30 volunteer’s subjects who were considered as control, their ages and gender were matched with patients; their ages ranged between (20-55) years. Blood samples were collected from study groups.

Approximately (6 ml) of human blood was collected intravenously from patient and control groups. Blood was divided into two parts; 2 ml of the aspirated blood was immediately transferred into EDTA tube for the genotyping of HLA-DR alleles and stored in deep freeze at (-70°C). The remaining blood (4 ml) was put in clot activator tubes, centrifuged at 3000 rpm for 10 minutes and the serum was immediately separated into small equal parts and stored in deep freeze at (-20 °C) till used.

The diagnosis of hypothyroidism was based on the clinical features and biochemical tests that depended mainly on elevated serum levels of TSH, low T4 level, and low or normal T3. All the patients had no complained of other chronic or systemic diseases, and pregnant women were excluded from the study. Determination of HLA-DR typing was done by PCR-SSO probe. This test was done using DNA-SSO kit (Innogenetic, Belgium).

The Statistical Analysis System- SAS (2012) program was used to identify the effect of different factors in study parameters. The quantitative outcome variables were normally distributed, and therefore conveniently described by mean, standard error (SE) and tested for statistical significance by t-test. To measure the strength of association between 2 categorical variables, such as the presence of certain HLA-allele and disease status the odds ratio (OR) was used (Sorlie, 1995).

RESULTS AND DISCUSSION

The results presented in this study are based on the analysis of 60 hypothyroidism patients. The age of hypothyroidism patients ranged between (20-64) years with a mean age of (39.88 ± 1.423) years. However, the majority (43.33%) of patients are in the age group of (35-45) years. Furthermore, there is a significant female's predominance among patients group, no statistically significant differences (P>0.05) in age or gender existed between patients and controls groups.

The results of this study revealed that serum TSH levels are increased significantly (P<0.001) in hypothyroid patients (10.26±0.36μIU/ml) as compared to control group (5.99 ± 0.2552μIU/ml). On the other hand, patient’s serum levels of tT4, fT4, tT3 and fT3 (3.54 ± 0.17μg/dl, 0.891 ± 0.04μg/dl, 0.572 ± 0.02 ng/dl, and 1.227 ± 0.06pg/ml) are decreased significantly (P<0.001) as compared to control for tT4, fT4, tT3 and fT3 (6.12 ± 0.37μg/dl, 1.489 ± 0.08μg/dl, 0.813±0.04ng/dl and 2.206 ± 0.16pg/ml) respectively, these results illustrated in the table 1. These results confirmed the diagnosis of hypothyroidism which is characterized by elevated TSH and declined T4 and T3 hormone levels compared with control.
The results of serum thyroid hormones levels (in table-1) are in accordance with the observations of the previous researchers, Senthilkumar et al., (2015) and Hasan et al., (2016), who demonstrated that hypothyroidism patients have elevated serum level of TSH and decrease levels of T4 and T3 than that in controls. Consistency, Li et al., (2014) revealed that there are significant differences between controls and hypothyroid patients in levels of fT4, fT3, tT3 and TSH. On the other hand, Jayan et al., (2015) suggested that elevation in serum TSH is an early indicator of decreased thyroid reserve and in conjunction with decreased fT4 and fT3 is diagnostic of primary overt hypothyroidism.

It is well known that patients with low thyroid hormone levels have increased TSH levels because of the negative feedback relationship between the different hormones. The majority of hypothyroidism cases result from primary thyroid failure. Consequently, pituitary gland responds to that failure by secreting more TSH, raising serum TSH levels fairly before there is a detectable decline in circulating thyroid hormones T4 and T3 afterward, the levels of fT4 and fT3 reduced (DeRuiter, 2002).

The frequency of distribution of various HLA-DR alleles for 30 hypothyroid patients group and 20 subjects as control group are presented in table 2. Comparison between patients and control groups showed alleles deviations in their frequencies. HLA-DRB1*03 and *04 alleles are observed with increased frequencies in patients (40.0% and 21.0%) than control (15.0% and 7.50%) with (P=0.001; P=0.024) and (OR=1.508; 0.739) respectively; whereas the frequency of HLA-DRB1*08 allele is higher in control group (10.0%) as compared to patients group (0.00%), with (P=0.036) and (OR=0.564).

Furthermore, the comparison between hypothyroid patients without periodontitis group and hypothyroid patients with periodontitis group showed a significant increase in the frequency of HLA-DR*03 allele among patients with periodontitis group (46.6%) as compared to patients without periodontitis group (33.3%), with (P=0.049) and (OR=0.154). On the other hand, the frequency of HLA-DR*04 allele increase in patients without periodontitis (26.6%) than that in patients with periodontitis (16.3%) but statistically non-significant (P=0.296), as shown in table 3.

<table>
<thead>
<tr>
<th>Table (1): Serum thyroid hormones level in studied groups</th>
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<tr>
<td><strong>Hormones in serum</strong></td>
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<tr>
<td>Total patients (N=60)</td>
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<tr>
<td>Control (N=30)</td>
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<td><em>t-test (P value)</em></td>
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SE: Standard Error; **: Highly Significant (P<0.001).

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<tr>
<th>Table 2: HLA-DRB1 genotypes in hypothyroidism compared to control group</th>
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<tr>
<td><strong>HLA-DRB1 alleles</strong></td>
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<td>DRB1*01</td>
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<td>DRB1*03</td>
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<td>DRB1*04</td>
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<td>DRB1*07</td>
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<td>DRB1*15</td>
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<tr>
<td>DRB1*16</td>
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<tr>
<td>Total</td>
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OR: Odds Ratio, **: Highly Significant (P<0.001); *: Significant (P<0.05); NS: Non-Significant.
Although progress has been made in determining the genetic predisposition toAITD, less is known about the genetic predisposition to non-AITDs, in part due to the use of small data sets and the assumption of the genetic homogeneity between the two diseases (hypothyroidism with periodontitis). This study has investigated the association of theHLA class II region (HLA-DR) with hypothyroidism patients and controls.

These results are consistent with other study done by Rekha et al. (2007) showed also increase in the frequencies of DRB1*04 allele in Indian patients with hypothyroidism when compared to the controls, and reported a positive correlation between DRB1*04 and goitrous hypothyroidism, whereas DQB1*05 is observed to be negatively correlated with this thyroid dysfunction. In Turkish population, Ersoy and colleagues (2007) found that HLA-DRB1*04 allele was significantly higher in the whole patients of thyroid diseases and patients with hypothyroidism (36.1%) compared to the control group, pose as risk factors for the development ofAITD, and HLA-DRB1*08 allele was defined as a protective locus due to its high positive rate in the control group compared to the whole patient population. Another study on Brazilian population with primary hypothyroidism (54 goitrous and 37 atrophic) revealed that DRB1*03 and DRB1*04 alleles were significantly increased in hypothyroidism. In atrophic form, the percentages of DRB1*03 and DRB1*04 were (14.84%; 21.45%) vs. control (10.44%; 9.71%); while in goitrous form the percentages (16.67%) for both alleles (Zautant-Wittmann et al., 2004).

Whereas, the data by Petrone et al., (2001) of polymorphic site at HLA in an Italian population reported that the DRB1*04-DQB1*03 haplotype do not play a major role in the susceptibility of hypothyroid disease, but in female gender with age over 50 years, HLA-DRB1*04-DQB1*03 haplotype significantly increased in patients and increased the risk of developing HT.

On the other hand, Patiroğlu et al., (2015) indicated that the HLA-DRB1*03 and *04 consider as susceptible alleles were higher in the HT Turkish patients than in the controls. Another study by Ueda et al., (2014) using a sample of Japanese HT patients, identified that HLA-DRB1*04 conferred susceptibility for HT. Conversely, Cho et al., (2011) indicated that in AITD and HT the allele frequencies of HLA-DRB1*04 was lower than in controls. Unlike in thyroids from normal individuals, thyroid epithelial cells from patients with HT have been shown to express HLA class II antigen molecules like to those normally expressed on antigen presenting cells (APC’s) such as macrophages and dendritic cells. The aberrant expression of HLA class II molecules on thyroid cells may initiate thyroid autoimmunity via direct thyroid autoantigen presentation, where the thyroid cells serve as facultative APC’s (Hanafusa et al., 1983). Specific HLA-DR alleles may permit an autoantigenic peptide to fit into their peptide binding pockets and to be recognized by the T-cell receptor, while other HLA-DR alleles would not be able to bind the same autoantigenic peptide (Faa and Trucco, 1996). The high percentage in the frequencies of HLA-DRB1*03 and *04 alleles in patients of this study may be associated with periodontitis also. So the comparison between two groups of patients in this study revealed that the frequency of HLA-DRB1*03 allele increase significantly among hypothyroid patients with periodontitis (46.6%); whereas the frequency of HLA-DRB1*04 increased non-significantly in group of hypothyroid patients without periodontitis (26.6%). Several studies confirm the association of periodontitis with these two alleles. Previous Iraqi study conducted by Mohssen.
et al., (2013) reported that HLA-DRB1*03 allele contributes to the increased susceptibility to periodontitis, and DRB1*04 allele confers protective effects against this disease. Another study on Brazilian periodontitis patients by Sippert et al., (2015) reported a high prevalence of HLA-DRB1*04 alleles in patients compared with healthy subjects.

The discrepancies observed among various studies could be caused in part by the influence of ethnicity and racial background in the distribution of HLA alleles, or might be attributed to differences in methodology and sample size. It is well known that HLA surface molecules have a key role in antigen presentation and activation of T-cells. The polymorphisms of HLA can directly affect the binding capability of Ag-peptides and thus affect the Ag-specific T-cell response. Hence, these polymorphisms could represent an important susceptibility or resistance factor to periodontitis (Sippert et al., 2015).

Conclusion: The present findings suggested the presence of HLA-DRB1*03 and HLA-DRB1*04 alleles may increase the susceptibility to hypothyroidism, while HLA-DRB1*08 allele could confer protective effects against this disease. Moreover, patients with HLA-DRB1*03 allele are more likely to develop periodontitis.

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