Anti-TPO and Anti-Tg Antibodies in Sudanese Patients with Thyroid Diseases and Association of HLA Class-II with Graves' disease

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Abstract: The objective of this study was to investigate some immunogenetic aspects of thyroid diseases and the possible association of HLA Class-II loci and their frequencies in Sudanese patients with Graves' disease. Among the 208 thyroid disease patients, 67 diagnosed as hypothyroidism, 57 hyperthyroidism, 56 goiters, 18 Graves' disease, and 3 Hashimoto's thyroiditis. All blood specimens from thyroid disease patients (n=208), and control group (n=60) were tested for anti thyroid (anti-TPO, and anti-Tg) antibodies by ELISA, and for TSH, T_4, and T_3 hormones by immulite autoanalyzer. HLA-class II, DR and DQ alleles were typed from the DNA samples of forty thyroid disease patients and twenty normal individuals. Analysis of the gel was done by using One Lambda DNA/LMT Software. Analysis of case-control data was performed using the Chi-square test with $P< 0.05$ considered significant.The result of anti-TPO antibody in serum of thyroid disease patients and control group was positive in 21.2% (44/208) and 5% (3/60) respectively, p. value (0.011). But 66.7% (12/18) of Graves' disease patients were give positive result of anti-TPO antibody, which is highly significant P value (0.000) when compared to control group. HLA-DRB1*0301 found to be carried by 50% of Graves' disease patients and by 15% of control group, P.value (0.020) and relative risk (5.7). HLA-DQB1*0201 allele carried by 55.6 % of Sudanese patients with Graves' disease, and in 20 % of control group, p. value (0.023), relative risk (5.0). In contrast, the allele DQB1*0601 found in 27.8 % of patients with Graves' disease, and in 60 % of control group, p. value (0.046). We concluded that anti-TPO antibody is better than anti-Tg antibody as an indicator of Graves' disease. HLA-DRB1*0301 and HLA-DQB1*0201 considered to be a risk candidates for developing Graves' disease, while DQB1*0601 is a protective allele in Sudanese individuals.

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1.Introduction

Autoimmune thyroid disease (AITD) is one of the most common autoimmune diseases (AID). Graves disease (GD) is typified by hyperthyroidism and autoantibodies directed against the thyroid stimulating hormone receptor (TSHR), Thyroid peroxidase (TPO) and thyroglobin (Tg) [(1)]. The presence of thyroid autoantibodies substantially contributes to the pathogenesis of a number of thyroid disorders, such as Hashimoto's thyroiditis, primary myxoedema, Graves' disease[(2).] They are also present in a smaller percentage of sera from other non-autoimmune thyroid disorders [(2)]. Thyroid autoantibodies are found more frequently in females and prevalence increases with age [(2)]. Thyroid autoantibodies in autoimmune thyroid diseases have been reported to range from 1-40% but its prevalence in non-autoimmune diseases is unknown [(3)].

Major histocompatibility complex (MHC) is a strong candidate locus for autoimmune thyroid disease (AITD), which contains the human leukocyte antigen (HLA) genes, is located on chromosome 6p21[(4)].The HLA class II gene region is one such area where susceptibility loci are less well defined. Due to the crucial role played by HLA class II molecules in peptide presentation to T cells in both the periphery and during thymic selection, components of the HLA class II region have been associated with most AIDs. In GD a predisposing effect for DRB1*03-DQB1*02-DQA1*05 (DR3) and a protective effect for DRB1*07-DQB1*02- DQA1*02 (DR7) have been consistently reported[(1)]. Autoimmune thyroid diseases (AITD), comprising Graves' disease (GD) and autoimmune hypothyroidism (AIH), are characterized by loss of immunological self-tolerance to thyroid antigens. These are complex diseases arising from combination of genetic and environmental factors. An understanding of the genetic susceptibility factors for AITD could help to target treatments more effectively and identify people at risk of these conditions [(5)]. For some pathological conditions, especially those believed to have an autoimmune etiology, an association between HLA phenotype and susceptibility to clinical disease has been found. For

complex disorders, HLA association can be positive (increased risk of disease) or negative (decreased risk of disease) [6].

2.Materials and Methods

This case-control study was carried out in Khartoum city, capital of Sudan. Patients with thyroid diseases attended Khartoum Teaching Hospital and, Fedail Medical Center, in the period 2007–2009, An informed consent procedure was approved by the local medical authority, and all patients were informed about the purpose of the study. The population under study consisted (208), those individuals who had been clinically 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), Graves' disease (hyperthyroidism with family history of thyroid disease and ophthalmopathy **).** 187of the patients were females, 21were males, with ratio 9: 1 females to males. The age mean of the patients is 39.3 years with range 11-80 years.

Ten mL of venous blood were collected by venipuncture from study population (thyroid disease patients and normal Sudanese individuals with no family history of thyroid disease), ethnicity, age and sex were matched. The blood placed into two separate containers. Five mL in plane container, which used for thyroid function test (TSH, T4, T3) and thyroid antibodies (anti-TPO, anti-Tg). The other five mL in EDTA containers (Greiner labortechnik GmbH, Austria) for DNA extraction by blood kit (QIAmp DNA blood Mini Kit, QIAGEN INC). Consequently DNA yield and quality determined by spectrophotometer, then used for the typing of HLA genes by DNA-based technique. Sequence Specific Primer (SSP) methodology used to amplify the target sequence with completely matched oligonucleotide primers. Primer pairs were designed to have perfect matches only with a single allele or group of alleles. After the PCR-SSP process, the amplified DNA fragments were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide.

3.Results:

A total of 208 patients with thyroid diseases were enrolled in this study. 89.9 %, (n=187) of the patients were females, and 10.1 %, (n=21) males, with ratio 9: 1 females to males. 67 diagnosed as hypothyroidism, 57 hyperthyroidism, 56 goiters, 18 Graves' disease, and 3 Hashimoto's thyroiditis**.** The age at onset of Graves' disease was 24 – 58 years. The concentration of thyroid antibodies (anti TPO and Anti Tg) were measured by quantitative ELISA, then transformed into negative (less than100 U/L), Equivocal (100-140 U/L), and positive(more than 140 U/L).

Table1: Anti -TPO antibody in the different categories of patients with thyroid disease.

Diagnosis	Anti-TPO		
	Positive	Negative	Equivocal
Graves	66.7%	16.7%	16.7%
Hyperthyroidism	17.5%	77.2%	5.3%
Hypothyroidism	19.4%	76.1%	4.5%
Goiter	9.5%	88.9%	1.6%
Control	5%	91.7%	3.3%

Table 2: Anti -Tg antibody in the different categories of patients with thyroid disease

Diagnosis	Anti-Tg		
	Positive	Negative	Equivocal
Grave	27.8%	72.2%	0%
Hyperthyroidism	14%	84.2%	1.8%
Hypothyroidism	7.5%	88.1%	4.5%
Goiter	15.9%	73%	11.1%
Control	10%	86.7%	3.3%

Table 3: Allele frequency in patients with Grave disease compared to normal individuals.

DRB1*03	Study Group		P. Value	Relative Risk
	Grave	Control		
DRB1*03 +ve -ve	50% 50%	15% 85%	0.020*	5.7
DQB1*0201 +ve -ve	55.6% 44.4%	20% 80%	0.023*	5.0
DQB1*0601 +ve -ve	27.7% 72.2%	60% 40%	0.046*	-

*P value considered significant at level less than or equal 0.05

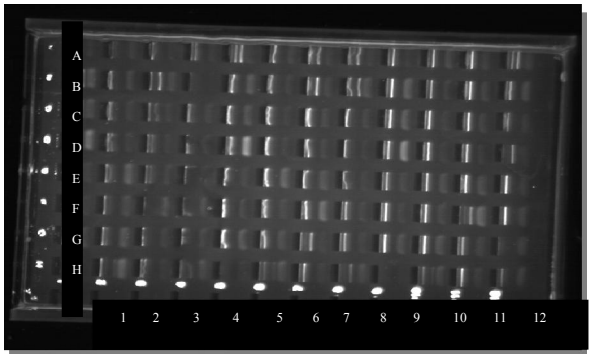

Figure 1: A 96 well gel electrophoresis result of three samples of the patients (4 rows, 8 columns, i.e. 32 wells for each patient sample). Each well showed one band of internal control (β-globin), except well number H1, H5, & H9 which are negative controls. Another band occurred in some wells indicates positive result for certain allele(alternative gene).

4.Discussion:

The age at onset of Graves' disease in this study was in the range 24 – 58 years, 100 % of these patients with age 24 -34 years had family history. Our observation is in agree with Brix who stated that, a family history of thyroid disease can be obtained in up to 50 % of patients with GD and family studies have repeatedly demonstrated a familial aggregation of GD[7].

Thyroid autoantibodies are the markers of autoimmunity in autoimmune thyroid diseases[2].In this study, Anti-TPO antibody was positive in 66.7% of graves' disease patients compared to 5% of control group. In patients with non-autoimmune diseases; hypothyroidism, hyperthyroidism, and goiter, the TPO antibody was of low frequency, it found to be positive in 19.4% , 17.5 %, and 9.5% respectively. All the above findings were higher than control group. A small number (3%) of people with no evidence of disease may have antibody [2]. Anti-Tg antibody in this study found to be positive in 27.8% of patients with Graves' disease. In our study, TPO Ab was more sensitive than ATG Ab in predicting hyperthyroidism in Graves' disease. The immunological process triggered by thyroperoxidase is reflected in the patient's antibody status. It is known that the prevalence of TPO and thyroglobulin antibodies increases with increasing age, and that the prevalence of TPO antibodies is higher in all age groups than that of Tg antibodies[8].

Grumet et al. first showed the association between GD and the alleles of MHC class I, with a higher frequency of HLA-B8 in GD patients (47%) compared with controls (21%) [9]. Stronger association of GD was found with the MHC class II allele, HLA-DR3, which is in strong linkage disequilibrium with HLA-B8 [10].

In this study, HLA-DRB1*03 & DQB1*02 were the most frequent alleles in Sudanese patients with Graves' disease compared to control group. HLA-DRB1*03 carried by 50% of Graves' disease patients and in control group carried by 15 %, P.value (0.020) and relative risk (5.7). Many case-control studies in white populations have since consistently shown the association of GD with HLA-DR3, with relative risks between 2.5 and 5 [11, 12]. In patients with autoimmune thyroid disease compared to non-autoimmune thyroid disease, the allele carried by 42.9 %, compared to10 % respectively, p.value (0.018) and relative risk (6.7). HLA-DQB1*02 allele carried by 55.6 % of Sudanese patients with Graves' disease, and in 20 % of control group, p. value (0.023), relative risk (5.0). In contrast, the allele DQB1*06 found in 27.8 % of patients with Graves' disease, and 60 % in control group, p. value (0.046). Relative risk (RR) is the chance that a member of a group receiving some exposure will develop a disease relative to the chance that a member of an unexposed group will develop the same disease. RR of 1.0 indicates probability is identical in 2 groups.

Heward et al. have confirmed the association of MHC with GD in a study that showed preferential transmission of the HLA DRB1*0304-DQB1*02-DQA1*0501 haplotype [13]. In nonwhite populations, GD has been found to be associated with different HLA alleles. For example, GD has been shown to be associated with HLA-DR1 and DR3 in South African blacks [14]. The DRB3*020/DQA1*0501 haplotype in African Americans [15]. and B46, DR9, DRB1*303, and DQB1*0303 in Hong Kong Chinese [16, 17]. However, strong linkage disequilibria within the MHC region have made it difficult to determine which of these loci is the most probable candidate for the primary susceptibility gene of Graves' disease.

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