RESEARCH

THE ROLE OF POLYMORPHISMS GENES HLA (DRB1, DQB1) IN HYPOTHYROIDISM PATIENTS

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Abstract

Background: Autoimmune diseases like auto-immune thyroiditis may be the cause of abnormal thyroid function. HLA is normally expressed on the surface of antigen-presenting cells, such as dendritic cells and macrophages, and plays an essential role in the induction of immune responses. The detection of HLA class II expressing thyroid follicular cells (TFC)' in thyroid autoimmune diseases. current studies had shown that components of the HLA class region have been associated with hypothyroidism. The Objective of this present study, we try to find out the association of the Polymorphism gene HLA (DRB1-DQB1-DQA1) with Hypothyroidism Patients. **Materials and Methods:** Present study comprises 200 participants of matched gender and age from the same population,110 participants served as healthy controls, and 90 participants as cases of hypothyroidism, with an age group ranging from 25-70 yrs. serum HLA was estimated by PCR Method. **Result:** The HLA alleles DRB1 and DQB1 were significantly ($p>0.05$) raised in clinically diagnosed hypothyroid patients as compared to age and gender match healthy control subjects. **Conclusion:** The HLA alleles DRB1 and DQB1 region exhibit complex associations with Hypothyroidism disease. Our shown that the Polymorphism Gene HLA is the main cause of derangement of thyroid function. The current cogitation proved that HLA Gene Polymorphism is highly significant in the case of Hypothyroidism.

INTRODUCTION

Hypothyroidism is a most common endocrine disorder developing from a deficiency of thyroid hormone. The thyroid hormone receptors are part of a nuclear receptor superfamily that also combines receptors for other small lipophilic hormones. Thyroid hormone receptors activity by binding to particular thyroid hormone-responsive sequence supporter of attacked genes and by regulating transcription. mutation in the associated gene also impacts the concentration of thyroid hormone and causes autoimmune thyroid disease. In India, there is 11 % of cases of hypothyroidism, compared to 2 % and 4.6 % in the UK and the USA, respectively. [1] Several studies projected the highest prevalence (13·1%) of hypothyroidism in aged 46–54 years, and 18–35 years old are less affected (7·5%). [2]

Class II HLA molecules are composed of non-covalently polypeptide chains, a 32 to 34 kD α chain, and a 29kD to 32 kD of β chain.[3] The genes encoded by both chains of class II molecules have polymorphic and are present in the HLA Locus.[4] HLA class II genes are located at position 6p21, in the D region. The D region consisted further three subregions of major functional encoding of the DP, DR, and DQ molecules. The DRB1, DQA1, and DQB1 genes have been seen as highly polymorphic and play a major role in maintaining tolerance to self-thyroid antigens. With abnormal expression seen on follicular cells (the target cells for GD) and on activated lymphocytes, the class II genes and their alleles act as primary candidates for involvement with AITD. In the Japanese population, HLA-B*35:01, -B*46:01, -DRB1*14:03, -DQB1*0604, and -DPB1805:01 were found to be positively

associated with GD, and HLA-A*02:07 and -DRB4 were found to be positively associated with HD. [5] However, few studies have been performed in India. Thus, the purpose of this study is to find out the association of the HLA gene and their alleles with hypothyroidism in the Indian population, which may be considered a novel gene for the early detection of disease.

MATERIALS AND METHODS

The study was conducted in IMCH & RC, Department of Biochemistry. Ethical clearances were obtained from the Institutional Ethical Committee and written informed consent was taken, before carrying out the study.

Subject selection: We studied 90 hypothyroidism patients and 110 matched control subjects with age groups, ranging from 25-70 years. We recruited consecutive hypothyroidism patients attending the OPD. Patients were diagnosed based on clinical criteria. The patients under treatment were included in this study. Patients with renal failure and malignancies have been excluded from this study.

Sample collection: Overnight fasting 5ml of blood was drawn from the antecubital vein for all investigations.

Sample analysis:

DNA extraction and genotyping: DNA extracted from peripheral blood leukocytes by kit method and stored at -20oC for further use. HLA-B (DQB1 and DRB1) alleles polymorphisms were genotyped using the PCR-RFLP method. Briefly, the region was amplified with the primers. The final product was digested with a restriction enzyme (TSP5091). The polymerase chain reaction products were analyzed. The results were recorded and analyzed as per standardized population genetics analytical tools.

HLA Alleles (DRB1 and DQB1) region was amplified with the following primers: -

Alleles	Forward	Reverse
DRB1	CCGCCTCTGCTC CAGGAG	TTGTGGCGCTTAA GTTTGAAT
DQB1	TGCACACCGTGT CCAACTC	GCTACTTCACCAA CGGGACC

Statistics analysis:

The odd ratio was calculated for each HLA allele in case and control by comparing the relative frequency of disease exposure. P-values considered significant were as follows: - $P < 0.05$- a Significant, $P>0.001$ - a highly Significant.

RESULTS

[Tables 1 and 2] show the difference in the ODD RATIO level and P-value of HLA alleles (DRB1*3*4*7*8*13 and DQB1*0301/4*04806) in Hypothyroidism found to be statistically significant. [Tables 1 and 2] show the HLA alleles level is significantly higher in Hypothyroidism patients who were suffering from the disease.

Table 1: The distribution of DRB1 alleles in patients with Hypothyroidism and control subjects.

DRB1	AT 2nd =90	AT%	Control 2nd=110	Control %	Odd ratio	95%CL	P-value
01	9	10	10	9.09	1.11	0.43 to 2.86	0.8274
03	17	18.89	6	5.45	4.03	1.51 to 10.72	0.0052
04	18	20	10	9.09	2.50	1.08 to 5.73	0.0305
07	11	12.22	4	3.63	3.68	1.13 to 12.01	0.0302
08	3	3.33	15	13.63	0.21	0.06 to 0.78	0.0192
09	2	2.22	4	3.63	0.60	0.10 to 3.36	0.5636
10	2	2.22	3	2.73	0.81	0.13 to 4.95	0.8203
11	8	8.89	16	14.55	0.57	0.23 to 1.40	0.2249
12	1	1.11	2	1.82	0.60	0.05 to 6.80	0.6853
13	7	7.78	22	20	0.33	0.13 to 0.83	0.0182
14	2	2.22	3	2.73	0.81	0.13 to 4.95	0.8203
15	9	10	13	11.82	0.93	0.38 to 2.23	0.8761
16	1	1.11	2	1.82	0.60	0.05 to 6.80	0.6853

Note: DRB1* 03 in case odd ratio (4.03, 95% CL 1.51-10.72), P -value 0.0052, DRB1* 04 in case odd ratio (2.50, 95% 1.08 to 5.73), P -value 0.00305, DRB1* 07 in case odd ratio (3.68,95% CL 1.13-12.01), P -value 0.000302, DRB1* 08 in case odd ratio (0.21, 95% CL 0.06-0.78), P -value 0.0192, DRB1* 13 in case odd ratio (0.33, 95% CL 0.13-0.83), P -value 0.00182, have shown highly significant.

Table 2: Shows the distribution of DQB1 alleles in patients with Hypothyroidism and control subjects.

DQB1	Case 2nd =90	Case %	Control 2nd=110	Control %	Odd ratio	95%CL	P-value
02	25	27.78	28	25.45	1.12	0.59 to 2.11	0.7112
0301/4	18	20	40	36.36	0.43	0.22 to 0.83	0.0122
0302	8	8.89	10	9.09	0.97	0.36 to 2.58	0.9604
3032	4	4.44	5	4.55	0.97	0.25 to 3.75	0.9727
305	0	-	0	-	-	-	-
04	3	3.33	13	11.81	0.25	0.07 to 0.93	0.0389
05	12	13.33	3	2.73	5.48	1.49 to 20.10	0.0102
06	20	22.22	11	10	2.57	1.15 to 5.70	0.0202

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Note: DQB1* 0301/4 in case odd ratio (0.43, 95% CL 0.22-0.83), P -value 0.0122, DQB1* 04 in case odd ratio (0.25, 95% CL 0.07-0.93), P -value 0.0389, DQB1* 06 in case odd ratio (2.57, 95% CL 1.15-5.70), P -value 0.0202, have shown highly significant.

Note: OR = Odds ratios, 95% CI = 95% confidence interval, case % and p-value is for significate, Number of hypothyroidism patients = 90, number of control subjects = 110.

DISCUSSION

This comparative study was done to analyze the polymorphism gene like HLA class-2 in Hypothyroidism patients and compare it with that of controls. In this case-control study total of 200 subjects were taken of which 100 were diagnosed with Hypothyroidism patients and 100 were age and gender-matched controls. Hypothyroidism occurs due to thyroid dysfunction or maybe altered thyroid stimulating hormone. According to the current study, radioiodine therapy and thyroid surgery are less prevalent causes of hypothyroidism, which is most frequently brought on by persistent autoimmune thyroiditis. Clinical hypothyroidism affects between 0.5 and 1.9 percent of women and 1 percent of men, while subclinical hypothyroidism affects between 3 and 13.6 percent of women and 0.7 to 5.7 percent of males. Our study shows HLA gene polymorphic alleles are the difference in the OR level and P-value of HLA alleles in Hypothyroidism found to be statistically significant. The table1 and 2 show the HLA alleles level (DRB1*3*4*7*8*13 and DQB1*0301/4*04806) significantly higher in Hypothyroidism patients who were suffering from the disease.

Our results confirm that genetic variation in the HLA gene is as strongly associated with hypothyroidism in India, as previously described in Japanese populations. Wan et al. [6] Studies had been reported by D Engelbrecht Zantut-Wittman et al. [7] a strong association between DRB1* 04and DQB1*03 in hypothyroidism patients.

Gouraud et al. found another disease also showed DRB variations, including *0401, DRB1*0404, DRB1*0405, and DRB1*0408 rheumatoid arthritis,[8] type 1 diabetes (T1D) Cucca et al. 2001, Thomson et al.[9,10] Schonland et al demonstrated that individuals with the DRB1*04 allele have shown telomeres ends shorting in both CD4+ T cells and granulocytes.[11] In addition, the study supports that ethnicity also impacts the HLA-DR gene polymorphism in the etiology of Graves' disease in Caucasians, and in other ethnic groups geographically. (Jacobson EM et al). [12]

A recent study done by M. Weissel also confirmed the strong association between HLA-DR antigens and autoimmune endocrine disorders. This denotes the "missing link" between these diseases and the HLA system. The frequency of HLA-DR5 is significantly increased in patients with Hashimoto's thyroiditis compared with controls. [13]

HLA-DRB1*030101, -DRB1*080201, -DRB1*0803, -DRB1*140301, and DRB1*1602 were found to be positively associated with hypothyroidism, In the Korean population.[14,15] Given Dong-Hwan Shin et al. early-onset AITD may be influenced by genetic factors more than late-onset AITD.[16] N. Tandon et al. studies show that hypothyroidism significantly play role in the polymorphism of HLA-DR3 and DQw2 in English Caucasia.[17] Genome-wide association studies focus on the identification of HLA alleles associated with disease susceptibility to the molecular structure of HLA,[18] HLA class I and class II genes amino acid polymorphisms independently contribute to disease risk.[19]

CONCLUSION

To conclude, we have replicated the strong association of variants in the HLA gene and shown it to be a susceptibility gene for hypothyroidism in the Indians. The epidemiological evidence is strong enough to suggest a significant genetic component in the emergence of hyperthyroidism, and in recent years, various loci and genes have been linked to or associated with hypothyroidism. It is clear that the HLA-DR and HLA- DQ genes play a major role in the etiology of hypothyroidism in India and possibly in other ethnic groups.

Strength and Limitations of the Present Study

There are a few drawbacks to the study. In the present study, only 25–70 years ages subjects participated in the research as well a reduced sample size. Hence, in the feature, we would like to include an increase in the number of participants to reach a concrete conclusion. The present study was given an impact to understand the increased concentration of the HLA polymorphism gene involved in the aetiopathogenesis of autoimmune thyroid destruction.

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