**Abstract:** Human Leukocyte Antigens (HLA) system is the loci of genes that encode for major histocompatibility complex (MHC) in humans. The expression of HLA Class II molecules on most cell types in later stages of Rheumatoid Arthritis (RA) is both a hallmark of activation and indicates the significance of these molecules in the onset and progression of RA. In the current study an attempt is made to check any possible association between HLA DQA1-0501 and DQB1-0201 alleles and progress of RA in South Indian population. The study was conducted on 154 subjects of which 84 are RA patients and 70 age and sex matched controls. HLA DQA1-0501 and DQB1-0201 alleles were amplified using sequence specific primers. The amplified product for different samples were separated by a 1.5% agarose gel stained with ethidium bromide and then photographed. All statistical analyses were carried out using SYSTAT 12 software. Fifty seven RA patients expressed HLA DQA1-0501 allele and 66 expressed HLA DQB1-0201 allele. In controls, of the total seventy, 42 expressed HLA DQA1-0501 and 53 expressed HLA DQB1-0201 allele. The results of X2 analysis suggests no significant heterogeneity between patients and controls. Odds ratio was also calculated for the two alleles which suggests no significant association between HLA DQA1-0501 & HLA DQB1-0201 alleles and RA. It is concluded that HLA DQA1-0501 & HLA DQB1-0201 alleles are not associated with progression of RA.

**Keywords:** Rheumatoid arthritis, Human Leukocyte Antigens, Major histocompatibility complex

**Introduction**

Rheumatoid Arthritis (RA) is an inflammatory disease characterized by pain, swelling, stiffness, and reduced function of the joints [1]. Onset is most frequent during middle age, but people of any age can be affected [2]. The exact cause of RA is still unknown. Several factors are responsible. Genes that play a role in the immune system seem to be involved in the development of RA, but they are not the only causative factor. The expression of HLA Class II molecules on most cell types in later stages of RA is both a hallmark of activation and indicates the significance of these molecules in the onset and progression of the disease [3].

**Human Leukocyte Antigens (HLA):** This contains three classes (I, II and III). Class I and II gene products have similar overall structure. There are no functional or structural similarities between class III gene products and class I and II gene products [4]. The HLA class I gene is located on short arm of chromosome 6 (6p21.31) telomeric to HLA class II region [5].

Studies on extended HLA haplotypes in RA have associated certain HLA class I antigens with it [6]. On the other hand these associations are mainly due to linkage disequilibrium between the two HLA Class I and Class II loci [7]. The HLA class II region is also located on short arm of chromosome 6. This contains three major independent gene clusters designated DP, DQ and DR and spans around 800 kilo bases (kb). The extra cellular region of HLA class II molecule consists of a peptide binding region and an immunoglobulin like region. The peptide binding regions of HLA class II are polymorphic and the immunoglobulin regions are non polymorphic. HLA class II antigens are primarily expressed on bone marrow derived cells, which serve as specialized Antigen Presenting Cells (APCs). Synthesis and expression of HLA molecules are highly regulated at the gene transcription level by cell type specific factors, inflammatory factors and cytokines such as interferon γ which can up regulate the expression of both HLA class I and II molecules. Epidemiological studies indicate that HLA genes are non-randomly associated with RA [8, 10].
These outcomes are not always consistent and differences exist between different racial and ethnic groups in the specific HLA alleles involved [10]. The effect of HLA class II region genes is more complicated than any of the existing hypotheses can explain [11].

In the present study, HLA DQA1-0501 and DQB1-0201 alleles were chosen because these are class II loci genes and are involved in the presentation of the foreign or self-antigens to the T-Lymphocytes and their polymorphisms can affect the antigens that are presented.

**Materials and Methods**

This study is carried out on 190 subjects of which 90 (65 females and 25 males) are RA patients and 100 age and sex matched controls. 5 ml of intravenous blood samples were collected from patients and controls into an EDTA vacutainer in aseptic conditions after obtaining informed consent from subjects. The laboratory work has been performed at Department of Human Genetics, Andhra University and Pediatric Research & Genetic Lab, Maulana Azad Medical College, New Delhi, India. All patients fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for classification of RA [12] and had a disease history of minimum 3 years.

**PCR Amplification:** The Genomic DNA is isolated by Salting out method [13] and quantified by using a Spectrophotometer. The absorbance ratio of 1.8:2.0 or higher is considered and the final solution is stored at -4°C. The set of sequence specific primers demonstrated in previous studies [14] were used to amplify the target DNA in HLA region as shown in the Figure 1.

<table>
<thead>
<tr>
<th>Allele</th>
<th>5'-GTGCCGTCTTGTGACGAGAAG-3'</th>
<th>5'-GCAAGGTCGTGCGGAGCT-3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQB1*0201-F</td>
<td>5'-TGCCCAAGCTGTGGAGAG-3'</td>
<td>5'-GGACACTGCTGAGGAGGT-3'</td>
</tr>
<tr>
<td>DQB1*0201-R</td>
<td>5'-TGC CAA GTG GAG CAC CCA A-3'</td>
<td>5'-GACA TCT TGC TCT GTG CAG AT-3'</td>
</tr>
</tbody>
</table>

**Figure 1:** List of HLA Primers

**Cycling File Parameters:** Hot start period for about 5 minutes at 95°C, followed by

- 95°C - 30 seconds
- 61°C - 35 seconds
- 72°C - 60 seconds

35 cycles

72°C - 5 minutes

An amplification product of 185-bp for HLA DQA1-0501 allele and 205-bp for HLA DQB1-0201 were detected and separated by using a 1.5% agarose gel, stained with ethidium bromide and photographed [Figure 2].

**Statistical analysis:**

Frequencies of patients and controls expressing HLA DQA1-0501 & HLA DQB1-0201 alleles were analyzed using 2 x 2 contingency table test to test the goodness of fit for any association of the said alleles with rheumatoid arthritis. A probability value of <0.05 was considered statistically significant.

**Results and Discussion**

**HLA DQA1-0501 and HLA DQB1-0201:** The frequencies of patients and controls expressing HLA DQA1-0501 and HLA DQB1-0201 alleles are presented in Table 1. Out of 90 patients, only 84 could be successfully analyzed for the two alleles. Fifty-seven (67.87) RA patients expressed HLA DQA1-0501 allele and 66 (78.57) HLA DQB1-0201 allele (percentage in parenthesis). In controls, of the total seventy, 42 (60.0) expressed HLA DQA1-0501 and 53 (76.0) expressed HLA DQB1-0201 allele (percentage in parenthesis). The results of $X^2$ analysis suggests no significant heterogeneity between patients and controls (p values = 0.090 and 0.671). Odds ratio was also calculated for the two alleles which suggests no significant association between HLA DQA1-0501 & HLA DQB1-0201 alleles and RA.

**Table 1:** Frequencies of Patients and Controls expressing HLA DQA1-0501 and HLA DQB1-0201 alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>RA Patients 84 n</th>
<th>Controls 70 n</th>
<th>X2</th>
<th>P-value</th>
<th>OR</th>
<th>CI for OR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n %</td>
<td>n %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA DQA1-0501</td>
<td>57 67.87</td>
<td>42 60.0</td>
<td>2.85</td>
<td>0.090</td>
<td>0.55</td>
<td>0.28-1.10</td>
</tr>
<tr>
<td>HLA DQB1-0201</td>
<td>66 78.57</td>
<td>53 76.0</td>
<td>0.18</td>
<td>0.671</td>
<td>0.85</td>
<td>0.40-1.80</td>
</tr>
</tbody>
</table>

n = number of individuals; p value = probability value of the statistical test, $X^2$=chi square, OR=Odds Ratio, S= Significant, NS= Not Significant.
Our study showed no significant association between these alleles and RA. Similar to our findings are reports from other studies. Ali et al., (2006) [15] demonstrated that HLA-DQB1-0201 is not associated with RA. Parthibhan et al., (2002) [16] stated that HLA DQA1 and HLA DQB1 alleles are not associated with disease progression in RA. Castro et al., (2001) [17], revealed that HLA DQA1-0501 and HLA DQB1-0201 are not significantly associated with RA and Taneja et al., (1996) [18] reported that DQB locus is not associated with RA. When other autoimmune diseases are considered the results are also inconsistent. Maciel et al., (2001) [19] and Yanagawa et al., (1994) [20] reported that HLA DQA1-0501 is associated with Graves disease. Philippou et al., (2001) [21] and Cuddihy et al., (1996) [22] stated that HLA DQA1-0501 is not an independent marker for Graves disease. HLA DQB1-0201 is associated with allergy and asthma (Movahedi et al., 2008) [23].

Conclusion
In Comparison with the previous studies and results obtained from this study it is concluded that HLA DQA1-0501 & HLA DQB1-0201 alleles are not associated with RA. The study has few limitations. This does not include patients with intermediate and mild severity and also the number of subjects studied is small. By resolving these limitations more encouraging results can be obtained.

References


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Conflict of interest: None Declared