ORIGINAL ARTICLE

HLA DRβ1 Alleles in Pakistani Patients with Rheumatoid Arthritis

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ABSTRACT

Objective: To determine frequencies of HLA DRβ1 alleles in rheumatoid arthritis in Pakistani patients.

Study Design: Cross sectional / analytical study.

Place and Duration of Study: Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi in collaboration with Rheumatology departments of Military Hospital, Rawalpindi and Fauji Foundation Hospital, Rawalpindi, from January 2009 to January 2010.

Methodology: HLA DRβ1 genotyping of one hundred Pakistani patients, diagnosed as having RA as per American College of Rheumatology revised criteria 1987, was done. HLA DRβ1 genotyping was carried out at allele group level (DRβ1*01-DRβ1*16) by sequence specific primers in RA patients. Comparison of HLA DRβ1 allele frequencies between patients and control groups was made using Pearson’s chi-square test to find possible association of HLA DRβ1 alleles with RA in Pakistani rheumatoid patients.

Results: HLA DRβ1*04 was expressed with significantly increased frequency in patients with rheumatoid arthritis (p<0.05). HLA DRβ1*11 was expressed statistically significantly more in control group as compared to rheumatoid patients indicating a possible protective effect. There was no statistically significant difference observed in frequencies of HLA DRβ1 allele *01, DRβ1 allele *03, DRβ1 allele *07, DRβ1 allele *08, DRβ1 allele *09, DRβ1 allele*10, DRβ1 allele *12, DRβ1 allele *13, DRβ1 allele *14, DRβ1 allele *15 and DRβ1 allele *16 between patients and control groups.

Conclusion: The identification of susceptible HLA DRβ1 alleles in Pakistani RA patients may help physicians to make early decisions regarding initiation of early intensive therapy with disease modifying anti rheumatic medicines and biological agents decreasing disability in RA patients.

Key words: HLA DRβ1 alleles, Rheumatoid arthritis, Pakistani population.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease with articular as well as extra articular manifestations. Bone erosions lead to joint deformities and disabilities in RA. The prevalence is reported to be 0.5-1% of world population.¹ There is a strong known genetic association of rheumatoid arthritis with the polymorphic HLA DRβ1 alleles. Role of DRβ1 alleles in disease susceptibility and disease activity have been studied extensively worldwide and known since 1970. The contribution of HLA DRβ1 as a risk factor is estimated to be 60% of the disease.² Different alleles of HLA DRβ1 have been found to be associated with RA in various population studies. The strength of the link between HLA DRβ1 alleles’ susceptibility and RA varies in different nations.³ HLA DRβ1 alleles associations in addition to recognizing susceptibility to RA also predict erosive disease in an individual.⁴ Shared epitope (SE) associated HLA DRβ1 alleles are indicative of high disease activity and poor radiological outcome in RA.⁵ The SE is an amino acid sequence consisting of QKRAA/QRRAA/RRAAA at position 70-74 in the HLA DRβ1 chain locus on short arm of chromosome 6. SE encodes an area in the third hypervariable region of the antigen binding groove of the beta chain of HLA Class II molecule. Citrullinated peptides produced in RA fit better in antigen binding groove of HLA Class II molecule with SE associated HLA DRβ1 alleles thus explaining underlying mechanism due to which HLA DRβ1 alleles predisposes to RA.⁶

The identification of susceptible HLA DRβ1alleles in Pakistani RA patients by this study will help physicians start treatment with disease modifying anti rheumatic drugs (DMARDs) and biological agents at disease onset in susceptible individuals, altering the disease course and reducing deformities and disabilities in Pakistani RA patients.⁷ An adequate treatment in genetically susceptible individuals can reduce burden on economy by reducing health care cost and loss of person’s productivity.⁸

However, sufficient data are lacking in this context in Pakistani population suffering from RA. Therefore, this study was conducted with the objective to determine the frequencies of HLA DRβ1 alleles in Pakistani RA patients.

METHODOLOGY

This was a cross sectional analytical study, carried out at the Department of Immunology, Armed Forces
Institute of Pathology, Rawalpindi in collaboration with Rheumatology departments of Military Hospital, Rawalpindi and Fauji Foundation Hospital, Rawalpindi, from January 2009 to January 2010. Sampling technique was purposive non-probability sampling. The study was approved by the local ethical committee at AFIP (Armed Forces Institute of Pathology, Rawalpindi Pakistan). A total of one hundred patients fulfilling American College of Rheumatology (ACR) revised criteria 1987 were included in the study. The patients with age less than 18 years were not included to exclude Juvenile RA. The patients were referred from Rheumatology Departments of Military Hospital Rawalpindi and Fauji Foundation Hospital Rawalpindi to Department of Immunology, AFIP. Informed consent was taken from all the patients. Fifty-three healthy individuals were included in the control group.

From each rheumatoid patient, 3 ml of venous blood was drawn in ethylene diamine tetra acetate (EDTA) tube. For DNA extraction, DNA purification kit Anagen's ultra Gene™ (Anagen Technologies, Inc., U.S.A) was used as per protocol in the kit for DNA purification from whole blood. For HLA DRβ1 genotyping, in-house method of polymerase chain reaction - sequence specific primers (PCR-SSP) was used, based on Olerup and Zatterquist study for DR “low resolution” typing by PCR-SSP. The protocol of the procedure has been established in a previous study carried out at Immunology department AFIP Rawalpindi. HLA DRβ1 alleles at low resolution, allele group level (DRβ1*01 - DRβ1*16) were identified by this procedure.

DNA extraction, PCR amplification and post amplification processing, including agarose gel electrophoresis and staining with ethidium bromide were performed as per protocol by Olerup and Zatterquist to identify HLA DRβ1 alleles in patients with rheumatoid arthritis and control group.

Frequencies and percentages were used for presentation of HLA DRβ1 alleles. Statistical Package for Social Sciences (SPSS) version 17 was used for statistical analysis of the data. Comparison of HLA DRβ1 alleles’ frequencies between patients and control groups were made using Pearson’s chi-square test. Fisher’s exact test was used when frequencies for any of the HLA DRβ1 alleles were less than 5. A p-value of < 0.05 was considered statistically significant.

RESULTS

HLA DRβ1 genotyping was employed by PCR-SSP typing in one hundred rheumatoid patients. A total of 195 alleles were detected in one hundred patients with rheumatoid arthritis. Five subjects were considered homozygous for HLA DRβ1 alleles. Fifty-three healthy individuals were included in the control group and 106 alleles were detected in this group.

The frequencies of HLA DRβ1 alleles in patients and control group are shown in Table I. HLA DRβ1 allele *04 was found to be expressed with increased frequency in patients suffering from rheumatoid arthritis (15%) when compared to the control group (4%) and the difference was statistically significant (p=0.0024). There was statistically significant difference in frequencies of HLA DRβ1*11 in patients and control group (p=0.0008). Alleles HLA DRβ1*11 was found to be occurring with significantly increased frequency in healthy control population indicating a possible protective effect. There was no statistically significant difference observed in frequencies of HLA DRβ1 allele *01, DRβ1 allele *03, DRβ1 allele *07, DRβ1 allele *08, DRβ1 allele *09, DRβ1 allele *12, DRβ1 allele *13, DRβ1 allele *14, DRβ1 allele *15 and DRβ1 allele *16 between patients and control groups. HLA DRβ1 *09 was absent in control group and found in low frequencies (2%) in RA group. Similarly, the frequency of HLA DRβ1 *08 was 2% in both control and patient groups indicating low prevalence of these two alleles in Pakistani RA population. HLA DRβ1 *15 was found with increased frequency in both patients (22%) and control (18%).

Table I: Comparison of frequencies of HLA DRβ1 Alleles in RA patients and healthy controls.

<table>
<thead>
<tr>
<th>HLA DR β1‡ Alleles</th>
<th>Rheumatoid patients No = 195 alleles</th>
<th>Control group No = 106 alleles</th>
<th>Statistical significance p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>Percentage</td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>DR β1*01</td>
<td>9</td>
<td>5%</td>
<td>6</td>
</tr>
<tr>
<td>DR β1*03</td>
<td>17</td>
<td>9%</td>
<td>6</td>
</tr>
<tr>
<td>DR β1*04</td>
<td>30</td>
<td>15%</td>
<td>4</td>
</tr>
<tr>
<td>DR β1*07</td>
<td>21</td>
<td>11%</td>
<td>15</td>
</tr>
<tr>
<td>DR β1*08</td>
<td>5</td>
<td>2%</td>
<td>2</td>
</tr>
<tr>
<td>DR β1*09</td>
<td>4</td>
<td>2%</td>
<td>0</td>
</tr>
<tr>
<td>DR β1*10</td>
<td>19</td>
<td>10%</td>
<td>18</td>
</tr>
<tr>
<td>DR β1*11</td>
<td>9</td>
<td>5%</td>
<td>17</td>
</tr>
<tr>
<td>DR β1*12</td>
<td>6</td>
<td>3%</td>
<td>2</td>
</tr>
<tr>
<td>DR β1*13</td>
<td>23</td>
<td>12%</td>
<td>7</td>
</tr>
<tr>
<td>DR β1*14</td>
<td>8</td>
<td>4%</td>
<td>8</td>
</tr>
<tr>
<td>DR β1*15</td>
<td>44</td>
<td>22%</td>
<td>19</td>
</tr>
<tr>
<td>DR β1*16</td>
<td>0</td>
<td>0%</td>
<td>2</td>
</tr>
</tbody>
</table>
group but the difference was not statistically significant (p = 0.3446). HLA DRβ1 *16 was absent in patients and observed to be present in low frequencies in control group also indicating low prevalence of this allele in Pakistani RA population.

**DISCUSSION**

HLA DRβ1 association with RA varies from population to population and among various ethnic groups. HLA DRβ1 genotyping was carried out in this study to find out genetic predisposition of RA in Pakistani population. This study has revealed an association of HLA DRβ1*04 with Pakistani patients suffering from RA. Various studies in Caucasians have shown HLA DRβ1*04 associations with RA.11,12 Previous studies on genetic similarities between Caucasians and Pakistani population have revealed that there is a strong genetic link to Caucasians. The study conducted by Anwar et al. on HLA frequencies in Pakistani population in comparison with Caucasians, Oriental and Negroid populations revealed that Pakistani population is genetically nearer to Caucasians and Orientals.13 Zafar et al. has also proved genetic linkage to Caucasians and Orientals.14 Mohyuddin et al. have also shown an influence in Pakistani population from Caucasians and Oriental populations.15 All these studies conducted on Pakistani population showing genetic similarities between Caucasians and Pakistani population are in favour of the results of this study and emphasize the fact that the RA susceptibility alleles in Pakistani RA patients are likely to be similar to the Caucasian rheumatoid patients.

In a previous study conducted by Hameed et al. on HLA DRB gene and the shared epitope association with RA in Pakistani population have reported increased frequency of HLA DR4 allele with SE in RA patients, however, the allele was not significantly expressed when compared with control group.16 In Indian population, HLA DRβ1*04 and HLA DQ*03 association has been reported in RA patients.17 A meta analysis done in Asian-Mongolid population (Korean, Japanese, Chinese and Thai) to assess association of HLA DRβ1 allele with RA in Asian population revealed that HLA DRβ1*0101, *0401, *0405, *0410 and *1001 were found to be associated with RA.18 These studies in Indian and Asian-Mongolid population has strengthened the results that HLA DRβ1*04 could be the susceptible HLA DRβ1 allele in Pakistani patients with RA.

HLA DRβ1 alleles with SE predisposes to RA. The SE is an amino acid sequence consists of QKRAA/QQRAA/RRAAA at position 70-74 in the HLA DRβ1chain on short arm of chromosome 6. SE encodes an area in the third hypervariable region of the antigen binding groove of the beta chain of HLA Class II molecule. Citrullinated peptides produced in RA fit better in antigen binding groove of HLA Class II molecule with HLA DRβ1 alleles. Hameed et al. have reported that HLA DR2 was found with increased frequency (52%) in rheumatoid patients as compared to control group (34%) and reported association of HLA DR15 (2) with RA in Pakistani RA patients.16 However, our findings revealed that though HLA DRβ1*15 frequency was (22%) in RA patients when compared with control group (18%) but this difference was not statistically significant. Ali et al. in another study have reported HLA DRβ1*01 and HLA DQβ1*06 alleles association with RA patients in Pakistan. In this study, the HLA DRβ1*01 frequency was not significantly expressed (p=0.6907) in patients (5%) as compared to control group (6%). In the study conducted by Ali et al. HLA DRβ1*15 (2) was more common (43.5%) in rheumatoid patients as compared to control group (30.8%) but the difference was not statistically significant.19 This finding is similar to the finding of the present study.

HLA DRβ1*16 was absent in patients and observed to be present in low frequencies in control group in our study. HLA DRβ1*09 was absent in control group (0%) and found in low frequencies (2%) in control group. The frequency of HLA DRβ1*08 was similar in both control (2%) and patients (2%) group. The frequencies HLA DRβ1 *08 and HLA DRβ1*09 were less indicating low prevalence of this allele too in Pakistani population. Ali et al. reported negligible number of HLA DRβ1*08 and HLA DRβ1*09 in their patient and control group.19 Hameed et al. have also reported low frequencies of HLA DRβ1*08 and DRβ1*09 in their patient and control group.16 Allele HLA DRβ1*11 was found to be statistically significantly increased in control group as compared to patients indicating a possible protective effect. Similar findings in part were reported by Hameed et al. who have shown a protective effect of HLA DR 5 (now reported as HLA DRβ1*11 and *12).16 A study conducted on Korean population conferred susceptibility to HLA DRβ1*04 and showed protective effects of HLA DRβ1*07, HLA DRβ1*08, HLA DRβ1*13 and HLA DRβ1*14.20 Meta-analysis of Asian - Mongolid population has showed protective effect of HLA DRβ1*0301, HLA DRβ1*0403, HLA DRβ1*0406, HLA DRβ1*0701, HLA DRβ1*1101, HLA DRβ1*1301 and HLA DRβ1*1405 partly supporting our finding of protective alleles in Pakistani RA patients.18 In Caucasians identified protective alleles were reported to be HLA DRβ1*07, HLA DRβ1*1201, HLA DRβ1*1301 and HLA DRβ1*1501.21 Further studies on large number of patients with high resolution typing of HLA DR alleles and research on conserved amino acid sequence in HLA DRβ1 alleles is recommended to confirm our findings.

**CONCLUSION**

This study has shown that HLA DRβ1*04 allele confers an increased risk for the development of RA in Pakistani
population. Allele HLA DRβ1*11 was expressed statistically significantly more in control group as compared to rheumatoid patients indicating a possible protective effect.

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