HLA Class II Allele and Haplotype Frequencies in Iranian Patients with Leukemia

Farideh Khosravi, Aliakbar Amirzargar, Abdolfatah Sarafnejad, Mohammad Hossein Nicknam, Kamran Alimoghadam, Saied Dianat, Ghasem Solgi, and Behrouz Nikbin

1 Molecular Immunology Research Center, Department of Immunology, Medical School, Medical Sciences/University of Tehran, Tehran Iran
2 Department of Pathobiology, Immunology Division, School of Public Health, Medical Sciences/University of Tehran, Tehran Iran
3 Department of Hematology, Oncology and Bone Marrow Transplantation, Shariaty Hospital, Medical Sciences/University of Tehran, Tehran Iran

ABSTRACT

Previous studies demonstrated significant differences in a number of HLA allele frequencies in leukemia patients and normal subjects.

In this study, we have analyzed HLA class II alleles and haplotypes in 110 leukemia patients (60 acute myelogenous leukemia “AML”, 50 chronic myelogenous leukemia “CML”) and 180 unrelated normal subjects. Blood samples were collected from all of the patients and control subjects. DNA was extracted by salting out method and HLA typing was performed using PCR-SSP method.

Significant positive association with AML was obtained for HLA-DRB1*11 allele (35% vs. 24.7%, P=0.033). Two alleles including HLA-DRB4 and –DQB1*0303 were significantly less frequent in AML patients than in controls. HLA-DQB1*0303 allele was never observed in CML patients compared with allele frequency in controls (4.2%). According to haplotype analysis, HLA-DRB1*0101/DQA1*0104/-DQB1*0501 frequencies were significantly higher and –DRB1*16/-DQA1*01021/-DQB1*0501 frequencies were significantly lower in CML patients than in controls. In conclusion it is suggested that HLA-DRB1*16 allele and HLA-DRB1*15/-DQA1*0103/-DQB1*06011 and –DRB1*16/-DQA1*01021/-DQB1*0501 haplotypes predispose individuals to AML and HLA-DRB4 allele predispose to CML.

In conclusion it is suggested that HLA-DRB1*16 allele and HLA-DRB1*15/-DQA1*0103/-DQB1*06011 and –DRB1*16/-DQA1*01021/-DQB1*0501 haplotypes predispose individuals to AML and HLA-DRB4 allele predispose to CML.

Future studies are needed to confirm these results and establish the role of these associations in AML and CML.

Key words: Acute myelogenous leukemia (AML); Chronic myelogenous leukemia (CML); HLA class II; Polymorphism

INTRODUCTION

Leukemias are classified by origin and pathogenesis into few categories. Acute myelogenous leukemia (AML) accounting for less than 1% of all cancers and 34% of all leukemias affecting human with peak of incidence between 15 and 39 years of life, but it is also observed in children. AML heterogeneity occurs during complex stages of differentiation of myeloid cells and it is distinct from chronic myelogenous leukemia (CML). Several genetic translocations occurring in AML have been reported which include: t(15;17),

Correspondence Author: Aliakbar Amirzargar, PhD; Immunogenetics Research Unit, Department of Immunology, Medical Sciences/University of Tehran, Tehran Iran. Tel: (+98 21) 8895 3009, Fax: (+98 21) 6642 2337, E-mail: amirzargar.ali@yahoo.com
t(4;11) and t(12;21) encoding PML-RARα, MLL-AF4 and ETV6-RUNX1, respectively. Mutated genes introduce two effects to human white blood cells including proliferation and/or survival of hematopoietic progenitors and impairment of hematopoietic differentiation.

Several environmental risk factors for the development of AML are benzene, radiation, alkylating agents exposure and history of chemotherapy for other cancers.

Chronic myelogenous leukemia (CML) is defined mostly by a genetic translocation t(9;22)(q34;q11) affecting human mainly in the fourth and fifth decades of life.

Genetic alteration in CML leads to Philadelphia chromosome formation.

Two genes which are fused together including BCR and ABL genes, encode P210 fusion protein with a tyrosine kinase activity. This protein not only serves as a proliferative factor for the cell cycle but also induces an antiapoptotic property. This may be due to some signals counterbalancing the apoptosis or blockade of apoptotic pathways. Some genes play a role in this process including phosphatidylinositol-3 kinase, She, GRB-2, p21RAS, p160BCR, Myc and C-Myb.

The first HLA study in human leukemia showed an increase of HLA-A2 allele frequency in ALL in 1967. Seremetis et al have reported the strongest association between AML and HLA alleles using a monoclonal antibody specific for the HVR3 epitope of HLA-DR53 (Pv<0.000005). In another study by Duncan Gowans in 150 AML patients from UK no significant association with HLA-DRB1 alleles was found.

However in CML, P210 fusion protein as a novel protein to the immune system is presented to T-cells in association with HLA molecules. In this case HLA molecules play an important role in immune response to the tumoral cells.

In a case-control study designed by Shailendra Mundhada to assess the association of HLA alleles with CML in 163 patients and 376 control subjects a significant positive association was observed between CML and some alleles including HLA-A*01, -A*66, -B*37, -B*38, -B*42, -B*45, -B*49, -B*53, -B*56, -B*62, -DQB1*0201, -DQB1*0402, -DQB1*0609, -DRB1*0301, -DRB1*0302, -DRB1*0901, -DRB1*1001, -DRB1*1201, -DRB1*1202, and -DRB1*1503. In two other studies HLA-A3, -B8, -DR4 and -Aw19 have been reported as protective factor against CML. In Chinese CML patients, HLA-DPB1*1301 and DPB1*20011 frequencies were higher in patients compared with control group. HLA-DRB6 frequency was significantly lower in Sicilian CML patients.

In the present study, we have analyzed HLA-DRB,-DQA1 and –DQB1 allele and haplotype frequencies in two groups of patients with AML and CML, we also compared alleles frequency in patients with AML and CML.

MATERIALS AND METHODS

Sampling and DNA Extraction

A group of 110 patients with leukemic disorders (CML (50 subjects) and AML (60 subjects)) referred to Hematology, Oncology and bone marrow transplantation (BMT) center at Shariaty Hospital (Referral center for hematological and leukemic disorders) Tehran, Iran, who were selected in this study. A hundred and eighty normal blood donor subjects as control group were randomly selected from healthy blood donors admitted to Iranian Blood Transfusion Organization (IBTO). CML Patients diagnosed on the basis of cytogenetic studies by G-banding method and all of them were positive for Philadelphia (Ph) chromosome. AML patients were diagnosed on the combination of morphologic, immunophenotype and cytogenetic studies. Informed consents were taken from all of the patients and control subjects participating in this study. Genomic DNA was extracted from 10 ml peripheral blood in EDTA by modified salting out method. HLA-DRB, DQA1 and DQB1 typing were performed by polymerase chain reaction based on sequence specific primers (PCR-SSP), according to Olerup and Zetterquist method. TAQ DNA polymerase was purchased from Roche (Basel, Switzerland). The PCR reactions were carried out in 10 µl volumes. Samples were amplified in Techne genius thermal cyclers, after initial denaturation at 94°C for two minutes, followed by 10 cycles of 94°C denaturation for 10 seconds, 65°C annealing and extension for 60 seconds, and finally 20 cycles of 94°C denaturation for 10 seconds, 61°C annealing for 50 seconds and 72°C extension for 30 seconds. After amplification, PCR products were run on an agarose gel, and then gel was interpreted for specific bands.
HLA Class II Allele and Haplotypes in Leukemia using a UV trans-illuminator. The haplotypes were calculated according to Iranian population specific linkage disequilibrium pattern among HLA-DRB, -DQA, and -DQB alleles.23

Statistical Analysis
The differences in allele and haplotype frequencies of HLA alleles among studied groups were analyzed using the Chi-square test after Yates correction.24 Each allele frequency in both patient groups was compared against the same allele in controls. Also, allele frequencies have been analyzed between AML and CML patients. The odds ratio (OR) and its 95% confidence intervals (CI) were calculated and a P-value of 0.05 or less was considered to be significant using InStat version 3.06 (GraphPad Software Inc, CA, 2003). All of P-values were Bonferroni's corrected (Pc).

RESULTS
Data on distribution of HLA class II alleles and haplotypes in AML, CML and control subjects are presented in Tables 1 and 2.

AML vs. Control
Our data show a significant positive association of HLA-DRB1*11 allele with AML (35% vs. 24.7%, Pc=0.033). The HLA-DRB4 allele is observed in AML patients with significantly lower frequency compared with controls (11.6% vs. 21.1%, Pc=0.021). The HLA-DQB1*0303 allele was never observed in AML patients while its frequency in control subjects was 4.2% (Pc=0.028). In AML patients, HLA-DRB1*07 and -DQ1*0201 alleles frequency were lower than in control group while statistically were insignificant (Pc=0.058). HLA-DRB5 frequency is higher in AML patients (21.6%) than in controls (13.8%), (Pc=0.059) (Table 1).

Table 1. HLA Class II alleles frequency in Iranian AML, CML patients and controls.

<table>
<thead>
<tr>
<th>DRB1 alleles</th>
<th>AML (n=60)</th>
<th>CML (n=50)</th>
<th>Control (n=180)</th>
<th>DQA1 alleles</th>
<th>AML (n=60)</th>
<th>CML (n=50)</th>
<th>Control (n=180)</th>
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<td>0101</td>
<td>11(9.2)</td>
<td>7(7)</td>
<td>24(6.6)</td>
<td>0101</td>
<td>9(7.5)</td>
<td>4(4)</td>
<td>35(7.9)</td>
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<tr>
<td>15</td>
<td>17(14.2)</td>
<td>11(11)</td>
<td>47(13.1)</td>
<td>01021</td>
<td>15(12.5)</td>
<td>7(7)</td>
<td>49(13.6)</td>
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<td>16(4.4)</td>
<td>0103</td>
<td>20(16.6)</td>
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<tr>
<td>0301</td>
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<td>9(9)</td>
<td>32(8.8)</td>
<td>0104</td>
<td>7(5.8)</td>
<td>10(10)</td>
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<td>0201</td>
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<td>9(9)</td>
<td>36(10)</td>
</tr>
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<td>04</td>
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<td>12(12)</td>
<td>33(9.2)</td>
<td>03011</td>
<td>8(6.6)</td>
<td>13(13)</td>
<td>38(10.5)</td>
</tr>
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<td>10(10)</td>
<td>36(10)</td>
<td>0401</td>
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<td>0(0)</td>
<td>6(1.6)</td>
</tr>
<tr>
<td>08</td>
<td>1(0.8)</td>
<td>0(0)</td>
<td>8(2.2)</td>
<td>05</td>
<td>52(43.4)</td>
<td>46(46)</td>
<td>130(33.3)</td>
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<td>0901</td>
<td>0(0)</td>
<td>0(0)</td>
<td>7(1.9)</td>
<td>DQB1 alleles</td>
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<td>1001</td>
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<td>1(1)</td>
<td>12(3.3)</td>
<td>0201</td>
<td>16(13.3)</td>
<td>18(18)</td>
<td>68(18.8)</td>
</tr>
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<td>11c</td>
<td>42(35)</td>
<td>33(33)</td>
<td>89(24.7)</td>
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<td>0(0)</td>
<td>0(0)</td>
<td>1(0.3)</td>
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<td>12</td>
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<td>0401</td>
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<td>6(1.6)</td>
</tr>
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<td>1401</td>
<td>2(1.6)</td>
<td>6(6)</td>
<td>18(5)</td>
<td>0501</td>
<td>26(21.6)</td>
<td>13(13)</td>
<td>66(18.3)</td>
</tr>
<tr>
<td>1402</td>
<td>0(0)</td>
<td>1(1)</td>
<td>0(0)</td>
<td>05031</td>
<td>10(8.8)</td>
<td>4(4)</td>
<td>9(2.5)</td>
</tr>
<tr>
<td>DRB3</td>
<td>63(52.5)</td>
<td>58(58)</td>
<td>177(49.1)</td>
<td>06011</td>
<td>14(11.6)</td>
<td>5(5)</td>
<td>25(6.9)</td>
</tr>
<tr>
<td>DRB44</td>
<td>14(11.6)</td>
<td>22(22)</td>
<td>76(21.1)</td>
<td>0602</td>
<td>12(10)</td>
<td>10(10)</td>
<td>37(10.3)</td>
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<td>DRB55</td>
<td>26(21.6)</td>
<td>12(12)</td>
<td>50(13.8)</td>
<td>0604</td>
<td>0(0)</td>
<td>0(0)</td>
<td>10(2.7)</td>
</tr>
</tbody>
</table>

A  AML vs. CML, OR 17.12 (0.98-298.2), Pc=0.004
b  AML vs. Control, OR 0.39 (0.14-1.02), Pc=0.058
c  AML vs. Control, OR 1.64(1.05-2.55), Pc=0.033
D  AML vs. CML, OR 0.46(0.22-0.97), Pc=0.045, AML vs. Control, OR 0.49(0.26-0.91), Pc=0.021
E  AML vs. Control, OR 1.71(1.01-2.90), Pc=0.059
f  AML vs. Control, OR 0.09(0.005-1.55), Pc=0.028, CML vs. Control, OR 0.11(0.006-1.87), Pc=0.049
Comparison of HLA polymorphism between patients and controls was analyzed using the Chi-square test for two by two tables. Fisher’s exact 2-tailed correction test was used when necessary.
Table 2. HLA Class II haplotypes frequency in Iranian AML, CML patients and controls.

<table>
<thead>
<tr>
<th>DRB/DQA1/DQB1 haplotypes</th>
<th>AML (n=60)</th>
<th>CML (n=50)</th>
<th>Control (n=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0101/0101/0501</td>
<td>8(6.6)</td>
<td>5(5)</td>
<td>20(5.5)</td>
</tr>
<tr>
<td>0101/0104/0501</td>
<td>2(1.6)</td>
<td>3(3)</td>
<td>0(0)</td>
</tr>
<tr>
<td>15/0102/0602</td>
<td>3(2.5)</td>
<td>4(4)</td>
<td>17(4.7)</td>
</tr>
<tr>
<td>15/0103/06011</td>
<td>12(10)</td>
<td>2(2)</td>
<td>23(6.4)</td>
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<tr>
<td>16/0102/0501</td>
<td>8(6.6)</td>
<td>0(0)</td>
<td>16(4.4)</td>
</tr>
<tr>
<td>03/0105/0201</td>
<td>9(7.5)</td>
<td>9(9)</td>
<td>32(8.8)</td>
</tr>
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<td>11/05/03011</td>
<td>39(32.4)</td>
<td>32(32)</td>
<td>81(22.4)</td>
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<td>04/03011/0302</td>
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<td>21(5.8)</td>
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<tr>
<td>13/0103/0602</td>
<td>8(6.6)</td>
<td>6(6)</td>
<td>17(4.7)</td>
</tr>
<tr>
<td>14/01/04/05</td>
<td>2(1.6)</td>
<td>5(5)</td>
<td>16(4.4)</td>
</tr>
<tr>
<td>07/02/01/0201</td>
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<td>30(8.3)</td>
</tr>
<tr>
<td>08/04/01/0401</td>
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<td>0(0)</td>
<td>4(1.1)</td>
</tr>
<tr>
<td>1001/0104/0501</td>
<td>3(2.5)</td>
<td>1(1)</td>
<td>8(2.2)</td>
</tr>
</tbody>
</table>

a CML vs. Control, OR 0.03 (0.001-0.75), Pc=0.01
b AML vs. CML, OR 5.44 (1.18-24.94), Pc=0.02
c AML vs. CML, OR 15.1 (0.8-266.6), Pc=0.008, CML vs. Control, OR 0.1 (0.006-1.74), Pc=0.02

CML vs. Control
The HLA-DRB1*0101/DQA1*0104/DQB1*0501 haplotype were significantly more frequent in CML patients than controls (3% vs. 0%, Pc=0.01) (Table 2). The only allele with significant different distribution was HLA-DQB1*0303 which was never found in CML patients but observed with a low frequency in controls (4.2%) (Table 1). HLA-DRB1*16/DQA1*01021/DQB1*0501 haplo-type was not found in any of CML patients while the frequency in control subjects was 4.4%.

AML vs. CML
Allele and haplotype frequencies of AML patients were compared with those of CML patients. The HLA-DRB4 allele was significantly more frequent in CML than AML patients (22% vs. 11.6%, Pc=0.045) (Table 1). The HLA-DRB1*16 allele frequency in AML patients was 7.5% while none of CML patient was positive for this allele (Pc=0.004).

Two haplotypes including HLA-DRB1*15/DQA1*0103/DQB1*06011 and DRB1*16/DQA1*01021/DQB1*0501 were also significantly more frequent in AML than CML patients (Table 2).

DISCUSSION

Studies in animal models including mice have shown the role of MHC class II in virus-induced leukemia.25

The first human study in association of certain HLA gene alleles with leukemia showed an increase of HLA-A2 allele frequency in ALL in 1967.12 In CML patients, a genetic alteration caused by t(9;22)(q34;q11) is expressed in the form of P210 fusion protein. P210 fusion protein as an endogenous protein in CML patients has been proposed to be presented to immune system in the context of HLA class II molecules. Recently it has been reported that HLA class II alleles such as HLA-DR1,-DR2,-DR3,-DR4 and -DR11 can present endogenous proteins like synthetic Bcr-abl peptides inducing the generation of T cell responses.26,27 The HLA alleles that present this protein to the immune system bring about a negative association with susceptibility to CML. Some alleles do not effectively present this protein, predisposing a positive association with CML. Only few studies have been reported in the case of AML. HLA-DR53 allele has been suggested by Seremetis et al to be associated with susceptibility to AML.13

In another study by Gowans et al in 150 AML patients from UK no significant association with HLA-DRB1 alleles was found.14 Accordingly, we investigated HLA-DRB1,-DQA1,-DQB1 alleles and haplotypes frequency in leukemia patients (AML and CML) and a normal control group. The results of our study showed that HLA-DRB1*11 was more frequent in CML patients than in control group (33% vs. 24.7%) (Table 1). Although this was not statistically significant, this is not consistent with in vitro studies by Pawelec et al26,27 In Turkish population, HLA-DR11 was considered as a protective marker against CML.28

We compared HLA-DR4 frequency with the previous studies designed by Posthuma et al who claimed that HLA-DR4 diminished the risk of CML.15 However, our study did not support the role of HLA-DR4 in diminishing the risk of CML. HLA-DR4 was more frequent in our CML patients than in controls (12% vs. 9.2%). In our study, the only significant difference in HLA class II allele distribution in CML patients comparing with controls is HLA-DQB1*0303 which was not observed in CML patients (Pc=0.049). A larger
study in the future may reveal some of the differences with marginal level of significance.

The only significant positive association with AML against control group was HLA-DRB1*11 (35% vs. 24.7, \(P_c=0.033\)). HLA-DRB4 allele which was significantly less frequent in AML patients comparing with controls (11.6% vs. 21.1%, \(P_c=0.021\)). HLA-DQB1*0303 was never found in any AML patients, suggesting that it might be a protective factor against AML (Table 1).

Two other alleles including HLA-DRB1*07 and –DQA1*0201 are negatively associated with AML (\(P_c=0.058\)).

Comparing AML with CML data, it is evident that HLA-DRB4 is more frequent in CML than AML patients. Thus, it is suggested that those individuals with HLA-DRB4 allele are more susceptible to CML than AML. One allele and two haplotypes including HLA-DRB1*16, HLA-DRB1*15/-DQA1*0103/-DQB1*06011 and HLA-DRB1*15/-DQA1*01021/-DQB1*0501 were more frequent in AML patients than CML patients.

At HLA-DQB1 locus two alleles including –DQB1*0604 and –DQB1*0303 alleles were never found either in AML or in CML patients (Table 2).

Genetic association studies in different diseases will help the clinicians with prediction of disease and the patient prognosis. One of the strongest HLA allele association studies is about HLA-B27 association in ankylosing spondilitis (AS) that is useful in differential diagnosis of this disease. Genetic association studies in leukemic disorders are also helpful in clarification of pathogenesis as well as prediction or treatment of leukemic disorders of poor prognosis.

In conclusion, it is suggested that the presence of HLA-DRB1*11 allele increase the risk of AML while HLA-DRB4 and –DQB1*0303 alleles are reported as protective genetic factors. It is also reported that HLA-DRB1*0101/DQA1*0104/DQB1*0501 haplotype as a genetic susceptibility factor is associated with CML while HLA-DQB1*0303 plays a protective role against CML (Tables 1 and 2). Moreover, our data showed that CML and AML are genetically different from each other since those with HLA-DRB4 allele are more susceptible to CML than to AML while HLA-DRB1*16 allele is proposed as a susceptibility factor for AML against CML.

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REFERENCES


