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Relation of HLA-A, -B, -DRB1 Alleles and Haplotypes in Patients with Acute Leukemia: A Case Control Study

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Background and Aims. A relationship between acute leukemia and HLA alleles has been demonstrated in several studies. However, the frequencies of HLA class I (A, B) and class II (DRB1) alleles and haplotypes has not already been determined in Turkish patients with acute leukemia.

Methods. We investigated the relation of the HLA alleles and haplotypes in 237 adult acute leukemia patients [103 acute lymphoblastic leukemia (ALL), 134 acute myeloid leukemia, (AML)] and 360 unrelated normal subjects by PCR-SSOP method using Luminex technology.

Results. Allele frequencies of HLA-A*03, and B*51 were higher in patients with AML compared with the controls ($p = 0.019$, and $p = 0.001$; respectively). Furthermore, HLA-A*11 and DRB1*01 allele frequencies were determined to be higher in patients with ALL ($p = 0.01$, $p = 0.001$; respectively), whereas DRB1*13 allele frequencies lower than controls ($p = 0.003$). The most observed haplotypes A*03 B*51 DRB1*11 (3.73 vs. 0%) in patients with AML; A*02 B*35 DRB1*01 (2.91 vs. 0%) and A*02 B*51 DRB1*11 (2.91 vs. 1.96%) in patients with ALL were determined. On the contrary, the most observed haplotype was A*02 B*35 DRB1*13 (2.19%) in the controls. We found A*02 B*39 DRB1*16 haplotype negatively associated with AML, whereas A*02 B*35 DRB1*13 was in ALL ($p = 0.015$, and $p = 0.017$; respectively).

Conclusions. These results suggest that HLA-A*03 and B*51 alleles may play a presumptive predisposing factor in AML. In addition, HLA-A*11 and DRB*01 alleles have been found to be associated with ALL, whereas DRB1*13 allele was determined to be negatively associated.

Key Words: HLA, Alleles, Haplotypes, AML, ALL, PCR-SSOP.

Introduction

Acute leukemia is a clonal disease developed by uncontrolled proliferation of immature cells (1). Etiopathogenesis of the disease is unknown. In acute myeloid leukemia (AML), alkylating agents, topoisomerase II inhibitors, environmental factors such as benzene, ionizing radiation, genetic and some hematological disorders may be precursors to the development of the disease (2,3). In addition to these factors, viral agents may play a role in acute lymphocytic leukemia (ALL) (4).

Major histocompatibility (MHC) genes are the most polymorphic genes in the human genome. The investigation of HLA gene polymorphism in various populations has been a useful tool to study the resistance and

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susceptibility to different diseases of unknown etiology (5). A relationship between acute leukemic disorders and HLA alleles has been indicated in previous studies (6,7). It was shown that HLA DRB1*13 allele and DRB1*04-DQB1*03 haplotype were observed less in ALL patients. HLA DRB1*07-DQB1*02 haplotype was linked in predisposition to development of AML (7–9).

In this study we aimed to determine the relations between HLA- A, -B, -DRB1 alleles, haplotypes and genetic susceptibility to acute leukemia.

Materials and Methods

Study Population

Two hundred and thirty seven adult patients with acute leukemia [103 ALL, (63 M, 40 F) and 134 AML, (70 M, 64 F)], diagnosed according to the French American British (FAB) criteria retrospectively in the Karadeniz Technical University, Farabi Hospital were included in to the study. The control group consisted of 360 healthy volunteer subjects matched by age, gender, and Turkish ethnic origin. This study was approved by the Institutional Ethics Committee on Human Research (2008/55 and 2009/61).

HLA-A, -B*, and -DRB1* Genotyping*

Genomic DNA of patients with acute leukemia and healthy controls were isolated from 200 μL aliquots of peripheral venous blood samples by using the Bio-robot EZ1 magnetic bead-based workstation (Qiagen, Hilden, Germany). Genotyping of HLA -A, -B, and -DRB1 alleles was performed in all subjects by polymerase chain reaction with sequence-specific oligonucleotide probes (PCR-SSOP) hybridization method using Luminex technology (Gen-probe Lifecodes, Stanford, CA). All studies were conducted in HLA Tissue Typing Laboratory of Karadeniz Technical University, which is accredited by the Turkish Ministry of Health and the external quality control tests of European Federation of Immunogenetics (EFI) and United Kingdom National External Quality Assessment Service (NEQAS) are routinely applied to the laboratory.

Statistical Analysis

The frequencies of HLA class I (A, B) and class II (DRB1) alleles and haplotypes in all subjects were performed by using Arlequin v3.5 population genetics software (10). The significance of differences in frequencies of HLA -A, -B, and -DRB1 alleles between patients and controls were compared by χ^2 test with Yates correction and Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated as a measure of relations between acute leukemia and HLA -A, -B, and -DRB1 genotypes. Bonferroni correction test for multiple comparisons was applied; p value of <0.025 was considered statistically significant.

Results

HLA-A, B and DRB1 allele frequencies of 237 patients with acute leukemia (103 ALL and 134 AML) and 360 healthy controls are presented in Tables 1 and 2. Due to the inheritance pattern of MHC-HLA alleles, HLA-A, B, DRB1 allele frequencies are given as 2n level in both patients and controls. Considering all three HLA loci together (HLA-A, -B and -DRB1), there were certain differences in allele distribution of AML and ALL patients compared with controls. In AML patients, HLA-A*03 and B*51 allele frequencies were found to be higher than the controls [OR; 1.62 (95% CI; 1.06–2.49), $p = 0.019$)], [OR; 1.87 (95% CI; 1.26–2.79), $p = 0.001$ respectively]. In ALL patients, HLA-A*11 and DRB1*01 allele frequencies were determined to be higher [OR; 2.28 (95% CI; 1.19–4.36), $p = 0.01$], [OR; 2.76 (95% CI; 1.45–5.23), $p = 0.001$ respectively], whereas DRB1*13 allele frequency is lower than controls [OR; 0.47 (95% CI; 0.27–0.79), $p = 0.003$]. HLA-B*35 allele frequency was higher in patients with AML compared with the controls. This was not statistically significant after Bonferroni correction test was performed (Table 1).

We evaluated the most observed haplotypes (HLA-A*B*DRB1*) in patients with acute leukemia and controls at frequency more than 1.00% (Table 3). A*03 B*51 DRB1*11 (3.73 vs. 0%) in AML patients; A*02 B*35 DRB1*01 (2.91 vs. 0%) and A*02 B*51 DRB1*11 (2.91 vs. 1.96%) in ALL patients were determined. On the contrary, the most observed haplotype was A*02 B*35 DRB1*13 (2.19%) in the controls. A*01 B*35 DRB1*07, A*02 B*40 DRB1*13, A*03 B*40 DRB1*15, A*03 B*51 DRB1*04, A*03 B*51 DRB1*11, and A*11 B*51 DRB1*14 haplotypes were detected positively associated with AML, whereas A*02 B*39 DRB1*16 haplotype was negatively associated with the disease. On the other hand, in ALL patients A*02 B*35 DRB1*01, A*03 B*35 DRB1*04, and A*03 B*40 DRB1*11 haplotypes were determined positively associated, whereas A*02 B*35 DRB1*13 haplotype was detected negatively associated with the disease (see Table 3 for p values).

In patients with acute leukemia (ALL and AML), a decrease in total heterozygosity and an increase in total homozygosity (at a 2–9% ratio) were determined in HLA genotype frequencies compared with controls, which was not statistically significant (Table 4). However, the total homozygosity ratio of HLA-B locus in the AML patients was twice of the controls (12 vs. 6%) and that was statistically significant ($p = 0.009$).