**Brief Communication**

# Typing of HLA Class I by Polymerase Chain Reaction-Sequence Specific Oligonucleotide Primer (*PCR-SSOP*) Technique in Iranian Cord Blood Donors

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## ABSTRACT

**Background:** HLA compatibility between transplant donor and recipient is one of the major determinants of transplant outcome. **Objective:** To determine HLA class I by PCR- Sequence-Specific Oligonucleotide Probe (PCR-SSOP) in cord blood donors. **Methods:** Genomic DNA of 142 cord blood samples registered at the Cord Blood Bank of Iran at Hematology, Oncology, and Bone Marrow Transplantation Research Center, was prepared and HLA class I was determined by the PCR-SSOP. **Results:** A total of 284 HLA-A alleles was identified of which A*02 and A*24 were the most common. Among 284 HLA-B and HLA-C alleles, B*35, B*51, Cw*4 and Cw*12 were the most frequent alleles in the studied population. **Conclusion:** Amplification of HLA loci with PCR-SSOP has proved to be a reliable method for HLA-A, -B and -C genotyping.

**Keywords: HLA Typing, PCR-SSOP, Cord Blood, HLA Class I**

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## INTRODUCTION

The Human Leukocyte Antigen (HLA) system is a complex, co-dominantly inherited system of antigens which has an important role in distinguishing self from non-self antigens by the immune system, thus allowing initiation of a targeted immune response to a foreign antigen. In organ transplantation, HLA compatibility between the donor and recipient is one of the major determinants of transplant outcome. For this reason, determination of an individual's HLA antigen combinations is used as a basis for donor-recipient matching. HLA typing has found further practical applications in the diagnosis of certain diseases, e.g. autoimmune diseases, in forensic and anthropological studies (1).

Histocompatibility testing plays an important role in the selection of bone marrow and kidney donors for transplantation. A correct assignment of HLA antigens is considered important, in contrast inadequate patient–donor HLA matching is associated with increased risk of rejection in solid organ transplantation or graft-versus-host disease (GVHD) in bone marrow transplantation. Although histocompatibility testing can be performed using various assays, histocompatibility locus antigen (HLA) class I polymorphisms have traditionally been detected at cell surface using serology techniques with alloantisera or monoclonal antibodies. Even though serology is a quick and convenient method for HLA class I detection, but it is hindered in many cases by serological cross reactivity and decreased expression of HLA antigens, particularly in immunosuppressed patients or in patients with haematological tumors (2).

Amplification of HLA loci with PCR-Sequence-Specific Oligonucleotide Probe (PCR-SSOP) has proved to be a rapid and accurate method for HLA-A, -B and -C alleles' genotyping and indicates that HLA typing by serology may not be sufficiently reliable.

In the present study we typed HLA class I from 142 cord blood donors by PCR-SSOP method.

## MATERIALS AND METHODS

This study was conducted between 2002 and 2005 at Hematology, Oncology, and Bone Marrow Transplantation Research Center at Dr. Shariati Hospital, Tehran University of medical sciences, Tehran, Iran. During this period, 142 cord blood donors were registered at the Cord Blood Bank of Iran. The samples were collected at the time of birth and their HLAs were typed by low-resolution DNA typing for HLA-A, -B, and -C.

Genomic DNA was prepared from anticoagulated cord blood using the salting out method as described (3).

The HLA-A, -B, and -C DNA typing were performed by Biotest ELPHA kit (Biotest AG, Dreieich, Germany) according to the manufacturer's recommendations. The hybridization between probe and target DNA was detected by a method adapted from the protein ELISA technique. Starting from genomic DNA, the polymorphic region in the second and third exons of the HLA class I genes were amplified by means of the polymerase chain reaction (PCR) using Biotin-labelled consensus primers. After amplification and denaturation of the PCR products the resulting biotinylated single stranded molecules were bound to Streptavidin-coated wells of a microtest plate. The wells contain FITC-labelled SSO-probes in dried form, which redissolved when the

diluted solution of the PCR product was transferred to the wells. Thus fixation of the PCR products to the wells and hybridization with SSO probes occur in one step. The specificity of the assay depends on the subsequent stringent washing step in which probes of insufficient sequence homology to the PCR products were removed. Hybridization probes were visualized in a colour reaction involving FITC-specific antibody fragments coupled with Horseradish peroxidase. The results were quantified photometrically in an ELISA reader at 450 nm. The combination of HLA alleles in the test material was determined from the pattern of positive reactions, by means of the reaction schemes in Biotest ELPHA program (software).

## RESULTS AND DISCUSSION

In the present study, HLA class I loci were determined in 142 Iranian Cord Blood donors. The most frequent alleles of HLA class I were A*24 (16.2%), A*2 (15.8%), A*11 (12.3%), A*1 (11.3%), A*3 (11.3%), B*35 (20.4%), B*51 (13.4%), Cw*4 (17.3%), Cw*12 (16.5%) and Cw*7 (13.7%) (Table 1).

**Table 1. HLA class I allele frequencies in the Iranian cord blood donor population**

| HLA-A allele | Frequencies (%) | HLA-B alleles | Frequencies (%) | HLA-Cw alleles | Frequencies (%) |
|---|---|---|---|---|---|
| A*01 | 32(11.3) | B*07 | 12(4.2) | Cw*01 | 13(4.6) |
| A*02 | 45(15.8) | B*08 | 12(4.2) | Cw*02 | 6(2.1) |
| A*03 | 32(11.3) | B*13 | 9(3.2) | Cw*03 | 13(4.6) |
| A*11 | 35(12.3) | B*14 | 6(2.1) | Cw*04 | 49(17.3) |
| A*23 | 3(1.1) | B*15 | 6(2.1) | Cw*05 | 3(1.1) |
| A*24 | 46(16.2) | B*18 | 11(3.9) | Cw*06 | 17(6.0) |
| A*26 | 14(4.9) | B*27 | 4(1.4) | Cw*07 | 39(13.7) |
| A*29 | 6(2.1) | B*35 | 58(20.4) | Cw*08 | 5(1.8) |
| A*30 | 13(4.6) | B*37 | 5(1.8) | Cw*12 | 47(16.5) |
| A*32 | 20(7.0) | B*38 | 10(3.5) | Cw*14 | 11(3.9) |
| A*33 | 15(5.3) | B*39 | 2(0.7) | Cw*15 | 25(8.8) |
| A*51 | 1(0.40) | B*40 | 12(4.2) | Cw*16 | 18(6.3) |
| A*66 | 3(1.1) | B*41 | 12(4.2) | Cw*17 | 9(3.2) |
| A*68 | 16(5.6) | B*44 | 14(4.9) | Cw*18 | 5(1.8) |
| A*69 | 2(0.70) | B*45 | 1(0.40) | | |
| A*80 | 1(0.40) | B*46 | 1(0.40) | | |
| | | B*49 | 7(2.5) | | |
| | | B*50 | 7(2.5) | | |
| | | B*51 | 38(13.4) | | |
| | | B*52 | 28(9.9) | | |
| | | B*53 | 3(1.1) | | |
| | | B*55 | 17(6.0) | | |
| | | B*57 | 2(0.70) | | |
| | | B*58 | 4(1.4) | | |
| | | B*78 | 1(0.40) | | |

The HLA system represents the greatest allelic and haplotypic polymorphism within the human genome. In a population with a mixed background it is expectable to have a higher genetic variability and polymorphism. As a result of different major ethnicities, immigration from neighbouring countries, and past historical events, Iranian people represent a population with mix and diverse HLA component.

In one study, molecular analysis of HLA-A, -B, and -C genes in unrelated healthy individuals from Baloch ethnic population of Iran showed that the most frequent HLA class I alleles are A*02011(20.2%) and A*01101(14.6%), B*35011(8.1%), B*4006 (11.1%), C*04011(28.6%) and C*15021(15.6%) (4). Although these results

show a close correlation with our results in some alleles, there are also some differences, which might be caused by sample selection methods from a different ethnicity. Determination of HLA class I profiles of other Iranian ethnic groups will help to shed further light on this matter.

A close match between HLA determinants of recipient and donors reduces the risk of allograft rejection (5). In other words, the outcomes of unrelated donor, hematopoietic cell transplants, engraftment, incidence of graft versus host disease (GVHD) and overall graft survival are strongly affected by the degree of compatibility between the recipients and donors. Therefore, establishment of central and regional cord blood banks in this country seems to be essential to support the HLA donor registry, which will give most of bone marrow transplant candidates a chance for survival.

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