

Allele Frequencies of HLA Class-I Loci in the Normal Iranian Population

Ghashghaie A.,¹ Alimoghaddam K.,¹ Ostadali MR.,¹ Ghaffari H.,¹ Khansari L.,¹ Sadraee M.,¹ Mirrasekhian E.,¹ Mohyedin I.,¹ Raoofi F.,¹ Noori Z.,¹ Yaghmaian H.,¹ Eshaghi H.,¹ Ghavamzadeh A.¹

¹Hematology- Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Dr. Shariati Hospital, Tehran, Iran

Corresponding Authors: Dr. Kamran. Alimoghaddam

Deputy Director of Hematology- Oncology and Stem Cell Transplantation Research Center, Shariati Hospital Kargar Shomali Street, Tehran, Iran14114

Phone Number: +982184902662

Fax Number: +98218800414

E-mail: alimgh@sina.tums.ac.ir

Abstract

Introduction: Human Leukocyte Antigen (HLA) genes are the most polymorphic loci in the human genome. They predict the outcome of transplantation by detecting self from non-self antigens. The best use of HLA typing is to find a HLA-match donor for BMT candidate patients. Evidence suggests that matching unrelated donors and recipients for HLA-A, -B and -DRB1 loci is a prediction for an improved outcome.

Methods and materials: HLA-A and -B of six hundred Iranian cord blood samples were typed by PCR-SSP method and the frequency of loci were estimated.

Results: The most frequent alleles were A*02(18.16), A*24(16.41), B*35(21.66) and B*51(13.35), respectively.

Discussion: Our HLA typing data show similarity between neighbor and related countries and Caucasians. Identifying HLA alleles helps to find suitable donors for patients in need of hematopoietic stem cell transplantation.

Keywords: HLA, Cord Blood, GvHD, Frequency Transplantation, Donor.

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Introduction

Human Leukocyte Antigen (HLA) genes are one of the most polymorphic loci in the human genome. They express on the surface of the T lymphocytes and, therefore, play a major role in the regulation of the immune system. HLA molecules serve have a protective role by detecting self from non-self antigens, graft success and therefore graft outcome. MHC is the major barrier to transplantation. Hematopoietic progenitor cell transplantation affirms the role of MHC in transplantation outcome by measuring both a patient's survival and GVHD. GVHD is caused by a reaction of the T cells in the graft to HLA antigens in the recipients. HLA typing is a search tool to identify allele frequencies. A patient is more likely to find an HLA-matched donor within the same ethnic group.(1) For 25% of patients, HLA-identical or one antigen mismatch donor can be identified in the family, but for 65% of patients, HLA-matched donor can be found in national or international registries of unrelated voluntary stem cell donors.(2) However, approximately 10% of patients lack HLA- matched donors due to the ethnical differences of HLA allele and haplotype distribution and can not be

transplanted. Therefore, searching for alternative solutions such as cord blood becomes imperative. The transplantation of cord blood stem cells is applied mainly to children, but recently also in adult patients with rare HLA alleles or in the case of emergent need for transplantation. Cord blood banks have increased the use of cord blood transplantation for patients with hematological disorders. Furthermore, the immaturity of lymphocytes in cord blood dampens GVHD reactions. Since 1993, following the opening of the first unrelated cord blood bank in New York, many other cord blood banks have been developed worldwide in order to support existing and planned hematopoietic stem cell transplantation by providing facilities, personnel and expertise for all the required activities associated with banking of this human material with clinical use. There are about 100 cord blood banks worldwide and present geographical distribution is as follows: 40% in Europe, 30% in USA and Canada, 20% in Asia and 10% in Australia. Cord blood banks have increased the use of cord blood transplantation for patients with hematological disorders.(3) Many factors (For instance histocompatibility) influence the outcome

of hematopoietic progenitor cell transplantation. HLA-A, -B and -DRB1 typing of all available members of the immediate family before transplantation helps to find a matched related donor. Evidence suggests that matching unrelated donors and recipients for HLA-A, -B and -DRB1 loci provides an improved outcome.(4) The ability of DNA typing methods to resolve HLA types to the allelic level now allows a transplant center to select an HLA allele-matched donor and decreases the probability of GVHD and graft rejection and increases survival.(5) In this study, we investigated class I allele frequencies for HLA-A and -B genes in 600 Iranian healthy cord blood donors.

Materials & Methods

Cord blood samples: Cord blood Samples were randomly collected from random mothers who were referred to Shariati Hospital for delivery. Six hundred cord blood samples were collected with informed consent from these healthy unrelated mothers. MNCs were separated by high centrifuge. Most of the mothers were volunteer donors who donated their cord blood to our bank although and some of them were directed donors who needed cord blood for their own patients.

HLA Typing: Genomic DNA was extracted from each sample using the salting-out method.(6) HLA-A and -B were typed to two digits by PCR-SSP method. The kits Biotest ABDR SSPTray (Biotest AG, Dreieich, Germany) and HISTO TYPE ABDR (BAG Health care, Lich, Germany) were used to amplify exons 2 & 3 of HLA-A and -B loci according to the manual's instruction. PCR products were separated on 2% agarose gel during electrophoresis. All the gels were documented and the pattern of positive bands was analyzed using the software in the each kit's.

Results

In this study, the results of the HLA class I typing of 600 unrelated cord blood samples of the Iran Cord Blood Bank, were reported. A total of 18 and 28 alleles were detected in HLA-A and -B loci. The allelic frequencies for HLA-A and HLA-B are given in Table 1. Eighteen alleles for HLA-A and twenty-eight alleles for HLA-B were detected in this Iranian population.

As expected, A*24 and A*02 (18.6 and 16.33%) were the most frequent alleles followed by A*03(12.08%) and A*11(10.41%).The A*43 and A*69(both 0.16%) were the least frequent alleles. B*35(20.75%) and B*51(12.16%) were the most frequent, which is much higher than the next

frequent alleles such as B*07, -*38, -*38 and B*08 (4.75%, 4.33%, 4.33% and 4.25%). Some alleles were homozygote, such as A*01(0.33%), A*02(1.33%), A*24(1.66%), A*11(0.66%), A*32(0.41%), A*30(0.58%). B*07(0.083%), B*13 and B*18(0.25), B*35(0.83%), B*51(0.41%), B*40(0.083%), B*44(0.016%) and B*58(0.083%).

Discussion

There are few studies focusing on HLA class I (-a, -B, -C) and HLA class II (-DRB1, -DP1, -DQ1) allele frequencies in Iranian populations. Most of these studies are concerning class II HLA loci (7, 8, 9, 10, 11, 12, 13, 14). In some reports, the most frequent alleles were A*0211(20.2%) and B*4006(11.1%),(8) A*24(16.2%), A*02(15.8%), B*35(20.4%) and B*51(13.4%),(15) and A*02(20.2%) and B*40(11.1%)(6). Our data show that A*02(18.16%), A*24(16.41%), B*35(21.66%) and B*51(13.33%) were the most frequent alleles, which is similar to the results of other populations. A*02 is frequent in Caucasians, especially in Italians (0.254),(17) and also in Greeks (Crete 0.255 and Greece North 0.270), Turkey (Class I 0.302),(18) Pakistan (Baloch 0.135),(17) and Morocco (0.178).(19)

Table1. HLA class I allele frequencies in Iranian Cord Blood Donors

| Allele | N | Frequency (%) | Allele | N | Frequency (%) |
|--------|-----|---------------|--------|-----|---------------|
| A*01 | 111 | 9.25 | B*07 | 57 | 4.75 |
| A*02 | 218 | 18.16 | B*08 | 51 | 4.25 |
| A*03 | 145 | 12.08 | B*13 | 47 | 3.91 |
| A*11 | 125 | 10.41 | B*14 | 36 | 3 |
| A*23 | 27 | 2.25 | B*15 | 38 | 3.16 |
| A*24 | 197 | 16.41 | B*18 | 52 | 4.33 |
| A*26 | 82 | 6.83 | B*27 | 31 | 2.58 |
| A*29 | 30 | 2.5 | B*35 | 260 | 21.66 |
| A*30 | 56 | 4.66 | B*37 | 12 | 1 |
| A*31 | 74 | 6.16 | B*38 | 52 | 4.33 |
| A*32 | 68 | 5.66 | B*39 | 11 | 0.91 |
| A*33 | 44 | 3.66 | B*40 | 43 | 3.58 |
| A*43 | 2 | 0.16 | B*41 | 33 | 2.75 |
| A*66 | 3 | 0.25 | B*42 | 2 | 0.165 |
| A*68 | 50 | 4.16 | B*44 | 50 | 4.165 |
| A*69 | 2 | 0.16 | B*45 | 2 | 0.165 |
| A*74 | 5 | 0.41 | B*47 | 2 | 0.165 |
| A*80 | 2 | 0.16 | B*48 | 6 | 0.50 |
| | | | B*49 | 30 | 2.50 |
| | | | B*50 | 43 | 3.58 |
| | | | B*51 | 160 | 13.33 |
| | | | B*52 | 42 | 3.50 |
| | | | B*53 | 3 | 0.25 |
| | | | B*54 | 2 | 0.165 |
| | | | B*55 | 42 | 3.50 |
| | | | B*56 | 6 | 0.50 |
| | | | B*57 | 12 | 1.00 |
| | | | B*58 | 23 | 1.915 |

A*24 has almost the same frequency in many countries like Italy (0.122),(18) Turkey class I (0.130),(18) Greece Crete (0.150).(18) A*11 is more frequently found in Thai (0.082%),(4) and Chinese (0.22).(20) B*51 is also frequent in relatively close populations like Turks (class I 0.130, pop2 0.158) and Italians (0.104), Greek (Crete 0.102, North 0.146) (18). B*35 is frequent in some neighboring countries like Turkey (Turkey class I (0.204)(18), Greece (Crete 0.244)(18), Italy (0.159)(18) and Pakistan (Baloch 0.138)(17). All these data show a similarity between neighboring and related countries and the most frequent alleles are common throughout the world. Identifying HLA alleles helps to find suitable donors for patient in need of hematopoietic stem cell transplantation.(21) Local registries are not responsible for the huge number of patients lacking family donors. Connecting the registries and access to international registries helps find ore suitable donors. Frequency studies have shown that the distribution of HLA alleles can vary among different racial and ethnic populations in unrelated stem cell registries and can impact searches for unrelated donors.(22)

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