The Royan Public Umbilical Cord Blood Bank: Does It Cover All Ethnic Groups in Iran Based on HLA Diversity?

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Keywords

Stem cell transplantation · Umbilical cord blood · HLA

Summary

Background: Umbilical cord blood (UCB) stem cells allow the transplantation of partially human leukocyte antigen (HLA)-matched grafts and are a valuable resource for the treatment of hematologic malignancies and heritable hematologic, immunologic and metabolic diseases, especially when a compatible bone marrow donor is unavailable. The aim of this study was to determine how many ethnic groups in Iran are covered by the available UCB units based on HLA diversity. **Methods:** From 2009 until mid-2013, 4,981 (30.3%) of the 16,437 UCB samples collected met the storage criteria and were cryopreserved at a public cord blood bank (CBB) in Tehran, Iran. HLA-A, -B and -DRB1 were typed in 1,793 samples. **Results:** The mean volume of the cryopreserved samples was 81.25 ± 20.3 ml. The range of total nucleated cells per unit was 51×10^7 – 107×10^7 . The most common HLA alleles were HLA-A*2 (17%) and HLA-A*24 (15.6%), HLA-B*35 (16.8%) and HLA-B*51 (13.9%), and HLA-DRB1*11 (20%) and HLA-DRB1*15 (14%). The predominant haplotypes were HLA-A*24-B*35-DRB1*11 (2%), HLA-A*02-B*50-DR*07 (1.8%), and HLA-A*02-B*51-DRB1*11 (1.5%). **Conclusions:** Based on the HLA-DRB1 profiles, the UCB units available at the Royan public UCB bank are a potentially adequate resource for hematopoietic stem cell transplantation for Iranian recipients belonging to particular ethnic groups. Regular educational programs to improve the public knowledge of UCB for transplantation can enhance the public CBB stocks for all Iranian ethnic groups in the future.

Introduction

Allogeneic hematopoietic stem cell (HSC) transplantation with cells from the bone marrow or the peripheral blood is a treatment of choice for hematologic malignancies as well as heritable hematologic, immunologic and metabolic diseases [1]. Unlike most solid organ transplantations, which are often predisposed to rejection, graft-versus-host disease (GvHD) is a risk in HSC transplantation [2]. Because human leukocyte antigen (HLA) genes are highly polymorphic, transplantation from an HLA-matched donor reduces the risk of bilateral immunologic reactions in HSC transplantation. In this regard, one of the best sources of bone marrow is a sibling donor, not only because of the high rate of HLA-identical donors among siblings, but also because the tolerance-like status afforded by noninherited maternal antigens (NIMAs) makes HSC transplantation possible from NIMA-bearing siblings [3]. When an HLA-matched sibling or haploidentical related donor is not available, unrelated HLA-matched HSC transplantation is suggested [4]. However, despite the millions of volunteer donors in bone marrow registries worldwide, many patients who need bone marrow grafts cannot find an appropriate donor [1].

Since 1988, umbilical cord blood (UCB) has been accepted as an alternative source of HSCs for bone marrow reconstitution [5]. Many samples of cord blood are discarded daily as clinical waste, although they are a valuable source of HSCs which is easy to collect with no risk to the mother or the newborn [6]. In addition to the high potential of UCB HSCs to generate different hematologic lineages [7], the risk of GvHD is reduced because these cells are immunologically immature or naive, and therefore partially HLA-mismatched UCB can be used for transplantation [8].

In recent years, reprogramming human somatic cells into induced pluripotent stem cells (iPSCs) has been considered a promising approach in regenerative medicine [9]. There are different sources like mesenchymal stem cells or fibroblasts for iPSC production, but UCB HSCs are more homogeneous than adult blood or marrow cells and less likely to harbor rare mutations, and can therefore be considered a preferential source for iPSC banking [10]. To improve the clinical outcomes of HSC transplantation, the HLA compatibility between the iPSCs and the recipients is a critical point. In this regard, a panel of UCB samples homozygous for common HLA alleles among the target population would be helpful for iPSC generation [11, 12].

Although private cord blood banks (CBBs) were initially founded for autologous or family use [13], the first public CBB was established in the USA in 1993, and currently about 400,000 UCB units are held worldwide in more than 50 public CBBs [14]. In Iran there are 1 private and 3 public CBBs. At present, the Royan Private CBB (www.rsct.ir) stores 23,000 UCB units, and the Royan Public CBB, founded in 2008, currently holds 4,981 UCB units. The units in this public bank are potentially available to all Iranians, and the present study was designed to determine the HLA diversity of this material and thereby to predict which ethnic groups in Iran are covered by the units currently available at the Royan Public CBB.

Material and Methods

Donor Selection

After approval of the study protocol by the Royan Ethics Committee, the importance of cord blood donation and the objectives of public CBBs were explained by nurses to healthy singleton pregnant women ranging in age from 18 to 45 years who were referred to hospitals to plan their obstetric care. The participating hospitals were located in different areas of Iran and included: Tehran (the hospitals of Mahdiah, Arash, Akbarabadi, Atieh, Laleh, Sevom Saban, Chamranan, Payambaran, and Bahman), Karaj (Kamali Hospital), Chalous (Taleghani Hospital), Borujerd (the hospitals of Kovsar and Chamran), Kurdistan (Tamin Ejtemae Hospital), Uromieh (Mottahari Hospital), Zanzan (the hospitals of Emam Hossein and Mousavi), Mazandaran (the hospitals of Shafa, Nimeh Shaban, Amir Mazandarani, and Mehregan), Qom (the hospitals of Al-Zahra and Valiasr), Ahvaz (Fateme Zahra Hospital), and Mashhad (the hospitals of Sina, BentolHoda, Mehr, Pasture, Emam Hadi, Mosabne Jafar, Razavi, and Hakim Neyshabouri). The procedure of UCB sampling and the conditions of sample preservation were explained to each woman, and a questionnaire was completed for each cord donor by CBB midwives, to identify and exclude any inherited hematologic, immunologic or metabolic diseases in either parent or their families. Then, written informed consent was obtained from the couples with no evidence of any known genetic diseases. During the last month of pregnancy, a 5-ml blood sample was taken from previously selected women with no history of high-risk sexual behavior, drug abuse, body piercing, tattoo, or blood transfusion within the last 12 months.

Donors with negative serologic tests for syphilis, HBV, HCV, CMV, HIV I/II, and HTLV I/II were selected, and cord blood was collected when a mature (full-term, 36 weeks or more) live fetus was born and the mother was nonfebrile and nonanemic.

Cord Blood Collection, Processing, and Preservation

Each cord blood sample was collected under aseptic conditions with a collection kit (Besaat Co., Tehran, Iran). All UCB samples were transferred to the Royan Public CBB at 4–15 °C. The volume, total nucleated cell (TNC) count, and percentage cell viability of each UCB unit were determined by standard methods, and a volume of 5 ml of each UCB unit was kept in aliquots for blood group, sterility, and viral testing. One of the aliquots was stored at –70 °C for HLA typing.

Cord blood units were processed 24 h after collection by the method of Harris et al. [15]. Briefly, red blood cells were removed with hydroxyethyl starch (Freeflex, Germany) from UCB units with no microbial infection and a volume of 60 ml or more. The TNC and percentage of viable cells were determined with a Sysmex XE-2100 hematology analyzer (Sysmex, Kobe, Japan) and a NucleoCounter NC-100 (ChemoMetric, Allerød, Denmark) according to the manufacturers' manuals. The percentage of CD34+ stem cells in each sample was determined with a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA) using specific antibodies against CD34 and CD45 and the International Society for Hematotherapy and Graft Engineering (ISHAGE) gating strategy [16]. Then eligible samples were cryopreserved with 10% dimethyl sulfoxide and 1% dextran while small aliquots were also taken for clonogenic assays and revival studies. Colony-forming cell (CFC) assays were done with methylcellulose-based media (Stem Cell Technologies, Vancouver, BC, Canada) according to the manufacturer's instructions. Colony-forming units of granulocyte-macrophage, erythroid, and multipotential colonies were scored after 14 days by light microscopy. To evaluate the cell viability and recovery rate, cryopreserved samples were tested periodically during the first year after freezing.

HLA Typing and Data Analysis

Genomic DNA was extracted from 1,793 UCB samples with the QIAamp kit (Qiagen, Hilden, Germany). HLA-A, -B, and -DRB1 were typed with the polymerase chain reaction-sequence-specific primers (PCR-SSP) method using a standard kit (Inno-Train GmbH, Kronberg, Germany). The PCR products were separated on 2% agarose gels and the results of electrophoresis were analyzed with software included in the kit.

HLA allele and haplotype frequencies were determined with Arlequin version 3.01 software [17]. To determine the genetic relationship among UCB samples from the Royan Public CBB and 884 unrelated healthy samples from 13 ethnic groups in Iran [18, 19], principal component analysis (PCA) was done with MVSP v. 3.1 software [20] using Nei's genetic distances (D_A) based on HLA-DRB1 allele frequencies, calculated with DISPAN software [21].

Results

From 2009 until mid-2013, 16,437 UCB samples were collected by the Royan Public CBB. 67.8% of the samples were not eligible because of insufficient volume or TNC, and 312 (1.9%) samples were excluded because of evidence of microbial infection in microbial detection tests ($n = 94$) or because of positive serologic tests (IgM anti-CMV, $n = 90$; anti-HCV, $n = 25$; anti-HBV, $n = 68$; anti-HIV I/II, $n = 16$; anti-HTLV I/II, $n = 19$). A total of 4,981 (30.3%) of the UCB samples met the necessary criteria for cryopreservation.

The mean volume of the cryopreserved samples was 81.25 ± 20.3 ml with a range of 51×10^7 – 107×10^7 TNC per unit. The characteristics of the cryopreserved samples obtained in each year of the study period are shown in table 1. The recovery rates during the first year after cryopreservation indicated a

Table 1. Characteristics of cryopreserved cord blood units in the Royan Public CBB from 2009 to mid-2013

Year	Units		TNC n × 10 ⁶	CD34+ cells		CFU, n × 10 ³				
	collected	preserved		recovery in 1 year, %	n × 10 ⁶	recovery in 1 year, %	BFU-E	CFU-GM	CFU-GEMM	CFC
2009	2,130	996	512.3 ± 112.38	78 ± 5.6	0.782 ± 0.52	85 ± 14.56	12.76 ± 2.57	16.65 ± 1.7	8.07 ± 3.01	37.50 ± 4.87
2010	4,020	1360	741.9 ± 151.33	83 ± 7.9	1.263 ± 0.78	88 ± 19.87	17.25 ± 3.19	19.62 ± 5.29	10.67 ± 4.33	47.55 ± 8.40
2011	7,919	1952	964.3 ± 212.97	88 ± 5.02	1.781 ± 0.65	85 ± 10.98	19.02 ± 3.78	22.25 ± 4.39	9.07 ± 4.05	50.34 ± 7.62
2012	2,099	476	975.9 ± 218.73	85 ± 10	2.033 ± 0.64	87 ± 18.56	19.12 ± 6.39	20.95 ± 8.18	13.98 ± 8.28	54.06 ± 10.93
2013	269	197	1,072.6 ± 215.97	80 ± 3.8	2.352 ± 0.90	90 ± 14.38	24.34 ± 11.08	23.09 ± 12.07	14.58 ± 7.72	62.02 ± 18.93

TNC, CD34+ cell counts, and CFU are presented as mean ± standard deviation (SD).
TNC = Total nucleated cell count, CFU = colony-forming units, BFU-E = erythroid burst-forming units, CFU-GM = granulocyte-macrophage colony-forming units, CFU-GEMM = granulocyte-erythroid-macrophage-megakaryocyte colony-forming units, CFC = colony-forming cells.

Table 2. HLA allele frequencies among 1,793 samples at the Royan Public CBB

HLA-A	F	HLA-B	F	HLA-DRB1	F
02	0.171	35	0.168	11	0.2011
24	0.156	51	0.139	15	0.1414
03	0.134	52	0.062	04	0.1291
01	0.111	18	0.056	07	0.1057
11	0.101	50	0.053	13	0.1057
26	0.063	44	0.050	03	0.0901
32	0.050	38	0.049	001	0.0669
68	0.049	07	0.047	14	0.0527
30	0.042	55	0.040	16	0.0402
33	0.041	40	0.039	10	0.0259
29	0.026	13	0.035	08	0.0198
23	0.023	49	0.035	12	0.0120
31	0.017	14	0.032	09	0.0073
69	0.007	41	0.032	58	0.0006
66	0.004	15	0.029	18	0.0003
25	0.002	08	0.029	53	0.0003
36	0.001	27	0.021	78	0.0003
80	0.001	58	0.018	83	0.0003
34	0.001	57	0.015	98	0.0003
74	0.001	37	0.012	missing	0.0003
		39	0.011		
		53	0.008		
		56	0.003		
		73	0.003		
		48	0.003		
		54	0.003		
		46	0.003		
		47	0.002		
		45	0.002		
		42	0.001		
		78	0.001		
		missing	0.001		

F = Frequency.

Table 3. Common HLA-A, -B, and -DRB1 haplotypes among 1,793 samples at the Royan Public CBB

A-B-DRB1	HF
24-35-11	0.020
02-50-07	0.018
02-51-11	0.015
11-52-15	0.013
33-14-01	0.013
03-35-11	0.013
03-44-04	0.010
02-51-04	0.010
30-13-07	0.010
24-52-15	0.007
26-38-04	0.007
03-51-11	0.006
01-52-15	0.006
23-49-11	0.006

HF = Haplotype frequency.

Fig. 1. PCA depicting the genetic relationship among 1,793 samples at the Royan Public CBB and 884 samples from 13 ethnic groups in Iran (Pa, Pars; Zo, Zoroastrian; Ku, Kurd; Az, Azeri; Tu, Turkmen; Qa, Qashqai; BI, Baloch; Ba, Bakhtiari; LL, Lur from Luristan; LY, Lur from Yasouj; AA, Arab from Ahvaz; AF, Arab from Famur; Jw, Jew) based on genetic distances for HLA-DRB1 allele frequencies.

reduction rate of 15–20%, and the mean recovery after 1 year was $82.8 \pm 3.9\%$.

The results of HLA-A, HLA-B, and HLA-DRB1 typing of 1,793 of the UCB samples showed that the most common HLA alleles were HLA-A*2 (17%) and HLA-A*24 (15.6%), HLA-B*35 (16.8%) and HLA-B*51 (13.9%), and HLA-DRB1*11 (20%) and HLA-DRB1*15 (14%) (table 2). The predominant haplotypes were HLA-A*24-B*35-DRB1*11 (2%), HLA-A*02-B*50-DRB1*07 (1.8%), and HLA-A*02-B*51-DRB1*11 (1.5%) (table 3). 20 samples were homozygous for all 3 loci, and 2 of these samples revealed high-frequency haplotypes. The PCA results are shown in figure 1. The HLA-DRB1 profiles of the UCB samples held at the Royan Public CBB were close to those reported for the Pars and Zoroastrian groups and were well separated from those of other ethnic groups in Iran.

Discussion

The growing number of applicants (according to national data) for UCB preservation in private banks for autologous or family use suggests that most people are still unaware of the limitations of private banks. In addition to the high cost to families, the shelf-life of these cells is still not well documented. Furthermore, the chance that an individual or one of his/her family members may need these cells during their lifetime is very low, whereas there are many other patients for whom no appropriate adult donor is available. In case of need, these patients may receive autologous or allogeneic HSCs from bone marrow or peripheral blood stem cells. Therefore, public CBBs can be considered a major source of HSCs for patients who cannot find appropriate donors in their family. To date, many CBBs have been established worldwide to support patients [22]. One such center, the Royan Public CBB, has been in operation since 2009 and has based its activities on standard international methods for donor selection and cord blood process-

ing and preservation [23–26]. During this study, about 30.3% of the collected UCB samples were preserved at the Royan Public CBB, with an average of 1,107 units per year – more than the number reported by a London CBB (786 units per year) [24] and a Guangzhou CBB (592 units per year) [25]. In our analysis, 67.8% of the cord blood samples were excluded because of insufficient volume or TNC. To improve the transplant outcome and prevent the storage of units that are not appropriate for transplantation [27], this center has annually reviewed its standards for cord blood collection based on Royan Quality Control Committee initiatives. In 2009, a blood volume of at least 60 ml and a TNC of $\geq 500 \times 10^6$ per unit were acceptable; during 2010, a volume of 75 ml and more and a TNC of $\geq 700 \times 10^6$ per unit were required; during 2011, a volume of 90 ml and a TNC of $\geq 900 \times 10^6$ per unit were required; and since 2012, a volume of 100 ml and a TNC of $\geq 1 \times 10^9$ cells per unit have been used as the minimum criteria for preservation.

Blood tests in samples from the mothers revealed a 1.9% prevalence of infectious disease markers for CMV, HCV, HBV, HIV, or HTLV, a rate similar to those reported by other centers [23, 24, 28].

The mean recovery rate of the preserved samples we analyzed was $82.8 \pm 3.9\%$, which is similar to the rate reported by a Valencia CBB in Spain that uses different volume reduction methods (from $72.8 \pm 7.2\%$ to $81.5 \pm 6.9\%$) [29], and to that at a Zhejiang Province CBB in China ($80.71 \pm 11.26\%$) [14]. However, our recovery rate was lower than those at a Progenics CBB in Canada ($97.7 \pm 2.5\%$) [30] and a CBB in Milan (between $82.8 \pm 12.3\%$ and $91.4 \pm 6.4\%$) [31].

Although the results of HLA typing of UCB samples from the Royan Public CBB showed that the most frequent HLA alleles were similar to those in reports from different parts of Iran [18, 19], the results of PCA revealed that the Royan Public UCB samples were closest to those of the Pars and Zoroastrian groups, and relatively far from those of other ethnic groups in Iran.

Public education to increase the awareness about the importance of public CBBs as a source of readily available HSC may encourage donations to public banks. Moreover, cord blood donation to public banks avoids the heavy cost to families of UCB collection, processing, and preservation. Currently, in Iran, the cost of private UCB storage including collection and processing is about USD 250 per unit, in addition to an annual storage fee of USD 100.

Donors to private banks might also be persuaded to share their samples with public banks if the maintenance costs could be covered by public banks. We found 2 homozygous UCB samples with highly frequent haplotypes in Iran, which may be potential candidates for the production of iPSC.

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Disclosure Statement

The authors declare no conflicts of interest.

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