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Hematopoietic Stem Cell Transplantation Using Preimplantation Genetic Diagnosis and Human Leukocyte Antigen Typing for Human Leukocyte Antigen–Matched Sibling Donor: A Turkish Multicenter Study

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A B S T R A C T

Preimplantation genetic diagnosis involves the diagnosis of a genetic disorder in embryos obtained through in vitro fertilization, selection of healthy embryos, and transfer of the embryos to the mother's uterus. Preimplantation genetic diagnosis has been used not only to avoid the risk of having an affected child, but it also offers, using HLA matching, preselection of potential HLA-genoidentical healthy donor progeny for an affected sibling who requires bone marrow transplantation. Here, we share the hematopoietic stem cell transplantation results of 52 patients with different benign and malign hematological or metabolic diseases or immunodeficiencies whose donors were siblings born with this technique in Turkey since 2008. The median age of the patients' at the time of the transplantation was 8 years (range, 3 to 16 years) and the median age of the donors was 2 years (range, .5 to 6 years). The most common indication for HSCT was thalassemia major (42 of all patients, 80%). The stem cell source in all of the transplantations was bone marrow. In 37 of the transplantations, umbilical cord blood of the same donor was also used. In 50 of the 52 patients, full engraftment was achieved with a mean of 4.6×10^6 CD 34^+ cells per kg of recipient weight. Ninety-six percent of the patients have been cured through hematopoietic stem cell transplantation without any complication. Primary engraftment failure was seen in only 2 patients with thalassemia major. All of the donors and the patients are alive with good health status. Preimplantation genetic diagnosis with HLA matching offers a life-saving chance for patients who need transplantation but lack an HLA genoidentical donor.

INTRODUCTION

Preimplantation genetic diagnosis (PGD) is a method for diagnosing genetic diseases in the early embryonic period so that implantation of affected embryos can be avoided and the potential need for termination of pregnancy is eliminated in families who have genetic diseases. The first application of

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PGD was in 2009 by Petrou, who reported that the biopsy of up to 2 cells from the 8-cell-stage embryo did not affect the development of the embryo to the blastocyst stage or the embryo metabolism [1]. PGD is also used with preimplantation HLA typing for treatment of affected siblings with genetic and acquired disorders who require HLA-matched stem cell transplantation [2]. The PGD and HLA-matching procedure is a form of in vitro fertilization (IVF) that requires a multiplex polymerase chain reaction amplification, in which the simultaneous analysis of gene mutations and HLA typing are done on a single blastomere. The first successful application of PGD combined with HLA typing was reported by Verlinsky et al. in 2001 [3] with the transplantation outcomes reported by Grewal et al. in 2004 [4] for a case of Fanconi anemia resulting in a successful tissue reconstitution after stem cell transplantation [3,4].

HLA typing without mutation analysis has also been used for acquired diseases, such as acute myeloid leukemia and acute lymphoid leukemia, which require allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-identical donor for the cure of the disease [5]. Because of limited availability of HLA-matched donors, even among family members, preimplantation HLA typing without mutation analysis appeared to be attractive for couples with children who have acquired diseases requiring HLA-matched bone marrow transplantation and do not have HLA-matched donor. It has been 15 years since the first successful application of PGD combined with HLA typing, and preimplantation HLA typing with or without mutation analysis has become 1 of the major pretreatment assessments for an increasing number of congenital and acquired diseases [6].

The present data include HSCT results of 52 patients in Turkey, who, since 2008, had benign or malign hematological diseases, metabolic diseases, or immunodeficiencies and whose donors were the siblings born via preimplantation HLA typing with or without mutation analysis. As far as we know, this is the largest case series on the subject performed to date in the literature and may, therefore, provide valuable information for clinical outcomes of the transplantations with donors born via this technique.

MATERIALS AND METHODS

Patient and Donor Characteristics

Between February 2008 and January 2014, 52 patients underwent 53 transplantations in 11 pediatric HSCT centers in Turkey with transplants from siblings born via preimplantation HLA typing with or without mutation analysis. One of the patient with thalassemia major (TM) had undergone a second transplantation from the same donor because of primary graft failure. The demographic characteristics of patients and donors are shown in Table 1.The median age of patients at the time of transplantation was 8 years (range, 3 to 16 years); 23 (44.2%) were female and 29 (55.8%) were male. One patient with TM and 1 patient with acute lymphoblastic leukemia had undergone transplantation from both of their twin siblings. Because of this, the total number of the donors was 54 and 28 of them (52.8%) were female and 26 of them (47.2%) were male. The most common indication for HSCT was TM (42 of all patients, 80%). Consent was obtained at PGD and HLA typing, at IVF cycle, at umbilical cord cryopreservation, and at the beginning of HSCT.

HSCT Characteristics

In all of the transplantations, bone marrow was used as the stem cell source. In 37 of the transplantations, umbilical cord blood of the same donor was also used. In all patients, except the 1 with TM who had undergone bone marrow transplantation twice, 1 bone marrow harvesting procedure was enough to collect the targeted total nucleated cell count. The 6 TM patients who were considered high risk according to classification proposed by Pesaro group were conditioned with Pesaro protocol 26 plus antithymocyte globulin. Of the 36 TM patients who were considered low risk, 34 were conditioned with regimens including busulfan and cyclophosphamide, while the remaining 2 were conditioned with regimens including treosulfan, thiotepa, and fludarabine. The conditioning regimens of the other patients are seen in Table 2. All patients were given different immunosuppressants, consisting of methotrexate, cyclosporine, antithymocyte globulin, or tacrolimus either alone or with different combinations for graft-versus-host disease (GVHD) prophylaxis (Table 2).

Table 1
Characteristics of Patients and Donors

Characteristic	Value
Age, yr	
Patients	8 (3-16)
Donors	2 (0.5-6)
Sex (female/male)	
Patients	23/29
Donors	28/26
Weight, kg	
Patients	24.5 (12-52)
Donors	13.1 (7.5-19)
Diagnosis	
TM, Pesaro low risk	36
TM, Pesaro high risk	6
Acute lymphoblastic leukemia	2
Acute myeloid leukemia	2
Wiskott-Aldrich syndrome	2
Juvenile myelomonocytic leukemia	1
Fanconi aplastic anemia	1
Sickle cell disease	1
Adrenoleukodystrophy	1

RESULTS

Transplantation Outcomes

In 49 of the 52 patients, full engraftment was achieved after first transplantation, with a mean value of total 4.6 (range, .6 to 14.8) $\times 10^6$ $CD34^+$ cells per kg of recipient weight. Three of the patients, all with TM (1 was assigned to high risk status and the others were assigned to low risk), failed to achieve engraftment. One of these patients underwent a second HSCT from the same donor, 1.5 years after the first transplantation, and full engraftment was achieved. Including this patient, full engraftment was achieved in 50 of 52 patients. HSCT outcomes are shown in Table 3. Among all patients, 1 patient developed grade IV, 1 patient developed grade II, and 2 patients developed grade I acute GVHD. Resolution was achieved in all patients with first-line treatments. None of the patients developed chronic GVHD or secondary graft failure. All 52 patients are alive and all, except the 2 with primary graft failure, are primary disease free without any complications. Forty-four patients are with full donor chimerism. Two of these patients have undergone transplantation from both of their twin siblings. They achieved full donor type chimerism and there was not predominance of cells from 1 twin. Eight patients, all with TM, achieved mixed chimerism but are transfusion independent.

PGD/HLA Typing Process and Total Costs

The median maternal age on the first IVF cycle was 32 years (range, 22 to 39 years). The median number of the IVF cycles to result with the birth of the donor was 2 (range, 1 to 8). Median time from the beginning of the first cycle to transplantation was 3.5 (range, 2 to 9) years. Median costs of the PGD/HLA typing and stem cell transplantation were €8500 (range, €1600 to €60,000) (US median, $9042; range $1702 to $63,826) and €44,000 (range, €35,000 to €60,000) (US median, $46,805; range, $37,232 to $63,826), respectively. The median total cost was €44,500 (range, €37,000 to €110,000) (US median, $44,347; range, $39,359 to $117, 014) (Table 4).

Table 2
Characteristics of HSCT

Characteristic	Value
Stem cell source, n	
Umbilical cord blood and bone marrow	37
Bone marrow alone	16
Umbilical cord blood, median (range)	
Viability, %	78.6 (43-94)
Volume, mL	47.7 (23-184)
Nucleated cell, $\times 10^7$	1.97 (.1-6.27)
$CD34^+$ cell, $\times 10^5$/kg	1.59 (.03-18.2)
Bone marrow, median (range)	
Nucleated cell, $\times 10^8$/kg	3.9 (.5-4.5)
$CD34^+$ cell, $\times 10^6$/kg	6.7 (.6-15.5)
Total no. of nucleated cells, median (range) $\times 10^8$/kg	4.09 (.5-5.12)
Total number of $CD34^+$ cells, $\times 10^6$/kg	4.6 (.6-14.8)
Conditioning regimen	
TM, Pesaro low risk	Busulfan, cyclophosphamide or treosulfan, thiotepa, fludarabine
TM, Pesaro high risk	Pesaro protocol 26 + ATG
Acute lymphoblastic leukemia	Busulfan, cyclophosphamide, etoposide
Acute myeloid leukemia	Busulfan, cyclophosphamide, melphalan
Wiskott-Aldrich syndrome	Busulfan, cyclophosphamide, fludarabine, ATG
Juvenile myelomonocytic leukemia	Busulfan, cyclophosphamide, melphalan
Fanconi aplastic anemia	Cyclophosphamide, fludarabine, ATG
Sickle cell disease	Busulfan, cyclophosphamide
Adrenoleukodystrophy	Busulfan, cyclophosphamide

ATG indicates antithymocyte globulin.

DISCUSSION

PGD provides an alternative to prenatal diagnostic testing, avoiding the distress associated with diagnosis, particularly if diagnosis is determined late in an established pregnancy. PGD is also an alternative to termination of pregnancy. If prenatal testing (through amniocentesis or chorionic villi tests) reveals a genetic abnormality, the options available to parents are to have a child with a genetic condition or undergo a pregnancy termination: this is a difficult and often traumatic decision. However, PGD is performed before pregnancy begins, thus eliminating this difficult decision. PGD also provides the opportunity to conceive a pregnancy that is biologically the parents' own and yet unaffected by a genetic condition. In the past, potential parents with a genetic condition or those who know that they are carriers frequently chose adoption, embryo donation, surrogacy, or not having children to avoid the risk of passing on the condition. PGD now allows these couples the opportunity to have a child free of the condition.

Table 3
Transplantation Outcomes

Outcome	Value
Engraftment, n	
Full engraftment	49/52
Primary graft failure	3/52
Secondary graft failure	0/52
GVHD,	
Acute GVHD	1
Grade IV	0
Grade III	1
Grade II	2
Grade I	0
Chronic GVHD	
Follow up, median (range), mo	46 (26-96)
Chimerism, n	
Full donor type chimerism	44
Mixed chimerism	8

Table 4
PGD/HLA Typing Process and Approximately Total Costs

Characteristic	Value
Maternal age at the time of the first IVF cycle, yr	32 (22-39)
No. of the IVF cycles resulting in birth of the donor	2 (1-8)
Time between the first cycle and transplantation, yr	3.5 (2-9)
Cost of the PGD/HLA typing process, $\times 10^3$, €	8.5 (1.6-60)
Cost of the transplantation,$\times 10^3$, €	44 (35-60)
Total cost, $\times 10^3$, €	44.5 (37-110)

Data presented are median (range) unless otherwise indicated.

Via the combination of PGD with HLA typing, PGD can be also used for the treatment of affected siblings with genetic and acquired disorders who require HLA-matched stem cell transplantation. The first successful application of PGD combined with HLA typing for a case of Fanconi anemia by Verlinsky et al. [3] in 2001 led to ethical concerns of PGD with the intention of enabling future tissue transplantation. The Human Fertilization and Embryology Authority, a nondepartmental public body in the United Kingdom that has regulated human embryo research since 1991, agreed at its meeting on November 29, 2001 that, in principle, PGD for HLA matching may be acceptable when the children born after PGD are themselves at risk for the condition to be treated in the existing child and if all other possibilities of treatment and sources of tissue for the affected child have been explored. However, there are ethical dilemmas regarding preimplantation HLA typing as a sole purpose, because it is done for the benefit of a potential recipient rather than for embryo itself, particularly when there is no need for testing of the causative gene, such as leukemia. Discarding embryos that are not HLA compatible with the candidate and the risk of the future child being valued only as a donor and neglected after birth raise these ethical concerns. However, the desire for saving a child's life from a severe disease may justify the action.

Because of these ethical, legal, and most likely financial/technical restrictions, there are only a limited number of PGD centers worldwide offering HLA typing on human preimplantation embryos. The use of PGD and HLA typing for HLA-matched sibling donor has become an important PGD

indication in those countries where the law permits it, such as Turkey. In Turkey, preimplantation HLA typing technique with or without mutation analysis is allowed if a family has an affected child who requires bone marrow transplantation and does not have an HLA-compatible related or unrelated donor. The cost is reimbursed by Turkish Social Security System since 2009. The number of the PGD centers offering HLA typing on human preimplantation embryos has increased in Turkey since 2003, with very successful results. According to the experience of Kahraman et al. from Turkey, the rate of availability of both HLA-compatible and healthy embryos is 20.8%, which is similar to the other European PGD experience of PGD with HLA matching. Once a mutation-free and HLA-compatible embryo has been found, the clinical pregnancy rate did not differ statistically significantly from other pregnancy rates with IVF [6].

The literature on this issue is commonly regarding PGD/HLA typing, selection, and implantation of the HLA-matched embryos, with only a few anecdotal reports about the outcomes of HSCT using HLA-matched sibling donors who were born after PGD with HLA typing [4,7,8]. To our knowledge, this is the largest case series on the subject reported in the literature with results of transplantations. The majority of the HLA typing combined with PGD cases were for TM patients and the majority of the preimplantation HLA typing–only cases were for malignant hematological diseases.

A high cell dose (3.7×10^7/kg) is considered crucial to sustain engraftment in patients, especially in those with nonmalignant diseases undergoing matched related donor umbilical cord blood transplantation [9]. The mean nucleated cell count of umbilical cord blood of our donors was 1.91×10^7/kg (range, .1 to 6.27). In most of the HSCTs, the number of umbilical cord blood–derived hematopoietic stem cells were merely enough for transplantation. Because of this, parents should be informed of the possibility of using bone marrow or bone marrow plus umbilical cord blood, as was our experience reported here, especially when insufficient umbilical cord blood–derived hematopoietic stem cells are available.

Transplantation outcomes were excellent, such that except for the 2 cases with primary graft failure, 50 of 52 patients (96%) are primary disease free without any complications, with a median follow-up periods of 46 months (range, 26 to 96 months). The median number of the IVF cycles that resulted with the birth of the donor was 2 (range, 1 to 8) and the median time between beginning of the first cycle and transplantation was 3.7 (range, 1 to 9) years. Hence, the foregoing methodology can be applied in patients requiring nonurgent HSCT, such as patients with a genetic disease or children who may require transplantation in the future because of progression of an underlying disease, such as high-risk leukemias in first complete remission. Although it is less costly in Turkey than elsewhere as reported in the literature, the approximate mean total costs of the HSCT with this procedure seems expensive [10]. However, if transplantation were not done, the costs of treatment for 40 years, which is the expected survival for TM patients, and the cost of relapse treatment for patients with malignant hematological diseases would be much higher.

Transplantation physicians should be aware of the feasibility and efficacy of the PGD/HLA typing method as well as the indications for its use. An immediate search for a suitable unrelated donor at the time of establishing the indication for HSCT should never be omitted. In cases where no suitable matched sibling donor is available and transplantation using an unrelated donor either carries significantly higher risks than the use of a sibling donor or is not possible because of the unavailability of a suitable matched unrelated donor, PGD/HLA typing technology should be considered and discussed with parents [11]; of course, the benefits and risks of IVF and PGD should be explained. Every couple may not be as fortunate as the couples in our study, who underwent IVF/PGD procedure and had healthy babies who were HLA compatible with their siblings. According to the report of Kahraman et al., among 242 couples who underwent IVF/PGD for HLA matching at their center, HSCT was completed in only 48 couples, leading to a success ratio of 19% (Kahraman et al., 2014 [2]; the center utilized this method for the majority of our patients). Information presented might inform how to counsel a pediatric patient's parents whose children need transplantation but lack a donor.

Besides the low rate of successfully completing HSCT, IVF/PGD for HLA matching has other risks, including risks of treatment and risks to babies born after PGD. Most of the risks involved in PGD are similar to those for conventional IVF, such as fertility drug reactions, multiple pregnancies (although some centers reduce this risk by only transferring 1 embryo), ovarian hyperstimulation syndrome, pelvic infection, miscarriage, or ectopic pregnancy. Regarding the risks to babies born after PGD, several studies have concluded that there do not appear to be any major side effects of the PGD treatment to babies born after PGD. Most of the risks involved in PGD treatment are similar to those for conventional IVF: babies are more likely to be born prematurely and to weigh less than naturally conceived babies born at the same age. After birth, the results of long-term follow-up of these children should be evaluated. In our series, there were no couples who abandoned the approach and there were no serious complications among mothers who had undergone PGD/IVF treatment or among babies born after PGD/IVF. Data on complications of IVF and PGD with HLA matching for HSCT purposes are scarce, and the current study could not provide detailed information about the complications of PGD and IVF because of the information collected. Further studies might elucidate these issues.

In conclusion, HSCT using PGD and HLA typing for matched sibling donors is a successful and feasible method. However, further studies are required to illustrate the clinical, social, psychological, and economic issues that might be related to the use of this technology.

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