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Journal of Hematology & Transfusion

Research Article

Preimplantation Genetic Testing – Practical Tool for Prevention and Radical Treatment of Hemoglobinopathies

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Submitted: 10 October 2020

Accepted: 27 October 2020

Published: 02 November 2020

ISSN: 2333-6684

Keywords

- Hemoglobinopathies; Preimplantation genetic testing; Transplantation

Abstract

Hemoglobinopathies are the most frequent indication for preimplantation genetic testing (PGT), performed for more than twenty years to provide an option for couplers at risk to avoid the birth of an affected offspring and have a healthy children of their own. We present here our experience of 684 PGT cycles for hemoglobinopathies, resulting in birth of 240 unaffected children, which is a part of our overall PGT series of 5883 PGT cycles for monogenic disorders (PGT-M), with 2,332 resulting births, free of genetic disorders. Although PGT-M was mainly offered to heterozygous carries, 15 cycles were performed for homozygous thalassemia patients, as PGT-M is the only option for them to have an unaffected children. Increasing number of PGT cycles (188 cycles) are performed together with preimplantation HLA typing (PGT-HLA), as a means for having an access to HLA compatible stem cell transplantation for the affected children in the family. The available experience on the results of stem cell transplantation treatment, using donors produced by PGT-HLA shows an extremely high success rate of radical treatment. The accumulated experience demonstrates considerable progress in using PGT for prevention and radical treatment of hemoglobinopathies.

INTRODUCTION

At the present time the appropriate health technology is readily available for hemoglobinopathies, so these patients may have a normal lifespan, good quality of life, and possibility to reproduce and have unaffected children of their own. Because of the long-term social, familial and financial consequences of these serious conditions, it is also a routine practice to prevent the new affected births, with also an option of preimplantation genetic testing (PGT), to avoid the previously required need for termination of pregnancy, which is the major limitation of the control measures, restricting significantly the choices of the at risk couples [1-3]. In those population and ethnic groups, or families that cannot accept the preventative approaches, reflecting their social and religious differences, it has also become realistic to provide a radical treatment through stem cell transplantation, using the exactly matched sibling donors obtained by preimplantation HLA typing (PGT-HLA) [4-9], currently performed for hundreds of patients [10-13]. The available framework of avoiding the birth of affected children involves a prospective carrier screening for identification of couples at risk, providing them with options of either to avoid the birth of an affected child using prenatal diagnosis, or utilize PGT in order to avoid facing 25% risk of termination of pregnancy, and also to combine PGT with HLA matching (PGT-HLA) for the opportunity to produce an unaffected HLA matched donor for stem cell transplantation treatment of their older affected siblings. This will allow performing a radical treatment by the 100% matched stem cell transplantation, which, as will be presented below, is an attractive approach in avoiding a life-long blood transfusion therapy which itself presents a high risk for life threatening complications.

Accordingly, it is reasonable that this information is available to the couples at risk detected through an expanded carrier screening [14], so they could make their choices from all the above mentioned available options. Thus, this paper will describe the present status of PGT for hemoglobinopathies, as a primary prevention tool, as well as an alternative approach for improving access to HLA-compatible stem cell transplantation treatment, based on our experience since the first PGT for thalassemia performed in 1996 [1].

MATERIAL AND METHODS

A total of 684 PGT cycles for 410 couples (Table 1), at risk for producing an affected progeny with hemoglobinopathies were performed (list of mutations for which PGT was performed is presented in Table 1). The most frequent were sickle cell anemia, HBS (227 cycles performed for 148 patients), and IVS I-110 thalassemia mutation, most prevalent in patients of Eastern Mediterranean origin (149 cycles performed for 75 patients). Among other less prevalent mutations tested were Gln39Stop codon, for which 79 cycles were performed, IVS1-5 - 37 cycles and IVS1-6 - 31 cycles. PGT for remaining mutations were performed in 23 or less cycles.

Table 1: List of thalassemia mutations for which PGT was performed in our experience.
1. Betta-Thalassemia
1.1 Transcriptional mutations
nt -90 C>T
nt -88 C>T
nt -87 C>G
nt -42 C>G
nt -32 C>T
nt -31 A>C
nt -30 T>A
nt -29 A>G
nt -28 A>G
CAP +1 (A->C) silent nt 1 A>C
Pro5Ala
Pro5Ser
Glu6Lys
Glu6Val
Leu11Pro
Arg12Ser
Trp15Stop
Lys18Stop
Glu26Lys
Ala27Ser
Gln39Stop
Glu121Ala
Glu121Val
Poly A (A->G)
1.2. Splicing Mutations
IVS-I-1 (G->T)
IVS-I-5 (G->C)
IVS-I-6 (T->C)
IVS-I-110 (G->A)
IVS-I-116 (T->G)
IVS-II-1 (G->A)
IVS-II-654 (C->T)
IVS-II-745 (C->G)
IVS-II-848 (C->A)
1.3. Insertions/Deletions
Codons 7/8 (+G)
Codons 27/28 (+C)
45 kb deletion; the Filipino deletion beta0
Codon 5 (-CT)
Codon 6 (-A);
Codon 8 (-AA)
Codons 36/37 (-T)
Codons 41/42 (-TTCT)
Codon 44 (-C)
Codon 76 (-C)
619 bp deletion beta0
Hb Lepore-Hollandia
Sicilian (deltabeta)0-Thal
Chinese ^{G}gamma(^{A}gammadeltabeta)0-Thal

2. Alpha Thalassemia
- -(MC); a deletion of at least 46 kb involving both alpha genes and zeta gene alpha-Thal-1
- -(THAI); a deletion of 34-38 kb involving the alpha1, alpha2, and zeta genes alpha-Thal-1
- -(FIL); a deletion of 30-34 kb involving the alpha1, alpha2, and zeta genes alpha-Thal-1
- -(MED-I); deletion of ~17.5 kb including both alpha-globin genes alpha-Thal-1
- -(SEA); deletion of ~20 kb including both alpha-globin genes alpha-Thal-1

PGT cycles were performed using a standard IVF protocol, coupled with micromanipulation procedures of polar bodis (PB), or embryo biopsy, described in detail elsewhere [15]. Details of PGT guidelines were reported previously [16,17]. The present standards of the procedure involve whole genome amplification (WGA), of biopsied PBs or embryos biopsy samples, followed by multiplex nested PCR analysis of the mutations in question, together with closely linked genetic markers in a multiplex heminested system. The majority of cases are currently performed by blastocyst biopsy followed by WGA [15,18].

HLA genes were tested simultaneously, using the short tandem repeats (STRs), in the HLA region. A multiplex heminested PCR system used closely linked polymorphic STRs located throughout the HLA region, described in detail elsewhere [15]. The choice of alleles and markers was based on information of the presence of maternal and paternal matching or non-matching chromosome 6 alleles. For each family, heterozygous alleles and haplotypes not shared by parents were selected. This allowed detecting and avoiding misdiagnosis due to preferential amplification and allele dropout (ADO), potential recombination within the HLA region, and a possible aneuploidy or uniparental disomy of chromosome 6, which may affect diagnostic accuracy of HLA typing of the embryo.

In PGT cycles, involving an advanced reproductive age of maternal partner, aneuploidy testing was also performed, initially by FISH analysis, and then by array-CGH, or next generation technologies (NGS) (Illumina Inc), for 24-chromosome aneuploidy testing [15,18,19].

RESULTS AND DISCUSSION

Hemoglobinopathies are one of the largest group of monogenic disorders for which PGT-M was performed in our experience. In some communities, such as in Cyprus, Greece, and Turkey, PGT has become a routine procedure for couples carrying thalassemia mutations, who cannot accept prenatal diagnosis and pregnancy termination**.** Introduced first in Cyprus [1], thousands of cycles have now been performed worldwide. At present, the proportion of PGT for hemoglobin disorders in our overall PGT-M experience exceeds 11% (684 of 5,883 PGT-M performed ovearall). Of 684 clinical cycles performed for different alpha and beta-globin gene mutations (see list of mutations in Table 1), 978 unaffected embryos (1.79 embryos per transfer on the average) were detected for transfer in 546 (79.9%), cycles, resulting in 244 (44.6%), clinical pregnancies and the birth of 240 unaffected children.

Table 2: PGT-for heterozygous carriers with the same or different HB genes Including PGT for affected HB patients.

Number of Mutation tested per couple	# Patient	# Cycle	# Transfers	# Embryo transferred	Pregnancy	Birth
SHARED SAME MUTATION IN THE FAMILY	**236**	**375**	**303**	**550**	**148**	**142**
TWO DIFFERENT MUTATIONS	**148**	**271**	**210**	**371**	**80**	**83**
THREE MUTATIONS (AFFECTED FAMILY MEMBER AND CARRIER)	**12**	**15**	**12**	**19**	**6**	**5**
SUBTOTAL BETA THALASSEMIA	*396*	*661*	*525*	*940* *1.79*	*234* *44.5%*	*230*
ALPHA THALASSEMIA	**14**	**23**	**21**	**38**	**10**	**10**
TOTAL	**410**	**684**	**546**	**978** **1.79**	**244** **44.6%**	**240**

While PGT cycles were mainly performed for heterozygous carriers, 15 cycles were done for couples with homozygous or compound heterozygous male or female patients at 50% risk of bearing an affected offspring. In these couples, PGT involved testing for either three different mutations or, in the majority of cases, for two different mutations, or the same maternal and paternal mutation. Analysis of these mutations was done either simultaneously or in sequence by testing the maternal mutations in PB1 and PB2, and the paternal ones by embryo biopsy (Table 2).

With progress in the treatment of hemoglobinopathies, PGT has been requested also by affected and well-treated patients who wish to reproduce (Table 2). Life expectancy has been significantly improved for hemoglobin disorders, which may be treated radically by stem cell transplantation. The strategy in such cases depends on whether the affected partner is male or female, because testing may be entirely based on the identification of mutation free oocytes if the affected partner is male, or in contrast, based on only by embryo testing when the female partner is affected. However, if a male partner is affected, male factor infertility required a special treatment prior to PGT, and patients were also undergone a testicular biopsy. On the other hand, in the affected females partner was affected, a limited number of oocytes were available after stimulation regiments, making PGT quite challenging [13].

Among conditions requiring HLA-compatible stem cell transplantation, hemoglobinopathies were one of the most prevalent. In our overall experience of 485 PGT-HLA cases, 188 (38.8%) were performed for hemoglobinopathies, as bone marrow transplantation is the only option for their radical treatment [20,21], which is still limited to the availability of HLA-matched donors. Of 188 PGT-HLA cycles, 159 (1.54 on the average) HLA matched embryos unaffected embryos were available for transfer in 103 (54.8%) cycles, resulting in birth of 32 unaffected HLA-identical children, serving a 100% compatible donors for the affected siblings. Umbilical cord blood or bone marrow from the children obtained from PGT-HLA were transplanted, resulting in a successful hematopoietic reconstitution of the affected siblings or pending. The other similar experience was reported from Istanbul Center, where 626 PGT-HLA cycles were performed, resulting in birth of 128 thalassemia-free HLA matched children [10,11]. Stem cells of 66 of these children have already been used for stem cell transplantation treatment, with successful bone marrow reconstitution almost in all of them. The other series of 127 babies produced by PGT-HLA were reported by ESHRE Consortium, with a 76.2% success rate of stem cell transplantation treatment [12].

The chance to identify unaffected embryos fully matched to siblings with hemoglobinopathies is 18.75%, based on 75% chance of having unaffected offspring, with additional 25% probability of HLA match. As there is also as much as 50% chance of chromosome aneuploidy in patients of advanced reproductive age, the chance of identification of A matched unaffected embryo is even as low as 9.4%. However, despite such a low probability, we were able to identify an unaffected HLA matched embryos for transfer in a PGT-HLA cycle for a couple with female partner of advanced reproductive age, whose 14 year old son was totally cured as a result of transplantation of stem cells obtained from the sibling produces in this cycle [8]. The other approaches used for couples with advanced maternal age were performing additional cycles to collect sufficient number of embryos for PGT-HLA, or obtaining donor oocytes from patient's younger HLA matched sister, if available [13].

Despite some remaining ethical issues, and other limitations mentioned, PGT- HLA remains an attractive option for couples who wish to have another unaffected child anyway, as it provides a realistic opportunity of HLA-compatible stem cell transplantation for the affected siblings. This makes PGT a realistic alternative to conventional prenatal diagnosis, as couples are provided with an important prospect not only to avoid an inherited risk without facing termination of pregnancy, but also to establish the pregnancy with genetic parameters, that may benefit the affected member of the family. Thus, couples at risk of having children with hemoglobin disorders will benefit from information provided them about presently available options not only for avoiding the birth of an affected child but also for selecting a suitable stem cell donor for their affected children.

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Cite this article

Kuliev A, Pakhalchuk T, Ramon GS, Rechitsky S (2020) Preimplantation Genetic Testing – Practical Tool for Prevention and Radical Treatment of Hemoglobinopathies. J Hematol Transfus 7(2): 1088.