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Case Report

Five Years Post Allogenic Transplantation and Seven Years Post Preimplantation Genetic Diagnosis for β-Thalassaemia Major Combined with HLA Matching

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Keywords

- Haemopoietic stem cell transplantation; Preimplantation genetic diagnosis; β-thalassaemia; Preimplantation human leukocyte antigen typing

Abstract

Seven years follow-up after the birth of a healthy infant, who became a bone marrow donor two years after delivery to his sick sibling, after successful preimplantation genetic diagnosis (PGD) testing by both cleavage stage and blastocyst biopsy for the purpose of diagnosis of β-thalassaemia and HLA compatibility has been described in the present case report.

Since allogenic hematopoietic stem cell transplantation (HSCT) is the only cure available for Beta-Thalassemia. However there is a limited possibility of finding human leukocyte antigen (HLA) matched donor, among relatives. HSCT from a HLA identical sibling provides the best treatment option which reduces the incidence of graft rejection and other serious complications associated with transplantation and the major part of these cases can be cured successfully.

Here, we present a successful pregnancy achieved after PGD for β-Thalassaemia combined with HLA typing in a family with β-thalassemia major carrier ship. A healthy girl with HLA compatibility with his affected brother was born in term and umbilical cord blood of the baby was frozen after delivery for possible future treatment. Two years after delivery the transplantation of bone marrow has been accomplished after complete immune suppression of the affected brother (5 years old). Another five years post allogenic transplantation two siblings are healthy and without complications in their development. Treatment and transfusions of the affected child were no longer needed. Being the first case in Bulgaria, this success demonstrates the feasibility of international collaboration for application of sophisticated techniques in different specialized hospitals and clinics.

ABBREVIATIONS

PGD: Preimplantation Genetic Diagnosis; HLA: Human Leukocyte Antigen; HSCT: Haemopoietic Stem Cell Transplantation; UCB: Umbilical Cord Blood; GVHD: Graft-Versus-Host Disease

INTRODUCTION

β-thalassaemia is a recessively inherited monogenic disorder where the affected individuals require blood transfusions on a regular basis. The incidence is quite high in certain parts of the world, such as the Mediterranean region [1,2]. The carrier pregnancy is around 4% but this rate reach 14% in some areas where consanguineous marriages are in common. Unfortunately, the only possibility of a cure lies in haemopoietic stem cell transplantation (HSCT). Stem cells of the umbilical cord blood from a HLA-compatible newborn have a great therapeutic value, as they can be used for transplantation without graft rejection, thus saving an affected sibling's life [3]. PGD technique together with HLA typing was first applied as a therapeutic tool in 2001 [4]. This technique could be used not only to avoid the birth of an affected child but also to conceive healthy children who may be HLA identical donors for HSCT [5].

Currently, allogenic HSCT is the only cure available for Beta-Thalassaemia. Unfortunately, in most cases there is only a limited possibility of finding HLA-matched donor, among relatives and from national or international sources. HSCT from a HLA identical sibling provides the best treatment option which reduces the incidence of graft rejection and other serious complications associated with transplantation and nearly 90% of the cases can be cured successfully [6]. As preimplantation genetic diagnosis (PGD) is a widely used technique for couples at genetic risk [7] and more than 10,000 healthy children have already been born the transfer of unaffected embryos generated through *in vitro* fertilization (IVF) techniques appear to be a solution.

For those couples who are at risk of transmitting a genetic disease to their offspring, preimplantation embryos can be selected according to their genetic status as well as Human Leukocyte Antigen (HLA) compatibility with the affected child.

Here we report a seven years follow-up after the birth of a healthy infant, who became a bone marrow donor two years after delivery to his sick sibling with β-thalassaemia major, after successful PGD testing by both cleavage stage and blastocyst biopsy for the purpose of diagnosis of β-thalassaemia and HLA compatibility. The specific feature of this work was not only to achieve a pregnancy and live birth after rigorous and difficult clinical and laboratory procedures such as two successive biopsies in the same embryo for the purpose of both mutation analysis and HLA compatibility. It also demonstrated how to achieve this via cross-border collaboration and the importance of follow-up processes and social adaptation in the family. Finally, the most important part of this case report is that two siblings (now at age 10 and 7) are healthy and without complications in their development. Treatment and transfusions of the affected child were no longer needed.

CASE PRESENTATION

The patient

The couple (age 39 male and female), both Beta-thalassemia carriers, are biological parents to a 2-year old boy and another older child, 11 years old daughter. Family members are Bulgarians which belong to the South Slavic ethnic group. Parents are highly educated with Doctors of Philosophy (PhD) degrees both. The son is suffering from β-thalassaemia major and receiving blood transfusion every week. The older child who is healthy girl who was not HLA identical with her brother. Molecular analysis revealed that the affected son is compound heterozygote and inherited IVS-1-6 mutation from his mother and Cod39 mutation from his father, respectively. The couple has indicated that HLA compatible donor could not be found after extensive search from national and international sources. Preimplantation HLA typing technique was the only option for the possible treatment of the affected boy, who has to receive blood transfusions every 20 to 30 days.

The couple was provided with proper genetic counseling about PGD with HLA typing processes and information regarding the success rates, the risk of misdiagnosis and possible genetic, clinical and social outcomes. A written consent form was obtained in which a possible risk of misdiagnosis was stated and confirmatory prenatal diagnosis is recommended for any achieved pregnancy after PGD.

Preclinical setup study

Before initiating ovarian hyper stimulation, DNA samples from parents and children were sent to the Reproductive Genetics Institute (RGI), Chicago, USA in order to confirm the mutations in β-globin gene and to design appropriate primers to amplify and select informative short tandem repeat (STR) markers for use in PGD and preimplantation HLA typing of single cells.

IVF treatment and blastomere biopsy

Controlled ovarian stimulation including regular ultrasound controls and hormonal monitoring were followed in SAGBAL Hospital, Sofia, Bulgaria. The oocyte pick up, intracytoplasmic sperm injection (ICSI), and embryo transfer procedures were all carried out in Istanbul Memorial Hospital IVF Laboratory, Istanbul, Turkey. A total of 17 oocytes were collected, 12 of them were mature (MII) and all of them were fertilized following ICSI. Cleavage-stage biopsy was performed to all 12 embryos which have more than 6-8 cells on day 3 of embryonic development. Since the number of fully informative STR markers was not enough to establish a confident result, a second biopsy from the carrier and HLA-matched embryo was needed in order to test the accuracy and increase the reliability of the previous data. Blastocyst stage biopsy was planned and the developmental capability of this embryo was followed for the following 2 days [8].

RESULTS

PGD and preimplantation HLA matching

According to the β-thalassemia mutation analysis one embryo was identified as a healthy carrier (Embryo 4 - heterozygote for IVS1-6) and HLA matched with the affected child by a previously described protocol and detailed mutation analysis [8]. All other remaining embryos were not HLA identical with the affected child; two embryos were identified as unaffected, one embryo was identified as affected (IVS1-6/Cod39) and 8 embryos were heterozygote carriers without HLA compatibility.

On day 5, re-biopsied embryo, which is at that time re-expanded and herniated through the biopsy opening, was transferred to the patient's uterus. Ten days after transfer, serum b-HCG levels were positive and a singleton pregnancy was confirmed at 6 weeks by ultrasound diagnosis of the sac presence and fetal heartbeat.

Confirmation by amniocentesis

For the purpose of confirmation of the diagnosis, amniocentesis was performed at 16th gestational week.. Molecular analysis revealed confirmation of the previous results; both mutation and HLA status of the fetus which was a heterozygote and totally HLA matched with the affected sibling. Furthermore, karyotype analysis showed that the fetus has a normal female karyotype (46, XX).

Clinical outcome

A healthy female baby girl (3200 g, 49 cm) was delivered at 40th gestational week by caesarean section in Bulgaria. The cord blood from the umbilical cord was collected and mononuclear cells were frozen as a source of stem cells for possible future transplantation.

Follow-up

Since HSCT is the only cure available for Beta-Thalassaemia major two years after delivery the transplantation of bone marrow has been accomplished after complete immune suppression of the affected brother (5 years old). Another five years post allogenic transplantation in Italy by the team of Lucarelli the healthy status of two siblings (girl donor age 7 and affected boy, age 10) is without any complications and no more blood transfusion for the affected child needed. The boy is adapting very well in class, life, studies, collaborative with his schoolmates and already has received all vaccines that he may need during his development according to the immunization schedule and national health regulation. The girl is also in good health, being already the first grade at school. This first case in Bulgaria demonstrates the feasibility of international collaboration for application of sophisticated techniques in different specialized hospitals and clinics. The contact with patients is also very important to follow all steps in the development and life style of those two children, their older sister and parents.

DISCUSSION

According to the worldwide experience the procedures of PGD and HLA typing are reliable and provide a realistic option for the couples in the treatment of an affected sibling. This case also shows that, once a HLA-compatible and healthy embryo is found, full term pregnancy, delivery and curing the affected child without compromising the health of the sister as a donor can be obtained.

However, there exist certain medical, ethical and social parameters that should be considered and extensively discussed with patients before starting the treatment.The legislation and regulation on PGD with HLA typing differ from country to country and ethical debates are ongoing. In many countries such as USA, UK, Italy, Turkey, Bulgaria is ethically acceptable. European Union and the Council of Europe have some arguments against PGD/HLA, but international opinion represented by European Society of Human Reproduction and Embryology (ESHRE Taskforce on PGD 2003) considers those procedures morally acceptable. There is still a need for national and international consensus.

The low probability of finding HLA matched embryo should also be discussed with the couple (18.7% or 3/16 is the chance to find both HLA identical 1/4 and diseases free embryo) especially in autosomal recessive diseases such as β–thalassaemia. The most important factor which increased the chance of having a transfer was the good ovarian reserve of the patient; although in this particular case the patient could be considered as advanced maternal age with all possible negative effects like pre-eclampsia. Other risks of severe ovarian hypestimulation syndrome and from PGD (e.g. amplification failure, allele drop out (ADO) or contamination with extraneous DNA) should be also taking in consideration [8].

After achieving pregnancy, amniocentesis should be recommended. Although some studies show promising results in paternally inherited mutation of thalassemia using analysis of cell-free fetal DNA with non-invasive tests, this method still could not replace conventional and invasive ones but it could have importance in future [9].

Also, despite that mononuclear cells were frozen from the umbilical cord blood (UCB) as a potential source of stem cells for possible transplantation, the engraftment was higher only after transplantation of enough UCB units at the time of infusion but still there are some risks of primary graft failure, acute graft-versus-host disease (aGVHD) and chronic GVHD (cGVHD) [10]. Therefore transplantation of bone marrow has been decided to be performed as an alternative.

Furthermore, special attention should be given to the prospective child and follow-up of the psychosocial environment.

CONCLUSION

The present case report shows 7 years follow-up after delivery and transplantation of bone marrow post PGD combined with HLA typing for the autosomal recessive disease Beta-Thalassaemia major. Nowadays two siblings are at school, first grade is the girl donor and the boyis in third grade. Bothof them are in good health and bblood transfusions are no longer applicable. The boy is adapting very well in class, life, studies, collaborative with his schoolmates and already has received all vaccines that he may need during his development according to the immunization schedule and national health regulation.

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