Case Report

Successful Preimplantation Genetic Diagnosis of Hb Bart's Hydrops Fetalis in Singapore after Fresh and Frozen Embryo Replacement Cycles

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Abstract

Introduction: We report the first successful preimplantation genetic diagnosis (PGD) for Hb Bart's hydrops fetalis in Singapore, involving both fresh and frozen embryo replacement cycles. Clinical Picture: Two couples who were carriers of the Southeast Asian type double gene deletion (--^{SEA} deletion carriers) requested for PGD. Couple A had 2 previous affected pregnancies, while couple B have a child of unknown genotypic status. Treatment: One PGD cycle was performed for each couple. The --^{SEA} deletion was detected using a gap-PCR strategy. Couple A had 1 fresh-embryo replacement cycle while couple B underwent 2 frozen-embryo replacement cycles. Outcome: Couple A achieved a twin pregnancy. Second trimester complications resulted in premature delivery, where 1 baby girl survived. Couple B achieved a singleton pregnancy resulting in delivery of a healthy baby boy. Genotype analysis of all babies confirmed the PGD results consistent with clinically unaffected status. Conclusions: We have successfully performed PGD to avoid Hb Bart's hydrops fetalis syndrome.

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Key words: Alpha-thalassaemia, Frozen embryo replacement, Hb Bart's hydrops fetalis, Polymerase chain reaction, Preimplantation genetic diagnosis

Introduction

Alpha-thalassaemia is one of the most common genetic disorders worldwide. It results from absent or reduced production of α-globin chains caused by mutations in the α-globin gene cluster. The α-thalassaemia mutations usually involve deletions of one (−α) or both (− −) α-globin genes, and are prevalent within Southeast Asia. In Singapore, the carrier frequency for α-thalassaemia mutations is about 6.4% in the Chinese, 4.8% in Malays, and 5.2% in Indians.[1] Couples who are both heterozygous for an α-globin double gene deletion (α^0-thalassaemia) have a 25% risk of conceiving a baby with Hb Bart's hydrops fetalis syndrome resulting from homozygous α^0-thalassaemia, who will either die *in utero* late in gestation or soon after birth. This syndrome is associated with an increased risk of maternal complications including hypertension, eclampsia and antepartum haemorrhage.[2] Therefore, genetic counselling and prenatal diagnosis are important for at-risk couples who wish to avoid having an affected baby. However, the positive prenatal diagnosis of an affected foetus can be emotionally painful, as parents have to grapple with the difficult decision of pregnancy termination.

Preimplantation genetic diagnosis (PGD) represents an alternative option to prenatal diagnosis. Genetic analysis is performed on preimplantation cleavage stage embryos, and only unaffected embryos are transferred to establish pregnancy thus eliminating or minimising the occurrence of affected pregnancies. We report here on the first successful application of PGD for the avoidance of Hb Bart's hydrops fetalis syndrome in Singapore.

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Couple A were a 28-year-old Chinese woman and her 33-year-old husband, both of whom are heterozygous for

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the common Southeast Asian double α-globin gene deletion (αα/--^{SEA}). They had two previous affected pregnancies (--^{SEA}/--^{SEA}) that were subsequently terminated. Couple B were a 36-year-old Chinese woman and her 39-year-old husband, both of whom were also carriers of the Southeast Asian double α-globin gene deletion. They have a 2-year-old son whose α-globin genotype is undetermined.

The PGD procedures were performed under a study protocol approved by the Domain-Specific Review Board of the National Healthcare Group (C/00/549) and the Ethics Review Board of the Singapore General Hospital (155/2002). Embryo biopsy, blastomere lysis and neutralisation procedures have been described previously.[3]

Lysed and neutralised blastomere DNA was subjected to a nested polymerase chain reaction (PCR) amplification protocol. A gap-PCR strategy was used to amplify the α-globin gene fragment and detect the --^{SEA} deletion (Fig. 1). In the first PCR round, both the α-globin gene and the D1S80 variable number tandem repeat (VNTR) locus were simultaneously amplified in a volume of 50 μL containing the lysed and neutralised blastomere, 0.2 μM of each first round PCR primer (Table 1), 0.2 mM of each deoxyribonucleotide triphosphate (dNTP) (Roche Biochemicals), and 2.5 units of HotStarTaq DNA Polymerase (Qiagen) in 1X PCR buffer containing 1.5 mM $MgCl_2$. Thermal cycling was performed in a Biometra T3 thermal cycler (Biometra), with an initial 15 minute enzyme activation at 95˚C, followed by 30 cycles of 98˚C denaturation for 45 seconds, 50˚C annealing for 45 seconds, and 72˚C extension for 1 minute and 30 seconds, culminating in a final 5 minute extension at 72˚C.

In the second PCR round, secondary amplifications of the α-globin gene and D1S80 VNTR were performed separately using 3 μL aliquots of first round PCR product. PCR reaction conditions were similar to the first PCR round, except that the primers were replaced with second round PCR primers for α-globin or D1S80 (Table 1). In addition, the amount of DNA polymerase was reduced to 1 unit. Thermal cycling conditions were similar to the first round PCR, except that annealing temperature was 55˚C and the cycle number was 25. Ten microliters of each amplified product was analysed by electrophoresis through a 2% agarose gel in 1 x Tris-Borate-EDTA buffer at 10 volts/cm for an hour. Absence/presence of the parental D1S80 VNTR amplicons was used to monitor for allele drop-out and/or exogenous DNA contamination of sample tubes. The α-globin genotypes were determined based on the amplicon size, examples of which are shown in Figure 1.

Results

One PGD cycle was performed on Couple A. In this cycle, 8 oocytes were recovered, of which 5 successfully fertilised after intra-cytoplasmic sperm injection (ICSI). On day 3 post-fertilisation, all 5 embryos developed to at

Table 1. Primer Sequences for Nested Multiplex PCR Analysis of the α-globin Gene and the D1S80 VNTR Locus

Name	5' → 3' sequence	GenBank ID: Nucleotides	Concentration	Amplicon (size)
First round PCR primers				
α-globin locus				
Common-1F	TCTGTGTTCTCAGTATTGGAG	HSGG1:26130-26150	0.2μM	
Deleted-1R	ATATGGGTCTGGAAGTGTATC	HSCOS12:3175-3155	0.2μM	697 bp
Normal-1R	GTACACCGTGAAGAGCCTG	HSGG1:27146-27128	0.2μM	1017 bp
D1S80 VNTR locus				
D1S80-1F	GAAACTGGCCTCCAAACACT	HUMD1SMCT:1-20	0.2μM	410-874 bp
D1S80-1R	GTCTTGTTGGAGATGCACGT	HUMD1SMCT:530-511	0.2μM	
Second round PCR primers				
α-globin locus				
Common-2F	TTCGCAGGAACTCGGTCGTC	HSGG1:26188-26207	0.2μM	
Deleted-2R	GTCTGTAGTGTCAGGTGGAAC	HSCOS12:3138-3118	0.2μM	602 bp
Normal-2R	AGGACCTTGGATGGCGTTGG	HSGG1:27038-27019	0.2μM	851 bp
D1S80 VNTR locus				
D1S80-2F	TGCATTCAGATAAGCGCTGGC	HUMD1SMCT:90-110	0.2μM	306-770 bp
D1S80-2R	CACGTGCCCCTTGCAGGCT	HUMD1SMCT:515-497	0.2μM	

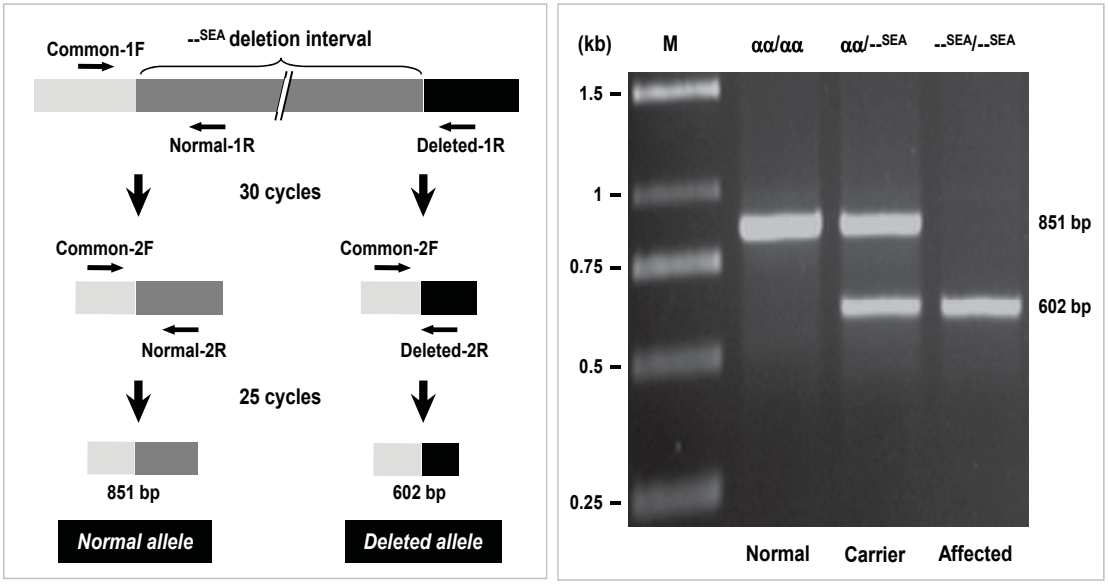

Fig. 1. Single cell gap-PCR genotyping of the Southeast Asian double α-globin gene deletion. Two rounds of PCR are employed to generate amplification products of different size from normal and deleted alleles (left), and genotypes are determined after agarose gel electrophoresis and ethidium bromide staining of DNA (right). M (kb) indicates Generuler 1kb DNA ladder (Fermentas, St Leon-Rot, Germany).

least the 6-cell stage and 2 blastomeres were removed from each embryo for analysis. Three embryos were diagnosed as affected (--^{SEA} /--^{SEA}) while the remaining 2 were carrier embryos (αα/--^{SEA}). Both carrier embryos were replaced back to the patient, resulting in a twin pregnancy. In the second trimester, severe polyhydramnios of unknown origin developed, resulting in premature labour. An emergency caesarean section was performed at 5 months of gestation, and twin baby girls of weight 700 gm and 630 gm were delivered. One baby girl (700 gram at birth) succumbed to infection after 19 days in the neonatal intensive care unit (NICU). The surviving baby girl is now well and developing normally. Post-natal DNA analysis confirmed the carrier genotype (αα/--^{SEA}) of both girls, indicating that the polyhydramnios was not caused by a Hb Bart's hydrops fetalis pregnancy, but was more likely due to complications associated with a twin pregnancy.

Couple B also underwent 1 PGD cycle, from which 40 oocytes were recovered. Of these, 23 oocytes were fertilised after ICSI and 20 embryos developed to at least 6-cell stage. Two blastomeres were biopsied from each embryo for analysis. Three embryos were diagnosed as normal (αα/αα), 5 embryos were diagnosed as carriers (αα/--^{SEA}) and 8 embryos were affected (--^{SEA}/--^{SEA}). The remaining 4 embryos yielded no analysable results. Because the patient exhibited symptoms of ovarian hyper-stimulation syndrome (OHSS), the PGD cycle was halted immediately without embryo transfer. Seven embryos (2 normal and 5 carrier) were cryo-preserved to enable replacement at a later date. Two months later, after resolution of the OHSS, 1 normal and 1 carrier embryos were thawed and transferred into the patient, but no pregnancy ensued. One month later, another 3 carrier embryos were thawed and replaced into the patient's uterus, resulting in a singleton pregnancy and birth of a healthy boy at term. Subsequent DNA analysis confirmed the carrier genotype (αα/--^{SEA}) of the newborn.

Discussion

This report documents the first successful performance of preimplantation genetic diagnosis to avoid Hb Bart's hydrops fetalis syndrome in Singapore. In this instance, PGD was employed to help 2 α^0-thalassaemia carrier couples to conceive clinically unaffected children. The thalassaemias (α and β) are the most common genetic disorders in Singapore.[4] For α-thalassaemia, double gene deletions that produce no α-globin chain account for more than 76% of the α-thalassaemia alleles in Singapore.[4] This high incidence of double gene deletions increases the risk of having pregnancies with homozygous α^0-thalassaemia, which not only causes Hb Bart's hydrops fetalis syndrome, but is also associated with maternal morbidity and mortality.[5] Prenatal diagnosis and PGD play an important role in preventing such cases. Although there are many reports of PGD for β-thalassaemia, only a few reports of PGD for α-thalassaemia have been published.[6-8] PGD is currently the only option for at-risk couples to begin with an unaffected pregnancy and thus avoid the need for termination.

We also illustrated the clinical benefits of performing frozen embryo replacement cycles on couples who were not able to complete the fresh embryo transfer in the same PGD cycle. This option allows them to proceed with further

rounds of embryo transfer without the need to go through more cycles of ovarian stimulation. This is especially useful should complications such as ovarian hyper-stimulation occur. In addition, with the increase in cumulative pregnancy rate per retrieval cycle, lesser numbers of embryos can be transferred to minimise the risk of multiple pregnancies.[9-10]

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