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Case Series

Pre-implantation genetic testing: a retrospective observational study of 13 cases of various genetic diseases successfully detected and managed at an IVF centre

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ABSTRACT

Pre-implantation genetic testing (PGT) is an advanced form of prenatal genetic testing that is done as a part of ART cycles. The purpose of PGT is to identify genetically normal embryos in a given cohort, in order to select the most desirable embryos for implantation. With the gaining popularity of day 5 trophectoderms biopsy over day 3 blastomere biopsy, the role of PGT has become more clinically significant. To report the data of 13 couples, with diagnosed genetic condition and having risk of transmitting this condition to their offspring's, who underwent Pre-implantation genetic diagnostics (PGT M/SR). PGT was performed in 13 couples with various rare chromosomal conditions like Hereditary Inclusion body myopathy, Col4a1 gene mutation etc at our Infertility Centre from January 2016 to January 2020. The clinical data of all these patients was reviewed and is reported in our study. A total of 193 oocytes were retrieved and 158 oocytes were fertilized by ICSI. 62 blastocysts were obtained and 55 blastocysts were biopsied for analysis. Among the 35 normal embryos, 17 embryos were transferred. 11 clinical pregnancies were established resulting in 8, disease free, live births. PGT (M/SR) is an effective molecular diagnostic test, that is a ray of hope for many genetically affected couples, as its prevents the transmission of their unwanted genetic condition to their offspring's.

Keywords: Pre-implantation genetic testing for monogenic disorder/structural rearrangement, *In vitro* fertilization, Genetic, Chromosomal

INTRODUCTION

Pre-implantation genetic testing (PGT) is an advanced form of prenatal genetic testing that is done as a part of ART cycles in order to select the most desirable embryos in a given cohort, produced during IVF, for implantation.[1] The aim of the study was to diagnose whether an embryo is affected by a monogenic disease and/or chromosomal impairment, thus limiting the risks and preventing the implantation of an abnormal fetus.[2] In recent years, PGT, has gained a lot of momentum. Various studies supporting as well as opposing its role in IVF have been published. An important aspect of this technique is the possibility that the biopsy itself may damage the developing embryo. In 2016, the pre-implantation Genetic Diagnosis International Society (PGDIS) has renamed PGD/PGS with its sub-classification (a) pre-implantation genetic testing for aneuploidy (PGT-A); (b) pre-implantation genetic testing for aneuploidy (PGT-A); and (c) pre-implantation genetic testing for chromosomal structural

rearrangements (PGT-SR) like translocations, inversions etc; (d) pre-implantation genetic testing for chromosomal structural rearrangements (PGT-SR) like translocations, inversions etc; and (e) pre-implantation genetic testing for single or multiple gene disorders (PGT-M) like cystic fibrosis etc.[3] We hereby report 13 cases, where PGT (M/SR) was successfully applied to detect rare genetic conditions like albinism, hereditary inclusion body myopathy, ColA4 gene mutation, spinal muscular atrophy in the embryos.

CASE SERIES

The study type was observational, retrospective. The study place was Akanksha Hospital and Research Institute. The cases of 13 indicated couples was studied from January 2016 to January 2020 at an infertility centre. Following detailed genetic consultation and discussion on all relevant aspects pertaining to PGT, the couples signed an informed consent form to undertake PGT. Each PG test was custom-designed based on the specific disease and mutation that was inherited in the family. The following steps were involved in the utilization of PGT in an IVF cycle: (a) step 1: case review and probe development- a detailed history and all the genetic reports of the couple was carefully reviewed. Next, DNA samples from the blood, saliva, egg source, sperm source, and that of (if needed) likely additional family members like parents or children was collected. These samples were used to develop the probe; (b) step 2: IVF cycle to create the embryos- once the probe was completed, an antagonist protocol of IVF cycle was planned and embryos were created. ICSI was typically the recommended method of fertilization; (c) step 3- culture and biopsy- extended blastocyst culture was performed on the embryos. Approximately 6-8 cells from the laser hatched blastocyst were drawn carefully by the embryologist for testing. All the blastocyst were vitrified and stored, while the cells from the biopsy were sent to the lab for PGT; (d) step 4: PGT- the testing was done using the probe that was developed prior. Using the same biopsy sample chromosomal errors in embryos using PGT-A was also done; and (e) step 5: embryo transfer- next, the results of their IVF+PGT cycle was discussed and normal unaffected embryos with the condition PGT was utilized for, were transferred into the patient's uterus. Frozen embryo transfer was done and hormone replacement therapy (HRT) was used to prepare the endometrium.

All the couples were coded from A to M so that case based analysis can be done effectively. The source of the DNA was blood (30 cc was drawn) from both the partners. For couples A, D, E, F and H, blood (30 cc) was also drawn from the affected first born child.

Technique used

Cells from trophectoderms biopsy were subjected to whole genome amplification using a commercial kit (PicoPLEX WGA Kit, New England bio labs, UK). Oligonucleotide probes targeting the specific mutation within the TSC1 gene was used to amplify a 220 bp amplicon with the target mutation location internal to it. Direct testing for mutations was performed by PCR amplification followed by automated DNA sequencing of the amplicon using BigDye Terminator Chemistry on an ABI Genetic Analyzer 3500DX platform to generate nucleotide sequences flanking the particular region of the disease in question. The raw data obtained was subsequently analysed for the presence or absence of mutation at the hot spot region.

The results obtained were confirmed by performing PGT-M for all the embryos using PCR amplification followed by Sangers sequencing. Considering an error rate of 1-5%, the results of prenatal diagnosis was confirmed using Chorionic villus sampling or amniocentesis.

Embryo gene profiling by single- cell sequencing was performed for couple K to detect c.1537G>A; pG513S mutation within the COL4A1 gene.[4]

Limitations of the assay

This assay will only detect the mutation targeted. Any other mutation present within the exons, promoter, the 5 and 3 untranslated regions and regions deeper (than 10-20 nucleotides) within the introns will not be detected. Large deletions that eliminate most or all of the coding sequence will also not detected by this assay. The mean maternal age in our study group was 32.46 years. Total 193 oocytes were retrieved, of which 158 were M2 oocytes and 105 were fertilized and 62 blastocysts were formed and 55 biopsies were taken for analysis. Couples C had history of recurrent abortions and couple G, L and M had history of previous 3, 4 and 2 failed IVF cycles respectively.

Couple J had 2 previous early pregnancy losses. There was a family history of neuropathy and convulsions to all sons born to the brothers of the male partner of couple K. Among the 35 normal embryos, 17 embryos were transferred. 8 clinical pregnancies were established resulting in 8 live births and no abortions were noted. All 8 live births resulted from term singleton pregnancies and were found to be normal and free from the genetic condition in question. Genetic testing was performed post birth for children born to couples A, B, F, I and K to confirm the PGT and CVS diagnosis and for child born to couple J who had refused CVS. Chorionic villus sampling test in antenatal period for pregnancies conceived using PGT M /SR is diagnostic for confirming fetal aneuploidy. These procedures carry risks, like procedure related miscarriage (approximate 1-2/1000 over the baseline risk). No finding of mosaicism was reported with a CVS sample in our study group. However, couple number H and J refused to perform any invasive tests like CVS. Amongst the 13 couples who underwent PGT (M/SR) 6 cases were of autosomal recessive, 4 cases of Autosomal dominant and 3 cases of Balanced reciprocal transmission as the inheritance pattern of the given genetic condition.

Interpretation of results

Normal

Embryo found not to inherit the 'at risk haplotype'. This embryo is expected to be unaffected by the indicated genetic mutation.

Carrier

Embryo found to inherit the 'at risk haplotype'. This embryo is expected to be carrier for the indicated genetic mutation same as the carrier parent(s).

Abnormal

Embryo found to inherit the parental 'at risk haplotype'.

This embryo is expected to be affected by the indicated disorder.

Non-informative

A reliable result could not be achieved due to factors such as Allele drop out (ADO), parental/external contamination, recombination and others.

Table 1: Highlights the clinical parameters of the couples in our study group.

	Age of female partner (yrs)	Age of male partner	Age of female partner (yrs)	Anti mullerian hormone (AM) (ng/ml)	Clinical presentation of previous affected child	Clinical presentation of previous affected child	Inheritance pattern	Female karyotype	Male karyotype
A	34	37	10	8.16	Hypopigmentation of skin, hair, iris. photophobia with refractory error	Albinism (with first affected child)	Autosomal recessive	Un-affected carrier	Unaffected carrier
B	35	37	8	1.4	Hypopigmented skin patches with seizures	Tuberous sclerosis	Autosomal dominant 2 to 3% chance of germline mosaicism	Normal	Unaffected
C	35	35	8	2.63	No previous live child	Balanced reciprocal translocation		Normal	Affected
D	31	35	6	0.41	Multiple blood transfusions, anaemia, with jaundice	Beta thalassemia major	Autosomal recessive	Un-affected carrier	Unaffected carrier
E	29	33	5	5.71	Muscle weakness with autism	Spinal muscular Atrophy	Autosomal recessive	Un-affected carrier	Unaffected Carrier
F	29	29	6	0.7	Difficulty in breathing with failure to thive	Methylmalonic academia	Autosomal recessive	Un-affected carrier	Unaffected carrier
G	35	43	11	2.19	No previous live child	Balanced reciprocal translocation		Normal	Unaffected
H	36	36	11	1.2	Multiple blood	Beta thalassemia	Autosomal recessive	Un-affected carrier	Unaffected carrier

Continued.

	Age of female partner (yrs)	Age of male partner	Age of female partner (yrs)	Anti mullerian hormone (AM) (ng/ml)	Clinical presentation of previous affected child	Clinical presentation of previous affected child	Inheritance pattern	Female karyotype	Male karyotype
					transfusions with anemia				
I	32	33	5	1.8	No previous live child	Inclusion Body Myopathy 2	Autosomal recessive	Un-affected carrier	Unaffected carrier
J	28	29	2	2.48	No previous live child	Polycystic Kidney disease	Autosomal dominant	Normal	Affected carrier
K	38	39	13	0.5	No previous live child	HANAC syndrome Col4A1 c.1537G>A;p.G 513S	Autosomal dominant	Normal	Affected carrier
L	31	32	5	6	No previous live child	Jacobs syndrome	Autosomal dominant	Normal	Affected carrier
M	29	29	7	3.6	No previous live child	Balanced Reciprocal translocation		Normal	Unaffected carrier

Table 2: Shows the embryology parameters of the couple.

Couple	Oocytes retrieved (n)	(MII) Oocytes (n)	Oocytes fertilized (n)	Blastocysts Formed (n)	Blastocysts tested for PGTM /SR	Blastocysts tested For PGTA	No. FET cycles done	Clinical pregnancy	Number of embryos transferred
A	6	4	1	1	B1-normal	B1-normal	1	Yes	1
B	23	16	14	7	B1-normal B2-normal B3-normal B4-normal B5-normal B6-normal B7-normal	B1-normal B2-normal B3-normal B4-normal B5-normal B6-normal B7-normal	1	Yes	2
C	14	13	13	6	B1-affected B2-affected B3-affected B4-normal B5-normal	B1-non informative B2-affected B3-affected B4-normal B5-normal	1	Embryo transfer pending	-
D	5	4	4	2	B1-normal B2-affected	B1-normal B2-affected	1	No	1
E	18	15	13	11	B1-normal B2-normal B3-normal B4- normal B5- normal	B1-normal B2-normal B3-normal B4- normal B5- normal	1	Embryo transfer pending	-
F	12	9	6	3	B1-normal B2-affected B3-normal	B1-normal B2-affected B3-normal	1	Yes	2
G	11	11	3	1	B1-normal	B1-normal	1	No	1

Continued.

Couple	Oocytes retrieved (n)	(MII) Oocytes (n)	Oocytes fertilized (n)	Blastocysts Formed (n)	Blastocysts tested for PGTM /SR	Blastocysts tested For PGTA	No. FET cycles done	Clinical pregnancy	Number of embryos transferred
H	5	3	3	1	B1-normal	B1-normal	1	Yes	1
I	11	10	7	5	B1-normal B2-normal B3-normal B4- normal B5- affected	B1-normal B2-normal B3-normal B4- normal B5- affected	11	10	7
J	13	13	8	4	B1-normal B2-affected B3-normal B4-normal	B1-normal B2-affected B3-normal B4-normal	2	Yes	2
K	23	20	12	12	B1-normal B2-affected B3-normal B4-normal B5-affected B6-affected B7-affected B8-affected B9-affected B10-affected B11-affected B12-affected	B1-normal affected B3-normal B4-normal B5-affected B6-affected B7-affected B8-affected B9-non-informative B10-non-informative B11-non-informative B12-non-informative	1	Yes	2
L	25	19	8	7	B1-normal B2-affected B3-normal B4-normal B5-normal B6-affected B7-affected	B1-normal B2-affected B3-normal B4-normal B5-normal B6-affected B7-affected	2	No	2
M	27	21	13	2	B1-normal B2-affected	B1-normal B2-affected	1	Yes	1

Table 3: Shows the outcome parameters.

Couples	Result of transfer	Antenatal screening performed	Outcome
A	Positive	CVS	Full term infant
B	Positive	CVS	Full term infant
C	Negative	-	Embryo Transfer pending
D	Negative	-	-
E	Negative	-	Embryo Transfer pending
F	Positive	CVS	Full term infant
G	Negative	-	-
H	Positive	CVS refused	Full term infant
I	Positive	CVS	Full term infant
J	Positive	CVS refused	Full term infant
K	Positive	CVS	Full term infant
L	Negative	-	-
M	Positive	CVS refused	Full Term infant

Note: CVS- Chorionic villus sampling.

DISCUSSION

PGT was first introduced by Handyside et al in 1990 by selecting female embryos in order to prevent the birth of male patients affected with X-linked recessive disorders.[5]

Since then the applicability and popularity of this technology has evolved in a massive way. Increasing number of ART cycles, recurrent IVF failures, late marriages and advanced maternal age at conception, consanguineous marriages, history of previously affected embryos or offspring, stressful lifestyle and more awareness among the couples, ultimately had led PGT to be sought and applied to numerous ART cycles.

Pre-implantation genetic testing allows testing at three levels: sex chromosome abnormalities/aneuploidy, structural chromosomal abnormalities, and single gene defects. Initially, PGT was used to prevent single gene disorders such as cystic fibrosis. Recently, whole genome amplification with comparative genomic hybridization has been used to derive the entire karyotype for PGT, but the results are still very preliminary and the procedure is lengthy.

Currently, the most common method of PGT involves the use of a single-cell multiplex PCR for the amplification of short tandem repeat (STR) polymorphic markers that are located in close proximity to the mutation site. Those polymorphic STR markers are repeats of DNA that are mostly heterozygous and whose sizes vary greatly among individuals. STR length values that are linked to the mutated allele can be determined via fragment analysis using paternal and maternal genomic DNA prior to PGT, and the genotypes of the generated embryos can be diagnosed by linkage analysis during PGT. The use of multiple STR markers that are linked to the mutation site is useful to overcome the diagnostic problems of ADO, which is the amplification failure of one of two alleles in a locus and is one of the major causes of misdiagnosis during PGT.[6]

Our study provides a comprehensive evaluation of 13 couples who underwent PGT for detection of various conditions. The clinical, embryological and outcome parameters of each couple is discussed in the Table 1-3.

8 out of 13 couples delivered healthy babies, 3 couples had a negative result and another 2 couples are awaiting an embryo transfer.

It is pertinent to note that an PGT is a multistep procedure which requires combined expertise in reproductive medicine and genetics. It requires a multidisciplinary approach that includes a team of a skilled fertility specialist, embryologist, genetic counselor, advanced laboratory facility and personnel, psychologist, obstetrician and neonatologist. Today, genetically determined disorders account for up to one third of admissions to pediatric wards and are a significant cause of childhood deaths. Although advances in molecular biology promise means for the long term curative treatment of many severe genetic disorders, the current approach for controlling these disorders remains prevention, including application of PGT which is an accepted standard protocol in many countries.[7]

The safety of PGT for children born is of major concern, but initial evaluation of about 250 babies born worldwide after PGT indicates that the procedure has no adverse consequences on early development.[6,8] It is important to note that non-mendelian disorders such as congenital heart disease, cleft lip/palate, some behavioral disorders like Autism or deafness are currently not conducive to PGT because there are polygenic, epigenetic and environmental contributions to such a phenotype. These conditions do not follow traditional monogenetic heritability. Sub-telomeric deletions, mosaicism, small structural rearrangements, microdeletions and micro-duplications may pose challenges and furthermore experienced laboratory personnel in IVF and genetics are important for success in such cases.[9]

Majority of centres are only willing to perform PGT to prevent the birth of children with severe genetic disorders, such as BRCA 1 and 2, rheumatoid arthritis, multiple sclerosis etc.[9] While there is also public concern about the use of PGT for social or eugenic reasons and there are a few centres which have begun to offer PGT for 'social' sexing (personal communication, through the ESHRE PGT Consortium). Thus, it is imperative to establish appropriate ethical guidelines and legislation as soon as possible.[10]

One of new and growing indications of PGT is detecting mitochondrial abnormalities due to mutations in nuclear DNA, which are only detected when a critical threshold of mutations are reached. Often, the mother is a carrier of an unknown small percentage of mutated mitochondrial DNA that is propagated to her offspring. The concept is that a high MtDNA copy number in euploid embryos is indicative of lower embryo viability and implantation.

A major technical hurdle in PGT is that it does not have a general formula for all mutations, thus different gene locus needs individualized, customized design to make the diagnosis accurate enough to be applied on PGT. Other limitations to the wider application of clinical PGT cycles include the necessity to involve IVF, even if the couple are not infertile, the relatively low pregnancy and birth rate, and the high cost of a complete PGT cycle.

Couple 1 in our study, underwent a comprehensive genetic testing, as a part of their workup and both were diagnosed to be carriers of inclusion body myopathy 2 disorder. Considering that almost 15% of genetic disorders are associated with infertility or recurrent pregnancy loss, comprehensive genetic testing, must be optimally offered to indicate couples as an advanced diagnostic test. Although a growing number of centres world over trust

PGT, however, as a clinical service, it is not widely performed by many since it requires combined expertise in the fields of reproductive medicine and molecular genetics and/or cytogenetics with an advanced laboratory set up. The limitation of the present study is that Statistical analysis was not performed.

CONCLUSION

In conclusion, we provide the first extensive overview of pre-implantation genetic testing for monogenic disorders. Over the years, major advancements have been introduced in the area of pre-implantation genetic testing and assisted reproduction, making PGT a well-established, accurate and safe clinical procedure. Our data, can aid in counseling prospective parents from families with monogenic disorders on the option of pre-implantation genetic testing. This will enable couples to make informed decisions in line with their personal, cultural and moral backgrounds and beliefs.

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