Abstract

Mitochondrial diseases are sporadic and very serious. The causes of these diseases are the mutations of the mitochondrial (mt) DNA (deoxyribonucleic acid) which is of maternal origin. The phenotypic variability of mitochondrial disease is determined by the simultaneous presence of normal and mutant mt DNA in the cytoplasm, a process called heteroplasmy. We present a case of Leigh syndrome in a twin pregnancy after in vitro fertilization (IVF). The two-month-old infant extracted by cesarean section at thirty-eight weeks of gestation was admitted to the hospital for worsening respiratory symptoms. The symptoms had started seven days before with fever and difficulty breathing; marked metabolic acidosis was discovered at the lumbar puncture that was done at admission in the hospital. We sent the patient to genetic testing due to congenital lactic acidosis. Leigh syndrome was strongly suspected. First, he had the sequencing analysis of the genes in Leigh syndrome and mitochondrial encephalopathy panel and a heterozygous variant of uncertain significance in the COQ8A gene, also known as ADCK3, c.521C>A p was found (Thr174Lys). Unfortunately, the infant died in the hospital due to cardiorespiratory arrest. The parents are now considering having another IVF procedure, and we are discussing all the possible variants with them.

Keywords: in vitro fertilization; Leigh syndrome; mitochondrial DNA (deoxyribonucleic acid); PGD (preimplantation genetic diagnosis).
1. Introduction

Mitochondria are cellular organelles that we find in all nucleated human cells. The role of mitochondria is in aerobic respiration, in oxidative phosphorylation that leads to cellular energy production in the form of adenosine triphosphate (ATP) [1].

The mitochondrial (mt) DNA is a circular double-stranded DNA of 16.6 kilobases, and it is found in each mitochondrion in several copies. Mt DNA is of maternal origin, and if it had mutations, this could determine mitochondrial disease in newborns [2]. Mitochondrial DNA is more susceptible to mutations than nuclear DNA. The phenotypic variability of mitochondrial disease is determined by the simultaneous presence of normal and mutant mt DNA in the cytoplasm, process that is named heteroplasmy. Now it is possible to test the mutant loads of embryos by preimplantation genetic diagnosis (PGD). There is a close relationship between the ratio of affected to normal mitochondrial DNA and the severity of the disease. PGD for the mitochondrial disease involves some ethical problems because it could be cycles of ovarian stimulation in which only affected embryos are available for transfer [3].

Mitochondrial diseases are widespread, the frequency in adults being higher than 1 in 5000, the neurological manifestations are typically [4]. These diseases can be fatal or cause severe chronic morbidity in the most energy-consuming tissues in the body, such as the central nervous system, muscle, heart, liver, and kidneys. Leigh syndrome, for example, typically appears in the first years of life and the patients had very high mutation load, typically more than 90% of mtDNA is mutated.

European Society of Human Reproduction and Embryology (ESHRE) and the European Society of Human Genetics (ESHG) worked together to evaluate the development of technology at the interface between assisted reproduction and molecular genetics [5].

There are three types of country categories depending on the legislation on mitochondrial manipulation techniques (MMT) in infertility clinics: countries where these procedures are banned like USA and China, countries where these are not legally regulated like Ukraine and Northern Cyprus, countries where legislative regulation is insufficient, unclear (Mexico and twelve other countries) [6].

Case presentation

We present the case of Leigh syndrome after in vitro fertilization (IVF). A 38-year woman with body mass index (BMI=24.5kg/m²) presented to our Reproductive Medicine Department for tubal infertility. The sperm was in normal parameters, and the antimullerian hormone was 1.3 ng/ml. We used a long antagonist micro flare protocol as previously presented [7]. She obtained ten oocytes fertilized by IVF (fig.1), resulting two medium quality blastocyst 3bc [8]. So, we decided to transfer two embryos. She got a twin pregnancy.
The pregnancy was evolute without significant problems, even if the mother was diagnosed with low-risk thrombophilia (double heterozygote MTHFR -C677T and MTHFR-A1298T) and group B streptococcus at thirty-two weeks of pregnancy which was treated with antibiotics. She delivered by cesarean section two babies, one girl perfectly healthy, one boy with birth weight 2490, Apgar8, that required CPAP (Continuous Positive Airway Pressure) resuscitation maneuvers at birth, then oxygen therapy under the cephalic tent.

The 2-month-old infant that had been extracted by cesarean section at thirty-eight weeks of pregnancy was admitted to the hospital for worsening of respiratory symptoms. The symptoms had started seven days before with fever and difficulty breathing; marked metabolic acidosis was discovered at the lumbar puncture that was done at admission in the hospital. At admission, there were also, high values of lactic acid 11-19 mmol/l; the
serum pyruvate lactate ratio was very high (127), so it was considered unlikely a deficiency of pyruvate dehydrogenase.

The patient was sent to a genetic test due to congenital lactic acidosis. Leigh syndrome was strongly suspected. First, he had the sequencing analysis of the genes in Leigh syndrome and mitochondrial encephalopathy panel, and a heterozygous variant of uncertain significance in the COQ8A gene, also known as ADCK3, c.521C>A p was found (Thr174Lys). A quantitative PCR assay (qPCR) was performed by using eight gene-specific amplicons encompassing the coding exons 2, 3, 5, 6, 8, 11, 14, and 15 (or part of it) of the COQ8A: NM_020247.4 gene(s). The genetic diagnosis of primary coenzyme Q10 deficiency type 4 was not confirmed. Deletion/duplication analysis performed to assess possible compound heterozygous state didn't find anything.

Unfortunately, the infant died in the hospital due to cardiorespiratory arrest. The parents are now considering having another IVF procedure, and we are discussing all the possible variants with them.

2. Discussion

Genetics has a greater or lesser role in all diseases [9]. Homozygous or compound heterozygous pathogenic variants in the ADCK3 gene cause primary coenzyme Q10 deficiency type 4, also known as spinocerebellar ataxia type nine. This is an autosomal recessive disorder characterized by childhood-onset of cerebellar ataxia and exercise intolerance. Some affected individuals develop seizures and have a mild mental impairment, indicating variable severity.

Given the recessive inheritance mode and because only one heterozygous variant was detected in the ADCK3 gene, a genetic diagnosis of primary coenzyme Q10 deficiency type 4 cannot be confirmed. However, because the patients' symptoms were significant, we decided to proceed with deletion/duplication analysis of this gene, but we did not find any modification.

In our case, the Leigh syndrome probably is determined by the mutant load of mtDNA, which was probably higher in one of the twins (the boy), confirming what is already known, the higher mutant load of mtDNA, the worsening of the symptoms. The mutant load of mtDNA of every embryo is different for the same person. However, the mutant load of a single blastomere is the same as the mutants' load of the entire embryo.

The reproductive options are CVS (chorionic villus sampling), amniocentesis or PGD. PGD involves embryo biopsy and selection for transfer of the embryo with the lowest mutation load.

There are two ways to avoid Leigh syndrome in the newborn, in case our patient decided to do another IVF cycle. First, to prevent the transmission of mutated mtDNA, we can perform PGD and select the embryos with the lowest level of mutant mtDNA or we could propose to the patients: mitochondrial donation, thus reducing or eliminating mutant mtDNA.

During embryo /fetus development, there could be a change in mutant load, and there is no cut-off point between mutant load and clinical symptoms. There is also a problem regarding PGD because we don't know if
the patient will have sufficient oocytes with a low mutant load during one IVF cycle. For repairing this situation, mitochondrial replacement therapy was developed [5]. The cytoplasmic transfer involves supplementing the cytoplasmic patient's oocytes from a donor [1]. There are two ways to achieve this, pronuclear transfer and spindle nuclear transfer. In nuclear transfer, both the patient's (recipient) and the donor's oocytes are fertilized with the father's sperm. Then the nucleus of each fertilized oocyte is removed, and the donor nucleus is replaced with the recipient nucleus. Very interesting, the first case of this type was performed in Mexico, with a Jordanian couple, to avoid the transmission of Leigh syndrome to the newborn [9]. This could be a solution also for our case.

In the case of spindle transfer, the patient's oocyte nucleus is removed and inserted into the unfertilized oocyte of the donor who previously had the nucleus removed [10].

3. Conclusion

Mutations in mitochondrial DNA could determine mitochondrial disease. Screening for family risk and genetic counseling is mandatory in mitochondrial disease.

Recommendations are to avoid clinical manifestations of the disease by preventing transmission of mutated mtDNA using mitochondrial donation or PGD and selecting the embryos with low levels of mutant mtDNA.

References


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