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Case Series

5, 10-methylenetetrahydrofolate reductase gene mutation and reproductive outcome: how much do we know? A case series in Indian population

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ABSTRACT

Recurrent pregnancy loss (RPL) defined as loss of two or more pregnancies is one of the reasons why couples visit an ART clinic. 2-5% of RPL cases show an abnormal parental karyotype. Folic acid is an essential B vitamin involved in processes of fundamental importance for cell division and embryo development. Folic acid deficiency can have effect on many processes involved in oocyte development, acquisition of endometrial receptivity, embryo implantation and also in the maintenance of pregnancy. The 5, 10-methylenetetrahydrofolate reductase (MTHFR) enzyme plays an important role in folate metabolism. The most investigated MTHFR gene mutations are single nucleotide polymorphisms (SNPs) at the mRNA positions 677 (rs1801133) and 1298 (rs1801131). MTHFR gene mutations are found less in Asian population and hence have not been studied or evaluated much. We presented a case series of 3 different cases of MTHFR gene mutation variants that were managed at our ART center. Testing for MTHFR gene mutation after 3 early pregnancy failures/missed abortions, PGT-A and surrogacy being our proposed interventions.

Keywords: MTHFR gene mutation, RPL, Recurrent IVF failures, Surrogacy, MTHFR c.677C>T, MTHFR c.1298A>C

INTRODUCTION

Folic acid is an important B vitamin essential for human reproduction.[3] The processing of folic acid and other dietary folates is vital for many key processes such as amino acid metabolism, purine and pyrimidine synthesis and methylation of nucleic acids, proteins and lipids.[4] These folate dependent functions are required for DNA synthesis and repair, control of gene expression and many other biological processes of fundamental importance for cell division and embryo development.[5,6]

Folate deficiency (genetically determined or due to dietary restriction) leads to higher frequency of uracil misincorporation into DNA, disruption of nucleic acid integrity, slower DNA replication and an increased risk of chromosome breakage. Insufficient folate or folic acid intake has also been shown to negatively affect specific reproductive functions; it has a detrimental effect on many processes involved in oocyte development, acquisition of endometrial receptivity, embryo implantation and also in the maintenance of pregnancy.[7-10]

Many variations in genes involved in folate metabolism have been identified.

In terms of prevalence and impact, genetic variations affecting the MTHFR are among the most biologically important. MTHFR is a key enzyme that plays an important role in catalyzing the conversion of 5, 10-methylenetetrahydrofolate into 5-methylenetetrahydrofolate, the predominant circulating form of folate. This molecule provides the single carbon needed for the synthesis of nucleotides, the re-methylation

of homocysteine to methionine, the synthesis of S-adenosylmethionine and the methylation of DNA, proteins, neurotransmitters and phospholipids.[11,12]

The most investigated are SNPs at the mRNA positions 677 (rs1801133) and 1298 (rs1801131). The well-characterised MTHFR c.677C>T transition, which results in an alanine to valine substitution (p.Ala222Val) in the predicted catalytic domain of MTHFR, renders the enzyme thermolabile and leads to a reduction in MTHFR activity. Homozygous and heterozygous individuals have *in vitro* MTHFR activity reduced by about 70% and 35%, respectively.[13] Homozygosity for the 677T allele is associated with elevated circulating homocysteine in some individuals, predominantly those who have a low plasma folate level.[14] In these individuals, the level of plasma homocysteine can be lowered by folic acid supplementation.[15] The other common polymorphism in the MTHFR gene, c.1298A>C transversion, results in a glutamate to alanine substitution (p.Glu429Ala) within a presumed regulatory domain of MTHFR.[16,17] The 1298C allele leads to decreased enzyme activity, although to a lesser extent than the 677T allele. Individuals who are homozygous for the 1298C allele have about a 40% reduction in enzyme activity in vitro, but do not appear to have higher plasma homocysteine levels than controls.[16-18] In few studies results demonstrated that maternal MTHFR c.1298A>C genotype strongly influences the likelihood of a pregnancy occurring, with the 1298C allele being significantly overrepresented amongst women who have undergone several unsuccessful assisted reproductive treatments also parental MTHFR genotypes were shown to affect the production of aneuploid embryos, indicating that MTHFR is one of the few known human genes with the capacity to modulate rates of chromosome abnormality.[26]

However, individuals who are compound heterozygous for the 677T and the 1298C alleles (MTHFR c.677C/T plus c.1298A/C genotype) have a 40-50% reduction in enzyme activity in vitro and a biochemical profile similar to that seen among 677T homozygotes, with increased homocysteine and decreased folate levels. The c.1298A>C polymorphism by itself may have clinically important effects under conditions of low folate intake or during times of high folate requirements such as pregnancy and embryogenesis.[16] Some authors have reported an association of certain genotypes with an increased risk of miscarriage, a potential consequence of poor vascularization of the placental area of individuals carrying minor alleles.[19-22] Others have described a link between c.677C>T and c.1298A>C polymorphisms and the likelihood of aneuploid conceptions, pointing out the possible influence of MTHFR on chromosome nondisjunction and other processes involved in chromosome segregation.[22-25] More recent reports have explored the impact of these polymorphisms in patients undergoing IVF treatment, suggesting an influence of some MTHFR variants on embryo implantation.[27,28] There was still a need of analysis of the genotypes of individuals affected by fertility problems and of the embryos they produce was required in order to obtain a better understanding of the effects of MTHFR gene variants on reproduction in general and on assisted reproduction in particular.

CASE SERIES

Basic history and investigations are discussed in a tabular form.

Table 1: History of all three patients.

Patient's name	Age	Marital status	Occupation	Menstrual history	Obstetric history
AAP	32	Married since 9 years (AML 4 years), non-consanguineous marriage	Interior Designer	Menarche 13 years PrMC- 3-4 days/1-2 pads/day/ irregular since 6 months PaMC 3-4 days/30 days/RMPL	A2 A1-6WGA/natural conception/ early pregnancy failure/dilatation and evacuation done A2-6WGA/natural conception/missed abortion/evacuated by medical method.
PM	36	MS 7 years AML 2 years non-consanguineous marriage	Housewife	Menarche 12 years PrMC- 5 days/1-2pads/day/25 days, PaMC- 5 days/1-2 pads/day/25 days	A2MTP2 A1-6WGA/natural conception/medical termination i/v/o personal reasons/dilatation and evacuation done A2-6WGA/pregnancy diagnosed with urine pregnancy test/natural conception/early pregnancy failure/evacuated by medical method. A3-12 WGA/natural conception/ medical termination i/v/o raised

Continued.

Patient's name	Age	Marital status	Occupation	Menstrual history	Obstetric history
					nt/dilatation and evacuation done/ product of conception tested positive for trisomy13 A4-8 WGA/natural conception/missed abortion/dilatation and evacuation done.
SPJ	27	MS 3 years AML 3 years non - consanguineous marriage	Housewife	Menarche - 12 years PrMC- 3-4 days/ 1-2pads/ day/ 30 days PaMC- 3-4 days/ 1-2pads/ day/ 30 days	A2 A1-6WGA/ natural conception/twin pregnancy/one twin vanishing and other missed abortion/dilatation and evacuation done/POC karyotyping - normal A2-chemical pregnancy/natural conception/early pregnancy failure/spontaneously aborted.

MS-married since, AML-active married life , PrMC-present menstrual cycle, PaMC-past menstrual cycle, A-abortion , WGA-weeks gestational age, MTP-medical termination of pregnancy , POC-products of conception.

Table 2: History of all three patients (continued).

Patient's name	Contraception history	Sexual history	Social history	Past medical and surgical history	Family history	Investigations and treatment done at other ART center
AAP	History of use of barrier contraception for 4 years after marriage	Coital frequency twice a week; no history of coital difficulties	Nonalcoholic, nonsmoker, stress factor+	Known case of hypothyroidism on 25 ug levothyroxine	Father-hypertensive, mother-diabetic	2 cycles of ovulation induction with T. letrozole, no dominant follicle was formed. Patient was advised thrombophilia profile i/v/o recurrent pregnancy losses. MTHFR 1298 A> C heterozygote variant present in patient. protein C, protein S, S. homocysteine, antithrombin 3, ACA, LA: Negative ANA-Weakly positive
PM	History of use of barrier contraception for 5 years after marriage	Coital frequency twice a week; no history of coital difficulties	Nonalcoholic, nonsmoker, stress factor+	Not significant	Not significant	None

Continued.

Patient's name	Contraception history	Sexual history	Social history	Past medical and surgical history	Family history	Investigations and treatment done at other ART center
SPJ	None	Coital frequency twice a week; no history of coital difficulties	Nonalcoholic, nonsmoker, stress factor+	Not significant	Not significant	None

Table 3: Husband's history.

Patient's name	Age	Occupation	Addiction	Semen analysis (at our centre)
AAP	32	Interior designer	None	Volume-2 ml, sperm count-72 million/ml, motility-50% (linear progressive-45%), morphology-48%, DNA fragmentation index-excellent (10%)
PM	37	Businessman	None	Volume-2.5 ml, sperm count-112 million/ml, total motility-80% (progressive motility 60%), morphology 65%
SPJ	28	Businessman	None	Volume-2 ml, sperm count-56 million/ml, total motility-60% (progressive motility 40%), morphology-5%, DNA fragmentation index-15% excellent

Table 4: Investigations done at our center.

Patient's name	Hb	FSH	LH	AMH	PRL	TSH	Thrombophilia profile	Karyotype	USG-TVS
AAP	12	5.5	7	4.8	17	4.8	MTHFR 1298 A>C; heterozygote variant present in patient; ANA weakly positive (patient had done at other centre)	Husband-46 XY+13 ps+(normal polymorphic variant) Wife- 46 XX, 9qh+ (normal polymorphic variant)	Uterus Av 5.6×3.7 cm ET-8.6 mm RO-4×2.25×3.7 cm, AFC 26 LO-3.5×3×3 cm, AFC 30 Bilateral PCOS
PM	11	7	5	1.37	9.3	1.37	MTHFR 677 C>T, 1298 A> C heterozygote variant present in patient. protein C, protein S, S. homocysteine, antithrombin 3, LA-negative	Husband-46 XY Wife-46 XX	Day 2 USG uterus AV 7×3.8 cm ET-3 mm RO-4×2.25×3.1 cm AFC 8 LO-3.5×3×3 cm AFC 7

Continued.

Patient's name	Hb	FSH	LH	AMH	PRL	TSH	Thrombophilia profile	Karyotype	USG-TVS
							ACA-weakly positive ANA-weakly positive (advised by us due to RPL)		
SPJ	11.8	4	3	4.2	19.7	2.6	Protein C, protein S, APS profile: negative ANA-weakly positive (advised by us due to RPL)	Husband-46 X inversion Y (normal variant) Wife-46 XX	Uterus AV 6.4×3.4 cm ET-7.5 mm RO- 4×2.25×3.1 cm AFC 25 LO-3×4×3 cm AFC 30 B/L ovaries PCOS.

FSH-follicular stimulating hormone, LH-luteinizing hormone, TSH-thyroid stimulating hormone, AMH-anti-Mullerian hormone, PRL-prolactin, LA-lupus anticoagulant, ACA-anticardiolipin antibody, ET-endometrial thickness, AFC-antral follicle count, RO-right ovary, LO-left ovary, PCOS-polycystic ovarian syndrome.

Case 1

Patient AAP was advised either for HSG/diagnostic hysterolaproscopy followed by controlled ovarian stimulation+IUI (2-3 cycles) or IVF, patient directly opted for IVF.

As patient had history of two miscarriages IVF-ICSI+PGT was planned.

Patient was downregulated with tablet norethisterone 5 mg thrice daily for 7 days and called on day 2 of menstrual cycle.

Stimulation was given with antagonist protocol and recombinant FSH, GnRH agonist was used as trigger.

Investigations on day of trigger were E2-3011, LH-0.74

Total 29 oocytes were retrieved (28 M2).

HSA on day of ICSI-count 102 million/ml, motility 50% (linear progressive-45%), morphology 41%.

Total fertilized was 24, total cleaved was 24.

16 embryos were kept for blastocyst out of which 10 progressed-on 5 blastocyst PGS was done.

8 day 3 embryos and 5 blastocysts were vitrified.

PGT was performed and all blastocysts were normal and recommended for transfer.

Patient was monitored for OHSS and advised high oral intake.

HRT cycle was started (estrogen valerate 2 mg TDS f/b injection micronized progesterone P+5) and FET was done (ET 10.1 mm on day of transfer) with one PGT blastocyst-patient tested negative.

2nd FET was done after 2 months with HRT cycle (same as above).

ET on day of transfer 9.7 mm, 2 PGT blastocyst were transferred.

Patient tested positive-beta hCG was 254.

Case 2

Patient PM was advised IVF i/v/o AMA with MTHFR positive status with bad obstetric history.

Patient was downregulated with tablet norethisterone 5 mg thrice daily for 7 days and called on day 2 of menstrual cycle.

Stimulation was given with antagonist protocol and recombinant FSH, rec hCG was used as trigger.

Investigations on day of trigger was 1004-3011, LH-0.766.

Total 8 oocytes were retrieved (4 M2).

HSA on day of ICSI-count 118 million/ml, motility 55% (linear progressive-45%), morphology 17%.

Total fertilized were 4, total were cleaved 4.

4 day 3 embryos were vitrified.

Patient was planned for embryo pooling.

2nd stimulation cycle was started after downregulation with tablet norethisterone for 7 days.

Patient was stimulated with ANTAGONIST PROTOCOL and recombinant FSH+hMG (275+150 stepped up to 300+150), dual trigger was given with rec hCG and GnrH agonist (injection buserelin 0.5 mg).

Total oocytes retrieved were 12, 7 M2.

HSA on day of ICSI-count 120 million/ml, motility 52% (linear progressive-45%), morphology 22%.

Total fertilized was 6, total cleaved was 6.

All embryos were kept for blastocyst out of which 1 progressed to blastocyst on day 5.

HRT cycle was started (estrogen valerate 2 mg TDS f/b injection micronized progesterone100 mg OD (P+6) and FET was done (ET 10.1 mm on day of transfer) with two-day 6 blastocyst-patient tested positive, beta hCG 700.

Case 3

Treatment history-i/v/o RPL hysteroscopy was planned.

Findings were short septum was present in uterine cavity, resection with scissors done.

Endometrial biopsy taken and sent for TB-PCR was negative.

Patient SPJ was advised ovulation induction+timed intercourse for 1-2 cycles.

1.1 cycle of OI given with tablet letrozole-patient was tested beta hCG positive (77).

Developed bleeding PV and spontaneously aborted.

Endometrial study and uterine artery Doppler were performed which showed normal findings.

I/v/o recurrent pregnancy losses-unexplained, patient opted for IVF.

Patient was downregulated with tablet norethisterone 5 mg thrice daily for 7 days and called on day 2 of menstrual cycle.

Stimulation was given with antagonist protocol and recombinant FSH, rec hCG was used as trigger.

Investigations on day of trigger-E2-1800, LH-0.66.

Total 9 oocytes were retrieved (9 M2).

HSA on day of ICSI-count 118 million/ml, motility 55% (linear progressive-45%), morphology 17%.

Total fertilized was 8, total cleaved was 8.

Sequential fresh embryo transfer was done on D3- O2, D5-01

Beta hCG was 1309 and ectopic pregnancy in the right tube was found.

Expectant management was observed-beta hCG was followed up till it was less than 1.

HRT cycle was started with tablet estradiol 2 mg TDS f/b injection micronized progesterone 100 mg once daily for 5 days.

Frozen embryo transfer-1 grade 1 blastocyst was done.

Patient tested positive-first beta hCG 41 with rising titres, TVS USG obstetrics was suggestive of intrauterine pregnancy of 5 weeks.

Patient developed bleeding PV-beta hCG was done with showed falling titres.

Repeat TVS-USG suggestive of missed abortion with empty uterine cavity.

Patient again conceived naturally after 3 months, beta CG 3540/TVS USG obstetrics s/o single live intrauterine pregnancy of 6.6 weeks.

Patient developed bleeding PV and follow up USG showed missed abortion.

Dilation and evacuation were done.

I/v/o 5 pregnancy losses (2 missed abortions and 3 early pregnancy failures) patient was advised MTHFR gene mutation testing-she tested homozygous positive for A1298C mutation.

A decision for surrogacy was taken-a healthy baby of 3 kg delivered at term.

DISCUSSION

The occurrence of MTHFR gene mutation in Indian population had not been widely studied.

All the more its role in recurrent pregnancy losses and assisted reproductive technique outcomes was yet to be concretely established in Indian population. The data on the exact role of MTHFR mutation on Indian female patients with recurrent miscarriage/RPL had been mostly lacking.[1]

MTHFR gene polymorphisms have been shown to be associated with vascular diseases, colon cancer, in pregnancy complications such as recurrent miscarriages, malformations in fetal development and non-disjunction of chromosomes.[2]

In our case series of three patients, all three were positive for some different variant of MTHFR gene mutation. All three patients had history of recurrent pregnancy losses with other causes ruled out.

MTHFR gene mutation testing should be included after 3 missed abortions/pregnancy failures.

One patient opted for PGT and it was found that all the tested embryos in her case were euploid.

Transfer of an euploid embryo also resulted in miscarriage depicting that may be MTHFR gene mutation does play an important role in endometrial receptivity, implantation and placentogenesis. Also in case 3, there were multiple pregnancy failures in the patient and finally it was decided to go ahead with surrogacy, a full-term baby of 3 kg was born.

CONCLUSION

Our case series gives a new pathway to management of cases with recurrent pregnancy losses or recurrent IVF failures where testing for MTHFR gene mutation can open new gateways for further management strategies for a successful pregnancy outcome like PGT to rule out aneuploidy caused due to disturbance in folate metabolism as a consequence of MTHFR gene mutation and transfer of euploid embryos. Surrogacy in cases of recurrent pregnancy failures even with assisted reproductive technique. MTHFR gene mutation (both variants) and their relation to reproductive outcomes need more studies and large prospective randomized controlled trials in Indian population.

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