Original Article

Association between MTHFR 1298A>C Polymorphism with RSA and IVF Failure

Sheikhha MH PhD[1], Kalantar SM PhD[1], Ghasemi N PhD[1], Soleimanian S MSc [1]

1. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran

Received: 9June2012
Accepted: 5Agust 2012

Abstract

Background

Polymorphism A1298C of the methylenetetrahydrofolate-reductase (MTHFR) gene has been implicated in spontaneous abortion. In this study, we determined the allele and genotype frequencies of this polymorphism in recurrent spontaneous abortion (RSA) and implantation failure after in vitro fertilization (IVF).

Materials and Methods

We performed a case–control study on 60 women with RSA and 72 women with implantation failure after IVF (both of the groups have a problem in embryo implantation, so each other compare to the health group) and 60 fertile women to investigate the association between MTHFR A1298G, and pregnancy loss by polymerase chain reaction restriction fragment length polymorphism (PCR-RLFP) technique.

Results

Among the RSA patients 29 (72.5%) were heterozygote and 7 (17.5%) of them were homozygote for MTHFR mutation. In addition, 46 (63.9%) of IVF failure patients were heterozygote and the frequency of homozygote was 17 (23.6%). While in the control group 28 (56.0%) were heterozygote but none of them were homozygote. So the mutation rate of MTHFR in patients with abortion was statistically different from that in controls. Also significant difference was found in the frequencies of MTHFR between the patients and IVF failure group ($p < 0.001$).

Conclusion

Our study revealed that the genotypes of MTHFR A1298C were significantly associated with increased risk of implantation failure of abortion and IVF failure.

Keywords

Methylenetetrahydrofolate Reductase, Polymorphism; Genetic, Abortion; Spontaneous, Fertilization in Vitro

Corresponding Author
Saeede Soleimanian, Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran, G-mail: saeedesoleimanian@gmail.com.

Introduction

Recurrent spontaneous abortion (RSA) is a considerable clinical problem of infertile couples with different etiologies that may have harmful effects on the emotional and social features of their lives. In addition, one of the most frustrating problems in infertility is IVF failure - also called implantation failure. This refers to infertile patients who have undergone many IVF cycles and produced good-quality embryos, but the embryos have consistently failed to implant for unexplained reasons. One of the considerable causes of RSA and IVF failure is thrombophilia (1). There are different inherited coagulation abnormal-ities that make patients susceptible to thrombophilia (2-4). For instance, 3 mutations in the factor V gene (5), methylenetetrahydrofolate reductase (MTHFR) gene (6), and prothrombin or factor II (FII) gene were recognized in thrombophilia. MTHFR is an enzyme that catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyl-tetrahydrofolate, the major methyl supporter for the remethylation of homocysteine to methionine. Genetic variation in this gene influences suscepti-bility to occlusive vascular disease; neural tube defects, colon cancer and acute leukemia, and mutations in this gene are associated with methylen-etetrahydrofolate reductase deficiency.

The polymorphism of A1298C in MTHFR gene is located within the apparently controlling domain. This polymorphism causes the substitution of a glutamate for valine, which effects on folate concentration and a reduction in the enzyme activity that is more impressive when the poly-morphism is homozygosis (7).

MTHFR deficiency has variable symptoms, from no symptoms to severe neurological and blood vessel disease. This deficiency leading to raised levels of homocysteine in the blood or urine (8). Consequently, elevated levels of homocysteine in the blood can damage the lining of the arteries, which enhances the risk of heart disease, including coronary heart disease, and stroke in adults. However, there are many other factors that play a part in determining a person's risk of heart disease and stroke and many people with a homocysteinemia may never have any symptoms or adverse complications (9). High homocysteine levels in the blood may also increase the risk of preeclampsia, abortion, and blood clots. In general, mild to moderate homocysteinemia has been associated with an increase risk developing blood clots in the veins often in the lower leg or calf, which can travel to the lung (i.e., pulmonary embolism) (10). A reduction in the MTHFR enzyme activity requires an increased folic acid intake to keep the remethylation of homocysteine into methionine normal (11). Therefore, low folate absorptions in humans with a reduced MTHFR enzyme activity lead to an increase in the homocysteine levels and a decline in plasma methionine (12). MTHFR is located at chromosome 1p36 and at least 3 polymorphisms that cause an amino acid change have been identified, which may lead to alter MTHFR enzymatic activities. Some researchers studied the effects of MTHFR polymorphism on IVF failure and found significant relationship (13,14), while some others couldn't achieve any significant findings (15,16). More studies have been done on polymorphism of 677, but few studies have been performed on polymorphism of 1298, which had controversial results. Thus, the aim of the present study was to analyze polymorphism of A1298C of the *MTHFR* gene in women with RSA and IVF failure and compare it with the control group.

Materials and Methods

This was a case-control study that included 60 women with RSA and 70 women with implantation failure after IVF who were sequentially attending to the Yazd Research and Clinical Center for

Infertility, and were randomly selected from September 2010 to July 2011 and 60 fertile women with at least one successful pregnancy. Both of groups have a problem in embryo implantation so, each other compare to the health group. The women in the control group had no history of RSA, stillbirth, arterial hypertension, bad obstetrics history or low birth weight. The patients in case groups were matched with the controls by age. Informed permission was obtained and the study was approved by the ethics committee of Yazd Research and Clinical Center for Infertility. Two or more pregnancy losses in the first or second trimester were considered as RSA. Also more than two implantation failure after IVF was suitable for our study. We achieved medical histories, routine laboratory tests, endocrinologic examinations, performed physical examinations and immunologic tests for auto antibodies for all patients. Exclusion criteria were: anatomic abnormalities, endocrinological dysfunction, autoimmune disease, liver function abnormalities, urogenital infection and inflammatory pelvic disease.

Five ml blood samples were collected in EDTA from both patients and controls. DNA was extracted using the Bioneer DNA mini kit based on manufacture protocol. MTHFR genotypes at A1298C sites were analyzed by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method as described in previous study with some modifications (10). The primers used were 5'-CTTTGGGGAGCTGAAGGACTACTAC-3' (sense) and 5'CACTTTGTGACCATTCCGGTTTG 3' (antisense). The PCR amplification procedure was as follows; an initial denaturation at 94^{o}C for 5 min, 35 cycles of 94^{o}C for 30 s, 62^{o}C for 30 s, 72^{o}C for 30 s, and a final extension at 72^{o}C for 10 min. Then, the PCR product of 163 bp was digested at 37^{o}C for 3 h with 10 units of MboII in a reaction mixture of 20 microlitres. Finally, the digested products were distinct by the electrophoresis on an ethidium bromide-contained 3% agarose gel, and the outcome was visualized under UV light. The 1298 AA wild type homozygote produced five fragments of 56, 31, 30, 28 and 18 bp; 1298 AC heterozygote yielded six fragments of 84, 56, 31, 30, 28 and 18 bp; and 1298 CC homozygote produced four fragments of 84, 31, 30 and 18 bp (Figure 1).

For quality control ,10% of samples from both patients and controls were regenotyped by other laboratory personnel, and no discrepancy in genotyping was distinguished.

Statistical Analysis

Data was processed with SPSS 11.0 software. The mutation frequencies of *MTHFR* gene between patient groups and the control group were analyzed using the Chi-square test. p of <0.05 were considered as statistically significant.

Results

In total 72 patients with IVF failure and 60 recurrent spontaneous abortion patients, all admitted in Yazd Infertility Clinic and 62 healthy fertile women as control group were evaluated for the presence of MTHFR A1298C mutation. The mutation rate of MTHFR in patients with abortion was statistically different from that in controls. Also significant difference was observed in the frequencies of MTHFR between the patients and IVF failure group ($p<0.001$). The details about mutation frequencies are shown in Table 1.Also Allele frequencies are shown in Table 2. In the RSA patients 45 (75%) of the patients were heterozygote and 7 (11.7%) of them were homozygote for MTHFR mutation. While 46 (63.9%) of IVF failure patients were heterozygote, and the frequency of homozygote was 17 (23.6%). In the control group, 26 (43.3.0%) were heterozygote but none of them were homozygote. When allele A&C in group one compared with control group, odd

ratio was 3.49 with CI (1.9-6.14) with p<0001. In addition, allele A&C in group two (IVF failure) compared with control group, odd ratio was 4.51 with CI (2.62-7.79) with significant difference (p<0.001).

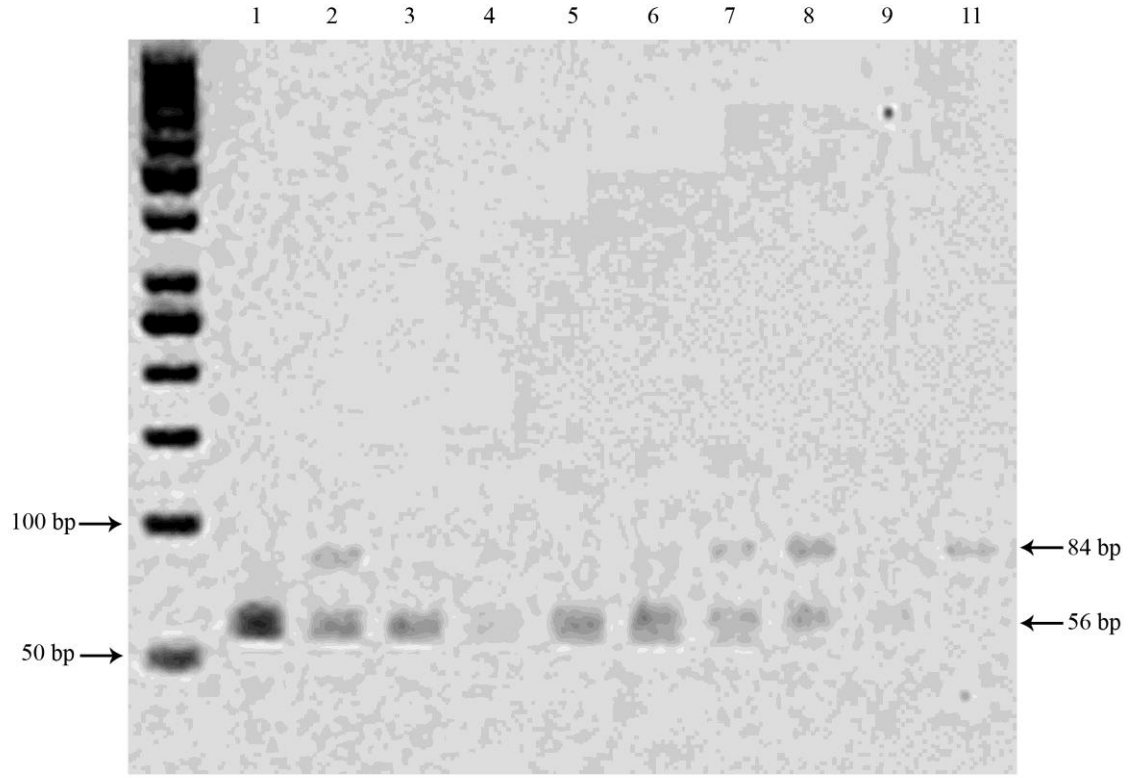

Figure 1: The 1298AA wild type homozygotes produced five fragments of 56, 31, 30, 28 an 18 bp; 1298AC heterozygotes yielded six fragments of 84, 56, 31, 30, 28 and 18 bp; and 1298CC homozygotes produced four fragments of 84, 31, 30 and 18 bp.

Table 1: Frequency of genotype of MTHFR1298A>C (heterozygous plus homozygous) in control group and patients

MTHFR 1298 Genotype	GROUP1:RSA n=60	Control n=60	GROUP2:IVF failure n=72	P Value
AA	8 (13.3%)	34 (56.7%)	9 (12.5%)	<0.001
AC	45 (75%)	26 (43.3%)	46 (63.9%)	<0.001
CC	7 (11.7%)	0 (0%)	17 (23.6%)	<0.001

RSA: recurrent spontaneous abortion, Cont: control group

Table 2: Frequency of alleles in control groups and patients(abortion;1 and IVF failure;2)

MTHFR 1298 **allele**	**RSA;1 n=120**	**CONTROL n=120**	**IVF failure;2 n=144**	**P Value**
A	61(50.8%)	94(78.3%)	64(44.4%)	<0.001
C	59(49.2%)	26(21.7%)	80(55.6%)	<0.001

Discussion

Studies in other populations have shown various results about relation between polymorphisms of MTHFR and RSA. Some studies demonstrated that decreased level of folate and also hyper-homocysteinemia and homozygosity for MTHFR 1298A>C are a risk factor for recurrent abortion and placental abruption, whereas some other studies didn't find any significant relation between this polymorphism of MTHFR and RSA (17-19). Wangs and his colleagues (2004) investigated the prevalence of polymorphism of MTHFR among the RSA patients and reported that it wasn't significantly different between RSA and control group. While genotype frequency of CC677/AA1298 in patients was significant lower than control (20). In another study Mtiraoui and his coworker (2006) analyzed these polymorphisms in RSA and found significant relationship between polymorphisms of 677and1298 of MTHFR and this problem. In another study, Cap et al (2002) investigated a relationship between thrombophilic factors and recurrent abortion and found that the prevalence of polymorphism of MTHFR (C677T) in RSA was more than control, but this difference was not significant. Overall, the discovery of the MTHFR A1298C mutation has altered the diagnostic approach of patients with thrombophilia (19-21). In the present study, our results indicated that the rate of homozygote CC (mutant type) in RSA and IVF failure is more than that of controls (11.7%, 23.6% respectively vs. 0% of control). This difference indicates that the prevalence of this polymorphism in implantation failure of the embryo is probably more common in Iran. Therefore, for helping couples to have a child, diagnosis of mutations that are involved in unexplained infertility and RSA and using an appropriate treatment plan, is necessary. It is recommended that the patients with infertility problem especially RSA to undergo screening tests for mutations related to thrombophilia. Our study is one of the few studies that has been presented concerning thrombophilia in Iranian patients with IVF failure, and more examinations should be performed for other correlated mutations in the future.

Conclusion

In conclusion, MTHFR genotypes are informative in explaining IVF failure. In addition; our results showed a skew towards higher mutation frequency of MTHFR in abortion group that may necessitate detection of such mutations in these Iranian patients. Further studies, however, examining birth outcomes and the other enzymes in the folic acid pathway are warranted.

Acknowledgment

The authors of this study expressed thanks, who participants that kindly accepted to include in the study.

Conflict of Interest

The authors have no conflict of interest.

References

1-Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. Arch Intern Med. 2004; 164(5):558-63.

2- Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: Part 1. Thromb Haemost. 1996; 76(5):651-62.

3-Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: Part 2. Thromb Haemost. 1996;76(6):824-34.

4-De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis,clinical syndromes, and management. Blood. 1996; 87(9):3531-44.

5-Vefring H, Lie RT, ØDegård R, Mansoor MA, Nilsen ST. Maternal and fetal variants of genetic thrombophilias and the risk of preeclampsia.Epidemiology.2004;15(3):317-22.

6-Wisotzkey JD, Bayliss P, Rutherford E, Bell T. Placental genotyping of the factor V Leiden, prothrombin 20210A and the methylenetetrahydrofolate reductase (MTHFR) C677T alleles in IUGR pregnancies. Thromb Haemost. 1999; 81(5):844-5.

7-Kelly Cristina de Oliveira; Bianca Bianco; Ieda T. N. Verreschi ; Alexis Dourado Guedes; Bianca Borsato Galera; Marcial Francis GaleraIII; et al .Prevalence of the Polymorphism MTHFR A1298C and not MTHFR C677T Is Related to Chromosomal Aneuploidy inBrazilian Turner Syndrome Patients: Arq Bras Endocrinol Metab 2008;53(1):1374-1381.

8-Pasińska M, Soszyńska K, Runge A, Dabrowska A, Juraszek A, Janiszewska T, et al. Molecular diagnostic tests for thrombophilia in patients referred to genetic counseling clinic because due to recurrent pregnancy failure. One center'sexperience. Ginekol Pol. 2012;83(3):178-82 .

9-Nair RR, Khanna A, Singh K. MTHFR C677T polymorphism and recurrent early pregnancy loss risk in north Indian population. Reprod Sci. 2012;19(2):210-5.

10-Settin A, Elshazli R, Salama A, ElBaz R. Methylenetetrahydrofolate reductasegene polymorphisms in Egyptian women with unexplained recurrent pregnancy loss.Genet Test Mol Biomarkers. 2011 Dec; 15(12):887-92 .

11-Bailey LB, Gregory JF 3rd.Polymorphisms of methylen etetrahydrofolate reductase and other enzymes: metabolic signifi - cance, risks , and impact on folate requirement. J Nutr. 1999; 129:919-22.

12-Melnyk S, Pogribna M, Pogribny IP, Yi P, James SJ. Measurement of plasma and intracellular S-adenosylmethionine and S-adenosylhomocysteine utilizing coulometric electrochemical detection: alterations with plasma homocysteine and pyridoxal5´-phosphate concentrations. Clin Chem. 2000; 46: 265-72.

13-Behjati R, Modarressi MH, Jeddi-Tehrani M, Dokoohaki P, Ghasemi J, Zarnani AH, et al. Thrombophilic mutations in Iranian patients with infertility and recurrent spontaneous abortion. Ann Hematol. 2006; 85(4):268-71.

14-Callejón G, Mayor-Olea A, Jiménez AJ, Gaitán MJ, Palomares AR, Martínez F,et al. Genotypes of the C677T and A1298C polymorphisms of the MTHFR gene as a cause of human spontaneous embryo loss. Hum Reprod. 2007;22(12):3249-54.

15-Chango A, Fillon-Emery N, Mircher C, Bléhaut H, Lambert D, Herbeth B,et al. No association between common polymorphisms in genes of folate and homocysteine metabolism and the risk of Down's syndrome among French mothers. Br J Nutr. 2005; 94(2):166-9.

16-Bosco P, Guéant-Rodriguez RM, Anello G, Barone C, Namour F, et al. Methionine synthase (MTR) 2756 (A --> G) polymorphism, double heterozygosity methionine synthase 2756 AG/ methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. Am J Med Genet A. 2003;121A(3):219-24.

17-van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural-tube defects? Am J Hum Genet. 1998; 62(5):1044-51.

18-Spiroski I, Kedev S, Antov S, Arsov T, Krstevska M, Dzhekova-Stojkova S,et al. Methylenetetrahydrofolate reductase (MTHFR-677and MTHFR-1298) genotypes and haplotypes and plasma homocysteine levels in patients with occlusive artery disease and deep venous thrombosis. Acta Biochim Pol. 2008; 55(3):587-94.

19-Bakker RC, Brandjes DP. Hyperhomocysteinaemia and associated disease. Pharm World Sci. 1997 Jun; 19(3):126-32.

20-Freitas AI, Mendonça I, Guerra G, Brión M, Reis RP, Carracedo A, et al. Methylenetetrahydrofolate reductase gene, homocysteine and coronary artery disease: the A1298C polymorphism does matter. Inferences from a case study (Madeira, Portugal). Thromb Res. 2008; 122(5):648-56.

21-Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: Part 1. Thromb Haemost. 1996 Nov;76(5):651-62.

22-De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. Blood. 1996;87(9):3531-44.

23-Wisotzkey JD, Bayliss P, Rutherford E, Bell T (1999) Placental genotyping of the factor V Leiden, prothrombin 20210A and the methylenetetrahydrofolate reductase (MTHFR) C677T alleles in IUGR pregnancies. Thromb Haemost 81(5):844−845.