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Methylene-Tetrahydrofolate Reductase 677 and 1298 Variations in Relation to Recurrent Abortion

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Abstract

Recurrent abortion is multifactorial involving clinical and biological risk factors. Evidence addressed the relationship of inherited thrombophilia with repeated pregnancy abortion and other serious pregnancy complications. However, the relation between thrombophilia associated gene mutations and adverse obstetric outcome is controversial and data in the literature are inconsistent. Main Purpose of this research was to exanimate the prevalence of Methylene-Tetrahydrofolate Reductase gene **(***MTHFR)* variations in association with recurrent abortion. A total of 92 samples were screened in the project: 52 women with multiple consecutive abortion and 40 normal controls. DNA of genomic was extracted from whole blood. For evaluate the presence of the both usual A1298C and C677T MTHFR gene variations in the women with recurrent abortion and controls, we employed real time polymerase chain reaction (RT-PCR). There is critical distinction in the pervasiveness of 677T/T genotype among ladies with repetitive premature birth and typical controls (P = 0.001). Consequently, the outcomes show significant difference in MTHFR C677T/A1298C genotype distribution among the healthy and women with recurrent abortion; hence, further examinations on bigger populace and other hereditary changes to more readily comprehend the molecular pathobiology of repeated pregnancy are required.

1. Introduction

Reapetead pregnancy loss is characterized as at least two continuous abortion before 20 weeks' of development. It is a multifactorial disorder like endocrine dysfunctions, uterine pathologies, hereditary disease, immune system ailments, inherited and obtained thrombophilia just as environmental variables are significant worry in gynecology (1,2,3,4). Numerous researches demonstrated that the inherited thrombophilic varieties are critical hazard factors for placental unexpectedness, stillbirth, pre-eclampsiaand fetal development limitation (5,6).

Recurrent abortion is furthermore seen as related with inherited thrombophilia that take in contrasting conditions including the thermolabile change of the 5,10-methylenetetrahydrofolate reductase (MTHFR) and the transformation is likewise connected with hyperhomocysteinemia. The compound MTHFR assumes a significant role in the folate absorption pathway, and coordinates the intracellular folate pool for association and methylation of DNA (7,8)

A number of previous study have talked about the issue that methylenetetrahydrofolate reductase gene mutations may be a hazard element for repeated pregnancy loss (1,5,8); henceforth, we explored the predominance of A1298C and C677T, two usual MTHFR gene mutations in Erbil,Iraq, to decide if these changes related with recurrent abortion. Defect in

MTHFR gene lead to diminished action of enzyme and hyperhomocysteinemia, which initiates platelet collection through advancement of endothelial oxidative harm (9). Although a few mutations inside the MTHFR gene were resolved, C677T and A1298C mutation are the two regular variations (10).

Over the most recent five years, a few examinations have assessed whether a connection between's the A1298C and C677T variations of the methylenetetrahydrofolate reductase gene and a higher danger of repetitive pregnant abortion exists (1,2). The C677T and A1298C variations of the MTHFR gene, in specific conditions, may prompt an expansion in plasma homocysteine (Hcy) and homocysteineemia, which can cause endothelial harm in veins (3). This may build thromboembolic chance, which in pregnant ladies can prompt a hindrance of the placental vessels bringing about repetitive premature births. In this way, hyperhomocysteinemia is viewed as a hazard factor for intermittent pregnancy abortion, and patients with recurrent abortion may show hyperhomocysteinemia; hence, as a major aspect of normal registration for recurrent abortion, serum homocysteine ought to be estimated. Once analyzed, treatment of hyperhomocysteinemia with folic corrosive and nutrient B12 can uniquely diminish homocysteine levels (3).

assortments in MTHFR gene lead to diminished activity of protein and hyperhomocysteinemia, which incites platelet amassing through headway of endothelial oxidative mischief (9). In spite of the fact that a couple of assortments inside the MTHFR genes were resolved, C677T and A1298C changes are the two regular mutations (10). C677T variety is a missense transformation in the exon 4 of this gene, which changes over an alanine to a valine codon (at codon 222) in the N-terminal reactant space of the protein inciting a thermolabile protein, with reduced enzymatic development (11). The second change is MTHFR A1298C, that is, relateed with decreased development of enzyme, however not with thermolability. A1298C transversion is a point variety in exon 7, propertied by a glutamate to alanine substitution (at codon 429) inside the C-terminal regulatory space of the protein (12).

The C677T and A1298C variations of the MTHFR gene, in specific conditions, may prompt an expansion in plasma homocysteine (Hcy) and homocysteineemia, which can cause endothelial harm in veins (3). This may build thromboembolic chance, which in pregnant ladies can prompt a hindrance of the placental vessels bringing about repetitive premature births. In this way, hyperhomocysteinemia is viewed as a hazard factor for intermittent pregnancy abortion, and patients with recurrent abortion may show hyperhomocysteinemia.

In the current project, which investigates the possible role of the MTHFR 677 and 1298 gene in women with recurrent abortion, we aimed to investigate the probable mutation in recurrent abortion patients by monitoring real-time polymerase chain reaction technique. We explored the role of MTHFR gene from 52 women with recurrent abortion and 40 normal controls, and differentiate the outcomes of samples in Erbil province, Iraq.

2. MATERIAL AND METHODS

2.1. Patients

The samples were collected from the Zheen International Hospital in Erbil, Iraq. A total of 92 samples were analyzed. The study included 52 recurrent abortion samples and 40 healthy control samples. The blood samples of the recurrent abortion stored at -20˚C until nucleic acid extraction.

2.2. DNA extraction

DNA samples from peripheral blood were extracted employed a company extraction kit based on the manufacturer's protocols; DNA obtained by AccuPrep Genomic DNA Extraction Kit (Bioneer, Korea). Quantification and qualification of DNA concentration was performed utilizing NanoDrop (ND- 1000, USA).

2. 3. PCR Optimization

A DNA source was utilized to do the gradient PCR for each primer pairs. Enhancement (Amplification) was performed in a Master-cycler ace PCR System (Eppendorf, German). The assurance of the ideal annealing temperature of all primers were depended on the aftereffect of agarose gel electrophoresis. The utilized blend for gradient PCR is demonstrated in the Table 4.3.

Table 4.1. The components of PCR reaction and their quantities in 25µL total volume.

Chemical Substances	**Quantity (µL)**
dH_2O	14.875µL
10X PCR buffer Ammonium sulfate $(NH_4)2SO_4$	2.5µL
25 mM $MgCl_2$	2µL
2 mM dNTP	1.5µL
20 mM Forward primer	1µL
20 mM Revers primer	1µL
5 U/ML Taq DNA polimerase	0,125 µL
cDNA template	2µL
Mixture	**25µL**

The thermocycling program was set to run 35 cycles according to the following parameters as showed in Table 4.2.

Table 4.2 Conditions of gradient PCR reaction.

Step	**Temperature (**°C)	**Time**
1 . Pre denaturation at	95	3 m
2 . Denaturation at	95	30 s
3 . annealing	55 - 60	30 s
4 .Extension	72	30 s
	35 cycles	
5 . Final extension	72 for	2 m
Hold	4	0

The final products were analyzed by 2.0% agarose gel electrophoresis and stained with ethidium bromide. The gel was run at 100 volts for 45 minutes. The DNA fragments were illuminated by UV-light.

2.4. Agarose gel electrophoresis

Electrophoresis is a procedure that utilizations distinctive electrical charges to seperate the molecule in a blend and some of the time purge macromolecules particularly proteins and nucleic acids. DNA molecules have negative charges. They are set in an electric field they move toward the positive post. The rate of relocation of a particle depended on two factors, its shapes and charges to mass proportion.

In this investigation, agarose gel electrophoresis was utilized for primer optimization reason. The examples were kept running in 2% agarose gel and recolored with an intensify that makes the DNA noticeable under UV light. Ethidium bromide (EtBr) is routinely used to recolor DNA in agarose gel. Our DNA tests were electrophoresed under electric field at 100 volts as long as 45 minutes. The length of the PCR items runs between 236- 300bp.

2.5. Real-time RT-PCR

DNA was amplified by real-time polymerase chain reaction (RT-PCR) and utilized the MTHFR mutation primers were designed by BLAST online tool (Table 1). Real-time PCR was performed using Rotor-Gene 6000 Real-Time PCR Machine (Qiagen GmbH, Hilden, Germany) with RT² SYBR Green ROX FAST Mastermix (Qiagen GmbH, Hilden, Germany) for *MTHFR* 677 and 1298.

Table 4.3. Sequence, PCR product size and annealing temperature of utilized primers.

Primer	Sequence	PCR product (bp)	Annealing temperature (˚C)
MTHFR677			
Forward	5'-AGGCCTGCTGAAAATGACTGAA -3'	221	55
Reverse	5'-AAAGAATGGTCCTGCACCAG -3'		
MTHFR1298			
Forward	5'-GCCTGCTGAAAATGACTGAAT -3'	195	55.5
Reverse	5'-TTGTGGACGAATATGATCCAACA -3'		

3. Result

Rresults of *MTHFR* gene in these 92 samples that obtained by Real-Time PCR. MTHFR mutations was indicated in figure 4.1.

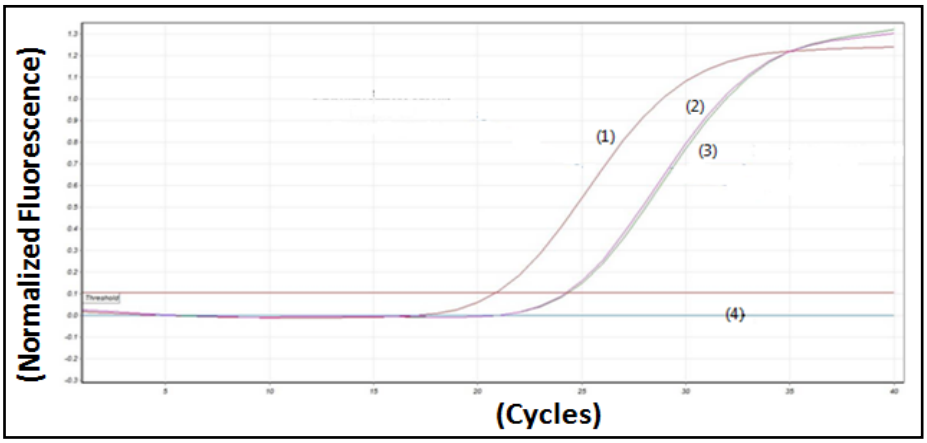

Figure 3. Normalized graph of high-resolution melting analysis conation normal and mutant alleles. (1) Mutant allele in patient, (2) Normal allele in patient, (3) Mutant allele in normal and (4) Normal allele in normal.

There is significant distinction in the predominance of 677T/T genotype among ladies with repetitive premature birth and normals. The outcomes demonstrate critical distinction in MTHFR C677T/A1298C genotype dissemination among the two gatherings. Statistically was significant (p = 0.0001, T-test; p > 0,05). The statistical result of MTHFR mutatio of both normal controls and recurrent abortion are shown in Figure 4.2.

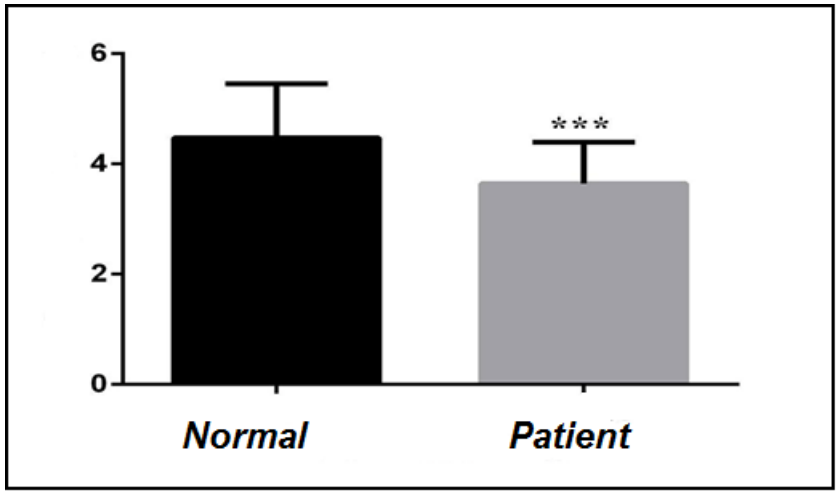

Figure 4.2. Statistic result of MTHFR 677 and 1298 gene mutation.

4. Discussion

we explored the relationship of MTHFR A1298C and C677T variations and hyper-homocysteinemia in ladies with recurrent abortion (11). As normal and patients were coordinated for a few hazard elements (age, smoking, stoutness and utilization of oral contraceptives), results got demonstrated that AP patients had a higher commonness of usual MTHFR variations as contrasted and generally normal parous women. The allele recurrence of MTHFR A1298C and C677T variations among control primigravid women was like that of healthy Tunisians, in this manner precluding likelihood predisposition in normal determination, and was similar with rates built up for European people group. In perspective on their essence among control women, and as not all MTHFR C677T and A1298C freak genotype bearers experienced APs, this showed the coordinated support of numerous non-inherited and inherited prothrombotic deformities set ladies at most serious hazard (12)

Several pieces of review have talked about the issue that methylene-tetrahydrofolate reductase gene mutations may be a hazard factor for repeated pregnancy loss (1,5,8); henceforth, we explored the predominance of C677T and A1298C, two common methylene-tetrahydrofolate reductase gene mutations in Erbil,Iraq, to decide if these changes related with recurrent abortion. Defect in MTHFR gene lead to diminished action of enzyme and hyperhomocysteinemia, which initiates platelet collection through advancement of endothelial oxidative harm (9). Although a few mutations inside the MTHFR gene were resolved, C677T and A1298C mutation are the two regular variations (10).

Inherited thrombophilia alludes to inborn conditions, generally genetic, that expansion the inclination to create venous thrombosis (VTE). The most successive reasons for an inherited hypercoagulable state are factor V Leiden transformation and prothrombin gene change, which together include half to 60% of cases in some populaces. Imperfections in protein S, protein C and antithrombin account for a large portion of the rest of the cases. Homozygosity for methylene-tetrahydrofolate reductase (MTHFR) polymorphisms (C677T, 1298C) is a moderately normal reason for somewhat raised plasma homocysteine levels (hyperhomocysteinemia) and expanded danger of thrombosis.

MTHFR variatin and its relationship to repeated pregnancy loss have additionally been concentrated in Iraqi population. MTHFR C677T polymorphism indicated practically 4.7-overlap expanded hazard of recurrent abortion in the Erbil population of north Iraq. Mutant genotypes (TT/CT) could have a huge effect on the metabolic phenotype as a result of extremely low folate and high homocysteine level in the Iraqi population (8). Selective preferred position of MTHFR C allele may be physiologically defensive and nearness of T allele would put the person at metabolic hazard in an Iraqi population (8). MTHFR CC genotype may support ordinary placentation and vessel improvement which is vital for legitimate trade of supplement from mother to baby (7). Ill advised maternal–fetal trade in patients with MTHFR TT and CT mutant genotypes on account of traded off angiogenesis, ill-advised placentation, and vessel advancement may at last lead to fetal abortion. It was additionally found in animal model investigation that supplementation of folic corrosive during the early trimester of pregnancy expanded the survival of embryo and furthermore the quantity of fetus (11) Hence, in patients with TT/CT genotype, the folic corrosive supplementation may improve effective pregnancy result.

Consequently, the outcomes demonstrate significant distinction in methylene-tetrahydrofolate reductase C677T/A1298C genotype distribution among the two groups; in this way, further investigations on bigger populace and other hereditary changes to all the more likely comprehend the molecular pathobiology of recurrent abortion are required.

References

1. Alijotas-Reig J, Garrido-Gimenez C. Current concepts and new trends in the diagnosis and management of recurrent miscarriage. Obstet Gynecol Surv.
2. 2013;68(6):445–66. 2. Ural ÜM, Tekin YB, Balik G, Şahin FK, Çolak S. Could platelet distribution width be a predictive marker for unexplained recurrent miscarriage? Arch Gynecol Obstet. 2014;290(2):233–6
3. Makino A, Nakanishi T, Sugiura-Ogasawara M, Ozaki Y, Suzumori N, Suzumori K. No association of C677T methylenetetrahydrofolate reductase and an endothelial nitric oxide synthase polymorphism with recurrent pregnancy loss. Am J Reprod Immunol. 2004;52(1):60–6.
4. Vanilla S, Dayanand C, Kotur PF, Kutty MA, Vegi PK. Evidence of paternal N5, N10-methylenetetrahydrofolate reductase (MTHFR) C677T gene polymorphism in couples with recurrent spontaneous abortions (RSAs) in Kolar District—a South West of India. J Clin Diagn Res JCDR. 2015;9(2):BC15.
5. E. Trabetti, "Homocysteine, MTHFR gene polymorphisms, and cardio-cerebrovascular risk," Journal of Applied Genetics, vol. 49, no. 3, pp. 267–282, 2008.
6. Brenner B, Sarig G, Weiner Z, Younis J, Blumenfeld Z, Lanir N. Thrombophilic polymorphisms are common in women with fetal loss without apparent cause. Thromb Haemost. 1999;82(1):6–9.
7. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci. 2001;22(4):195–201.
8. N. Mtiraoui, W. Zammiti, L. Ghazouani et al., "Methylenetetrahydrofolate reductase C677T and A1298C polymorphism and changes in homocysteine concentrations in women with idiopathic recurrent pregnancy losses," Reproduction, vol. 131, no. 2, pp. 395–401, 2006.
9. G. Unfried, A. Griesmacher, W. Weism¨uller, F. Nagele, J. C. Huber, and C. B. Tempfer, "The C677T polymorphism of the methylenetetrahydrofolate reductase gene and idiopathic recurrent miscarriage," Obstetrics and Gynecology, vol. 99, no. 4, pp. 614–619, 2002.
10. C. V. Ananth, M. R. Peltier, C. De Marco et al., "Associations between 2 polymorphisms in the methylenetetrahydrofolate reductase gene and placental abruption," American Journal of Obstetrics and Gynecology, vol. 197, no. 4, p. 385, 2007.
11. Van der Molen EF, Arends GE, Nelen WL, van der Put NJ, Heil SG, Eskes TK, Blom HJ. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene as a new risk factor for placental vasculopathy. Am J Obstet Gynecol. 2000;182(5):1258–63.
12. Tiwari D, Bose PD, Das S, Das CR, Datta R, Bose S. MTHFR (C677T) polymorphism and PR (PROGINS) mutation as genetic factors for preterm delivery, fetal death and low birth weight: a Northeast Indian population based study. Meta Gene. 2015;3:31–42.