J Surg Med. 2020;4(7):536-539.
DOI: 10.28982/josam.768231

Research article
Araştırma makalesi

Journal of Surgery and Medicine
e-ISSN=2602-2079

Pregnancy outcomes in patients with MTHFR gene polymorphism: A case series

MTHFR gen polimorfizmi olan hastalarda gebelik sonuçları: Vaka serisi

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Abstract

Aim: Based on the literature, MTHFR polymorphism is common among the general population and is controversial in terms of treatment, as it is poorly associated with pregnancy complications. In this study, we aimed to investigate the relationship between treatment and pregnancy outcomes in patients with MTHFR polymorphism.

Methods: The data of 48 patients who were diagnosed with MTHFR C677T and A1298C polymorphism between June 2012 and April 2020 and followed up during their pregnancy were reviewed retrospectively. Demographic and clinical features of patients, pregnancy history, diagnosis, and perinatal complications were examined. Pre- and post-treatment clinical features of the patients were compared.

Results: Comparison of pre- and post-treatment pregnancy data of the patients revealed that live birth rates were significantly higher (pre-treatment: 9.4% post-treatment: 68.7%, *P*=0.001) and abortion rates were significantly lower after treatment (pre-treatment: 81.2%, post-treatment: 32.1%, *P*=0.001). Pregnancy complications were observed in 9 (18.3%) patients. It was observed that among patients with MTHFR gene mutation, live birth rates increased by 24.12-fold and by 3.76-fold for each year of decrease in the age of conception following treatment.

Conclusion: In pregnant women with MTHFR polymorphism, methionine-poor diet and medical treatment had a positive effect on pregnancy outcomes. It was also observed that among those with MTHFR gene polymorphism, young patients with MTHFR A1298C heterozygotes had the best treatment results.

Keywords: MTHFR C677T, MTHFR A1298C, Treatment, In-vitro fertilization, Abortion, Complication

Öz

Amaç: Literatürde MTHFR polimorfizminin genel popülasyonda sık görüldüğü, gebelik komplikasyonları ile zayıf ilişkili olduğu için tedavi konusunda tartışmalı olarak görülmektedir. Bu çalışmada MTHFR polimorfizmi olan hastalara tedavi ile gebelik sonuçlarının ilişkisinin araştırılması amaçlanmıştır

Yöntemler: Haziran 2012-Nisan 2020 tarihleri arasında *MTHFR C677T* ve *A1298C* polimorfizmi tanısı olan, gebeliği boyunca takip edilen ve tedavi uygulanan 48 hastanın verileri geriye dönük olarak taranmıştır. Hastaların demografik ve klinik özellikleri; demografik özellikleri, gebelik öyküsü, tanı perinatal komplikasyonlar incelenmiştir. Hastaların tedavi öncesi ve sonrası verileri karşılaştırılmıştır.

Bulgular: Hastaların uygulanan tedavi öncesi ve sonrası gebelik verileri karşılaştırıldığında canlı doğum oranlarını tedavi sonrasında anlamlı yüksek olduğu(tedavi öncesi: %9,4, sonrası: %68,7, *P*=0,001), abort oranlarının ise tedavi sonrasında anlamlı düşük olduğu gözlenmiştir(tedavi öncesi: %81,2, sonrası: %32,1, *P*=0,001). Hastaların toplam 9'unda (%18,3) gebelik komplikasyonu gözlenmiştir. *MTHFR* gen mutasyonu olan hastalardan tedavi sonrasında *MTHFR A1298C* heterozigot olanların 24,12 kat, gebe kalma yaşında her bir azalma için 3,76 kat gebeliğin canlı doğum ile sonuçlandığı gözlenmiştir.

Sonuç: MTHFR polimorfizmi olan gebelerde metiyoninden fakir diyet ve medikal tedavinin gebelik sonuçlarına olumlu etki ettiği kanaatine varılmıştır. Aynı zamanda MTHFR gen polimofizmleri içinden MTHFR A1298C heterozigot olan genç hastaların en iyi tedavi sonuçlarına sahip olduğu gözlenmiştir.

Anahtar kelimeler: MTHFR C677T, MTHFR A1298C, Tedavi, İn-vitro fertilizasyon, Abortus, Komplikasyon

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Ethics Committee Approval: The study protocol was approved by the Clinical Research Ethics Committee of Alanya Alaaddin Keykubat University, Faculty of Medicine (Date: 05/06/2020, No: 19-23). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.
Etik Kurul Onayı: Çalışma protokolü Alanya Alaaddin Keykubat Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu tarafından onaylanmıştır (Tarih: 05/06/2020, No: 19-23). İnsan katılımcıların katıldığı çalışmalardaki tüm prosedürler, 1964 Helsinki Deklarasyonu ve daha sonra yapılan değişiklikler uyarınca gerçekleştirilmiştir.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Published: 7/30/2020
Yayın Tarihi: 30.07.2020

How to cite/Atıf için: Deniz A. Pregnancy outcomes in patients with MTHFR gene polymorphism: A case series. J Surg Med. 2020;4(7):536-539.

Introduction

Methylenetetrahydrofolate reductase (*MTHFR*) is one of the enzymes involved in the amino acid metabolism and catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a substrate for homocysteine remethylation to methionine [1]. The *MTHFR C677T* and *A1298C* single-nucleotide polymorphisms of the *MTHFR* gene is common in the general population and their prevalence varies among populations [2]. These common mutations of the *MTHFR* result in a thermolabile variant of the enzyme with reduced activity in elevated temperatures, leading to increased homocysteine levels [3].

The increase in the homocysteine levels may cause impairment in the vascular smooth muscle cells and endothelium, disrupting the coagulation cascade and promoting thrombosis [4]. In addition, it induces a rapid autooxidation process, producing free radicals. Hyperhomocysteinemia becomes evident in the presence of reduced *MTHFR* enzyme activity, or folate, vitamin B6 and B12 deficiencies [5]. Folate deficiency causes cell cycle arrest in the S phase and an uracil misincorporation into deoxyribonucleic acid (DNA), leading to DNA double-strand breaks [6].

Recent studies have demonstrated that the *MTHFR C677T* and *A1298C* polymorphisms are common in women with recurrent pregnancy loss and the *MTHFR* gene structure has effects on fetal growth [7]. Also, folic acid supplementation before and during pregnancy with methionine-restricted diet has been shown to be beneficial to prevent pregnancy complications [8-10].

In the literature, there is a limited number of studies showing an association between the *MTHFR* gene polymorphism and pregnancy complications and its treatment is still controversial [11-12]. In the present study, we aimed to investigate the possible relationship between *MTHFR* gene polymorphism and pregnancy outcomes.

Materials and methods

This study was conducted at a private Obstetrics and Gynecology clinic between June 2012 and April 2018. A written informed consent was obtained from each patient. The study protocol was approved by the Clinical Research Ethics Committee of Alanya Alaaddin Keykubat University, Faculty of Medicine (Date: 05/06/2020-No. 19-23), and it was conducted in accordance with the principles of the Declaration of Helsinki.

Study design and study population

In this single-center, retrospective case series study, medical data of pregnant women were reviewed and those who were diagnosed with *MTHFR C677T* and *A1298C* polymorphism were included. The *MTHFR* gene polymorphism analyses were performed for myriad reasons (miscarriages, preeclampsia, birth defects, family history etc.) using the real-time polymerase chain reaction (PCR) method. Patients with antithrombin III, protein C, or protein S deficiency and factor V Leiden, prothrombin *20210A* mutations, autoimmune disorders or severe systemic diseases which could affect the study results were excluded from the study. Finally, a total of 48 patients with *MTHFR C677T* and *A1298C* polymorphism were included in the study.

All patients underwent a thorough examination and treatment before planning the next pregnancy. After diagnosis for MTHFR mutations, at least three months before the next pregnancy, methionine-restricted diet was initiated with folic acid supplementation at a dose of 5 mg twice a week for patients with *MTHFR* gene polymorphisms. Once the pregnancy was confirmed, treatment was modified as methionine-restricted diet, folic acid (5 mg twice a week), enoxaparin sodium 40 mg/day, vitamin B1, B2, B6, B9, and B12 throughout pregnancy and acetylsalicylic acid 100 mg/day until the 36th weeks of pregnancy. Pre-treatment and post-treatment clinical features of the patients were compared.

Demographic and clinical characteristics of the patients including age, parity and gravida, history of pregnancy, laboratory test results, gestational age, birth weight, perinatal complications such as preeclampsia, fetal growth restriction (FGR), premature rupture of membranes (PROM), placental abruption, and stillbirth were noted.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows version 21.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in median (min-max) or number and frequency, where applicable. Conformity of the variables to normal distribution was assessed visually (histogram and possibility graphs) and with analytical methods using the Kolmogorov–Smirnov/Shapiro–Wilks tests. For paired nominal data, the McNemar test was used for the comparison of the groups. Logistic regression analysis was carried out by the retrospective elimination method to the groups with stillbirth using genetic and clinical data. A *P*-value of <0.05 was considered statistically significant.

Results

The median age of the patients was 32.5 (range 23 to 41) years. A total of 54.2% of the patients were treated with *in vitro* fertilization (IVF). The most common *MTHFR* gene mutations were heterozygous *MTHFR C677T* in 62.2% (n=30) and heterozygous *MTHFR* A1298C in %47.9 (n=23). Demographic and clinical characteristics of the patients are presented in Table 1.

Table 1: Baseline demographic and clinical characteristics of patients

		Median(min-max) (n=48)
Age, years		32.5 (23-41)
Duration of marriage		9.5 (3-14)
Spontaneous pregnancy		45.8% (n=22)
IVF pregnancy		54.2% (n=26)
Follow-up, months		16.0% (8-32)
MTHFR C677T	Homozygous	31.1% (n=15)
	Heterozygous	62.2% (n=30)
MTHFR A1298C	Homozygous	2.1% (n=1)
	Heterozygous	47.9% (n=23)

Data are given in number and percentage or median (min-max), unless otherwise stated. IVF: in vitro fertilization, MTHFR: methylenetetrahydrofolate reductase

The number of parity and live births was significantly higher (*P*=0.001) and abortion rates were significantly lower after the treatment compared to pre-treatment rates (*P*=0.001). A comparison of pre- and post-treatment values are summarized in Table 2.

Nine (18.3%) patients had pregnancy complications. After the treatment, the rates of preeclampsia and FGR were both 8.4%. Post-treatment pregnancy complications are shown in Table 3.

The logistic regression analysis was used to predict risk factors for live birth in patients with *MTHFR* gene mutation. The analysis results were statistically significant (χ^2: 12.151, P=0.011) which explained 42.6% of all cases. The likelihood of live birth increased by 24.12 folds after treatment in patients with heterozygous *MTHFR A1298C* gene polymorphism. In addition, each decline in the maternal age resulted in a 3.76-fold increase in live births after treatment in these patients. In the present study, the highest and lowest maternal age were 41 and 23 years, respectively. The logistic regression analysis results are summarized in Table 4.

Table 2: Pre- and post-treatment values

(n=48)	Before treatment		After treatment		*P*-value*
	% (n)	Median (min-max)	% (n)	Median (min-max)	
Gravida	85.4 (44)	2.00 (0-9)	97.9 (47)	2.00 (0-4)	0.410
Parity	18.8 (9)	0.00 (0-1)	70.8 (34)	1.0 (0-2)	0.001
Abortion	81.2 (39)	2.00 (0-8)	32.1 (17)	1.0 (0-2)	0.001
Live birth	9.4 (5)	0.00 (0-1)	68.7 (33)	0.0 (0-2)	0.001

Data are given in number and percentage or median (min-max), unless otherwise stated. McNemar test was used for statistical analysis.

Table 3: Pregnancy complications after the treatment

Variable	% (n)
Preeclampsia	8.4 (4)
FGR	8.4 (4)
PROM	4.2 (2)
Oligohydramnios	4.2 (2)
Intrauterine death	2.1 (1)

Data are given in number and percentage, unless otherwise stated. FGR: fetal growth restriction, PROM: premature rupture of membranes

Table 4: Logistic regression analysis results for the factors predicting live birth

Variable*	χ^2	R^2	*P*-value	OR	95%CI
	12.151	0.426	0.011		
MTHFR A1298C (heterozygous)			0.019	24.12	2.30-105.3
Age (annual decrease)			0.021	3.76	1.21-8.40

* Only included variables are shown. OR: odds ratio, CI: confidence interval, MTHFR: methylenetetrahydrofolate reductase

Discussion

In this study, 48 patients diagnosed with *MTHFR C677T* and *A1298C* polymorphism were evaluated and the most common *MTHFR* gene mutations were heterozygous *MTHFR C677T* and heterozygous *MTHFR A1298C* mutations. The live birth rates were significantly higher and abortion rates were significantly lower after treatment. Pregnancy complications were observed in nine patients. The likelihood of live birth increased by 24.12-fold after treatment in patients with heterozygous *MTHFR A1298C* gene polymorphism. In addition, each decline in the maternal age resulted in a 3.76-fold increase in live births after treatment in these patients. In this study, the highest maternal age was 41 years and the lowest maternal age was 23 years.

In a study, Turgal et al. [10] employed a similar treatment protocol before and after pregnancy in 617 patients with *MTHFR* gene polymorphism. The authors classified the patients according to subtypes of *MTHFR* gene polymorphism and compared them with a control group without *MTHFR* gene polymorphism. They found no statistically significant difference in the gestational weeks at birth among the groups. However, similar to this study, the abortion rates significantly decreased in patients with *MTHFR* gene polymorphism from 40% at baseline to 10.8% at the end of treatment, indicating nearly four-fold decrease. In addition, increased severity of *MTHFR* gene polymorphism was associated with increased abortion and perinatal mortality rates and decreased term delivery rates. In another recent study including 121 patients, the effect of low-molecular-weight heparin (LMWH) on obstetric outcomes of recurrent miscarriage patients complicated with *MTHFR* gene polymorphism was investigated. The patients were divided into two groups as those receiving only folic acid and iron and those receiving folic acid, iron, and prophylactic doses of enoxaparin sodium. The live birth rate was higher in patients receiving enoxaparin sodium. Similarly, Brenner et al. [13] examined the efficacy and safety of enoxaparin sodium at a dose of 40 to 80 mg/day and enoxaparin resulted in live birth in 46 (75%) of 61 patients.

In the current study, pregnancy complications were observed in nine patients. In the study of Turgal et al. [10], the most common pregnancy complications included FGR, oligohydramnios, PROM, preeclampsia, and placental abruption. However, the rate of these complications was lower than previous pregnancies and there was no significant difference in the complication rate between the patients with and without *MTHFR* gene polymorphism. In another study, no significant differences were observed in the rates of stillbirth, preterm delivery, chorioamnionitis, preeclampsia, and FGR between the patients receiving and not receiving enoxaparin sodium during pregnancy [14]. Of note, some authors concluded that it was not necessary to add LMWH to the routine treatment regimen to prevent pregnancy complications [15]. In a meta-analysis including 3,559 cases with unexplained recurrent pregnancy loss and 5,097 healthy controls, *MTHFR C677T* gene polymorphism, but not *MTHFRA1298C* gene polymorphism, was found to be associated with preeclampsia [16]. In a study conducted in Denmark with 91,661 pregnant women, the *MTHFR C677T* gene polymorphism increased the risk of preeclampsia by 1.27-fold; however, there was no significant increase in the rate of other pregnancy complications [17].

Review of the literature reveals that the most favorable results for predicting live birth can be obtained in the presence of heterozygous *MTHFR A1298C* gene polymorphism (odds ratio: 3.76 per year). Turgal et al. [10] found significant differences in the perinatal complications between the previous pregnancies of the patients in the homozygous polymorphism, heterozygous polymorphism, and healthy control groups. The patients with *MTHFR C677T* gene polymorphism had a significantly higher abortion rate than those with *MTHFR A129C* gene polymorphism (56.9% *vs.* 44.8%, respectively; P=0.039). In another study including 439 pregnant women, the correlation between the *MTHFR C677T* gene polymorphism and IVF outcomes was investigated [18]. The presence of heterozygous *MTHFR C677T* gene polymorphism was associated with an improved embryo quality and increased likelihood of pregnancy than homozygous genotypes. Similarly, Ahangari et al. [19] evaluated the possible association between the *MTHFR C677T*, *A1298C*, *F2G20210A*, and *F5G1691A* genetic variants in Iranian women with recurrent miscarriage and found that the *MTHFR C677Tm* and *A129C* gene polymorphisms increased the recurrent pregnancy loss risk by 5.5 fold and 3.3 fold, respectively. In another case-control study conducted in India, recurrent early pregnancy loss was evaluated among 106 patients with the history of three or more recurrent early pregnancy loss and 140 healthy fertile controls with successful pregnancy outcomes [20]. The homozygous and heterozygous *MTHFR C677T* gene polymorphisms were associated with 6.30-fold and 1.96-fold

increased risks of idiopathic recurrent early pregnancy loss. Although the link between the *MTHFR* gene polymorphism and age in predicting live birth has not been clearly understood yet, some authors have proposed that advanced age is associated with reduced live birth rates [21]. In particular, age is of utmost importance in pregnancies in which IVF is used. The live birth rate has been reported as 43.7% in pregnancies in which IVF is used in the first cycle before 30 years of age; however, this rate tends to decrease to 10.7% in women aged 40 to 44 years [22].

Limitations

There are some limitations to our study. The single-center and retrospective design of the study with a relatively small sample size are the main limitations. Despite this limitation, the findings of this study are important because MTHFR polymorphisms are potential risk factors for negative pregnancy outcomes, and our findings contribute to the literature, which is controversial in terms of treatment.

Conclusion

These study results suggest that methionine-restricted diet, folic acid (5 mg twice a week), enoxaparin sodium 40 mg/day, acetylsalicylic acid 100 mg/day, vitamin B1, B2, B6, B9, and B12 throughout pregnancy exert positive effects on pregnancy outcomes in patients with *MTHFR* gene polymorphisms. In addition, the most satisfactory results can be achieved in younger patients with *MTHFR A1298C* heterozygous polymorphism. However, further large-scale, prospective studies are needed to establish a definite conclusion.

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