Medicinski podmladak

Medical Youth

ORIGINAL ARTICLE

INFLUENCE OF METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM C677T ON THE RISK OF RECURRENT SPONTANEOUS ABORTIONS

UTICAJ POLIMORFIZMA C677T GENA ZA METILEN-TETRAHIDROFOLAT REDUKTAZU NA NASTANAK SPONTANIH POBAČAJA

Miloš Svirčev[1], Nela Maksimović[1]

[1] University of Belgrade, School of Medicine, Institute of Biology and Human Genetics, Belgrade, Serbia

Correspondence: milossvircev92@gmail.com

Abstract

Introduction: Spontaneous abortion (SA) is a frequent obstetric complication. Up to 15% of pregnancies end by spontaneous abortion in the first trimester. Five percent of women have two, and 1-2% of women have more than three abortions. The risk of spontaneous abortion is influenced by variety of genetic and environmental factors. Enzyme methylenetetrahydrofolate reductase (MTHFR) is primarily involved in homocysteine remethylation to the methionine. Gene polymorphism C677T in the MTHFR gene causes enzyme thermolability which leads to hyperhomocysteinemia, that might increase the risk of developing placental thrombosis and spontaneous abortion.

Aim: The aim of our study was to determine whether the MTHFR C677T polymorphism is associated with the increased risk of SA.

Material and methods: This study included 157 women who had at least two unexplained spontaneous abortions and 135 healthy women with at least one child, without previous SA. Detection of MTHFR C677T polymorphism genotypes was performed by PCR-RFLPs method. Investigation of differences between genotype and allele frequencies, in the group of women with SA and the control group, was completed using the $\chi 2$ test.

Results: In the group of patients with recurrent spontaneous abortions, 58 of them had CC genotype (36.94%), 74 had CT genotype (47.13%), and 25 had TT genotype (15.92%). In the control group, 59 of them had CC genotype (43.07%), 55 had CT genotype (40.74%), and 21 had TT genotype (15.56%). The frequency of C allele in the group of patients was 60.51%, and T allele 39.49%; while the frequencies in the control group were 64.07% and 35.93%, respectively. The difference in the genotype (p=0.470) and allele (p=0.393) frequency between the two groups of patients was statistically insignificant.

Conclusion: Our results do not show any influence of the analyzed polymorphism on SA.

Key words:
MTHFR C677T polymorphism, spontaneous abortion

The authors declare no conflicts of interest.

doi:10.5937/mp69-15694
Editorial board: podmladak.med.bg@gmail.com

Sažetak

Uvod: Spontani pobačaj predstavlja čestu komplikaciju u akušerstvu. Oko 15% trudnoća završi se spontanim pobačajem u prvom trimestru. Kod 5% žena javlja se dva, a kod 1-2% više od tri spontana pobačaja. Uzroci spontanih pobačaja mogu da budu brojni genetički faktori i faktori spoljašnje sredine. Enzim metilen-tetrahidrofolat reduktaza (MTHFR) učestvuje u remetilaciji homocisteina do metionina. Polimorfizam C677T u genu za MTHFR dovodi do nastanka termolabilne forme enzima i pada enzimske aktivnosti, što za posledicu ima hiperhomocisteinemiju koja može da predstavlja faktor rizika za nastanak tromboze placente, a time i spontanog pobačaja.

Cilj: Cilj naše studije je da utvrdimo da li polimorfizam C677T u genu za MTHFR predstavlja faktor rizika za nastanak spontanih pobačaja.

Materijal i metode: Naša studija je obuhvatila 157 pacijentkinja koje su imale dva ili više neobjašnjenih spontanih pobačaja i 135 zdravih žena sa najmanje jednim detetom, koje nisu imale nijedan pobačaj. Polimorfizam C677T gena za MTHFR otkriven je uz pomoć metode polimorfizma dužine restrikcionog trajanja lančane reakcije polimeraze-PCR-RFLP metode. Razlika između učestalosti genotipova i alela u grupi žena sa spontanim pobačajima i u kontrolnoj grupi ispitivana je $\chi 2$ testom.

Rezultati: U grupi pacijentkinja sa rekurentnim spontanim pobačajima, s CC genotipom ih je bilo 58 (36,94%), s CT genotipom 74 (47,13%) a s TT genotipom 25 (15,92%). U kontrolnoj grupi s CC genotipom bilo ih je 59 (43,70%), s CT genotipom 55 (40,74%), a s TT genotipom 21 (15,56%). Učestalost C alela u grupi pacijentkinja je 60,51%, a T alela 39,49%, dok je u kontrolnoj grupi učestalost C alela 64,07%, a T alela 35,93%. Razlike u učestalosti genotipova ($p = 0,470$) i alela ($p = 0,409$) između grupe pacijentkinja i kontrolne grupe nisu bile statistički značajne.

Zaključak: Naši rezultati ne ukazuju da bi analizirani polimorfizam mogao da ima uticaj na nastanak spontanih pobačaja.

Ključne reči:
MTHFR C677T polimorfizam, spontani pobačaji

Introduction

Spontaneous abortion is a common complication in obstetrics (1). It is defined as clinically recognized abortion before the 20th gestational week (2). Up to 15% of pregnancies end in the first trimester by spontaneous abortion. In 5% of women there are two, and in 1-2% more than three spontaneous abortions (3). The etiology of the disorder is complex. Numerous genetic factors and external environmental factors may cause spontaneous abortion. Except for chromosomal, anatomic, endocrine and infectious factors, there are about 50% of cases in which etiology remains unexplained. Such cases are called unexplained recurrent spontaneous abortion – URSA (4).

Hyperhomocysteinemia has been recognized as possible risk factors for recurrent spontaneous abortions. (5). Methylene-tetrahydrofolate reductase (MTHFR) enzyme plays an important role in homocysteine metabolism. The MTHFR enzyme catalyses reduction of 5,10-methylenetetrahydrofolate into 5-methylentetrahydrofolate, a predominant circulatory form of folate, which plays an important role in remethylation of homocysteine into methionine. Methionine is essential for DNA synthesis and reparation (1). Vitamins B2 (riboflavin), B6 and B12 are important cofactors in folate metabolism. Folate is necessary for normal fetal development, and during pregnancy women have an increased physiological need for this vitamin (6). It has been shown that hyperhomocysteinemia can be caused by impaired MTHFR function or by a deficiency of folates and vitamin B6 or B12 supply. (6) The polymorphic variant in the MTHFR gene caused by substitution of cytosine, with thymine at nucleotide 677 (C677T), produces a thermolabile form of the enzyme with reduced enzyme activity resulting in the accumulation of homocysteine (7). In pregnancy, elevated homocysteine levels are associated with defective chorionic villus vascularization that interferes with embryonic development (8).

The aim of our study is to investigate whether the C677T polymorphism is a risk factor for development of a spontaneous abortion.

Research Design and Methods

Our group included 157 patients of 29.5 ± 5.98 years of age, with at least two spontaneous abortions in the first trimester of gestation, without any successful pregnancy. Spontaneous abortion was confirmed by the analysis of human chorionic gonadotropin (hCG), ultrasound, and / or physical examination. Kariotype analysis was performed for all patients and their partners and cases with chromosomal aberrations were not included in the study. Also, none of the patients had deficiencies of antithrombin, protein C or protein S.

The control group consisted of 135 healthy women, 30.9 ± 8.54 years of age, without a history of spontaneous abortion and with at least one healthy child. Molecular-genetic analysis was carried out at the Institute of Human Genetics, Faculty of Medicine, University of Belgrade. Each patient and control signed informed consent that will be included in the study.

For the needs of genetic analysis, 5ml of peripheral blood

was taken from each examinee and then genomic DNA was isolated using salting out method (9). Concentration and quality of isolated DNA were checked spectrophotometrically. Detection of MTHFR C677T polymorphism genotypes was performed by PCR-RFLPs method. The reaction mixture had the following composition: 10 × PCR buffer 2.5 µl; 10mM dNTPs 0.5 µl; primers (300mg / µl) of 0.5 µl; 25 mM MgCl2 1.5 µl; Taqpolymerase 5 U / µl (Thermo Fisher Scientific, USA) 0.2 µl; genomic DNA (300mg / µl) 1 µl; H2O 17.3 µl. For the amplification of the gene fragment that contains a polymorphic site, the following primers were used: Fw 5'-TGA AGG AGA AGG TGT CTG CGG GA - 3 'and RV 5'- AGG ACG CTG CGG TGA GAG TG - 3'.

PCR reaction conditions were: initial denaturation of 95°C / 5 min, then 35 cycles with 3 steps: denaturation 95°C / 1min, primers hybridization 60°C / 1min and elongation of 72°C / 1min and at the end final elongation 72°C / 7 min. The PCR product is 198 bp long. PCR products were incubated with restriction enzyme HinfI (Thermo Fisher Scientific, USA) at 37 ° C for 16 hours.

The HinfI restriction enzyme cuts an amplified gene segment for MTHFR if T allele is present, giving fragments of size 179 and 19 bp. Products of restriction enzyme digestion were analyzed by electrophoresis at 8% PAA gel, and the restriction fragments were visualized on the UV transilluminator.

The difference between the frequencies of genotypes and alleles, in the group of women with spontaneous abortions and in the control group, was tested with χ^2 test.

Results

In the group of patients, 119 of them had two spontaneous abortions, 22 had three, 8 had four, 5 had five spontaneous abortions and 3 patients had six spontaneous abortions.

Following molecular-genetic analysis, the frequency of genotypes was determined in the group of patients: CC genotype had 58 (36.94%), CT genotype 74 (47.13%) and TT genotype 25 (15.92%) (**Figure 1**). In the control group of healthy women, CC genotype had 59 (43.70%), CT genotype 55 (40.74%) and TT genotype 21 (15.56%). The difference in the frequency of genotypes between the group of patients and the control group was not statistically significant (χ^2= 1.51, p = 0.470).

The frequency of C allele in the patient group was 60.51% and T-allele was 39.49%, while in the control group frequency of C allele was 64.07% and T allele 35.93% (**Figure 2**).

Differences in the incidence of alleles between the patient group and the control group also did not reach statistical significance (χ^2= 0.68, p = 0.409).

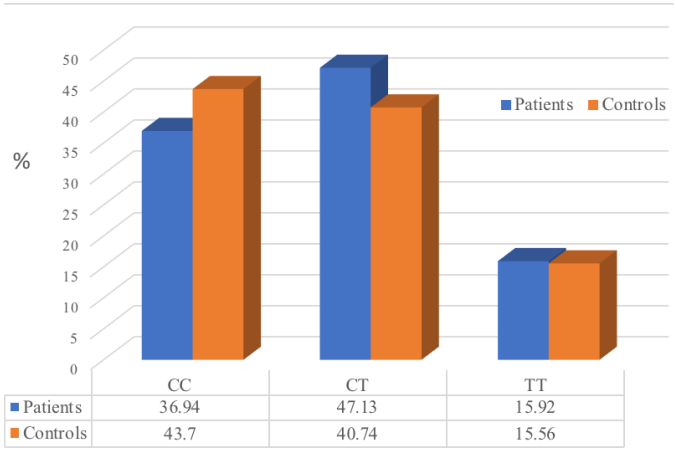

Figure 1. The frequency of genotypes in the group of patients and in the control group

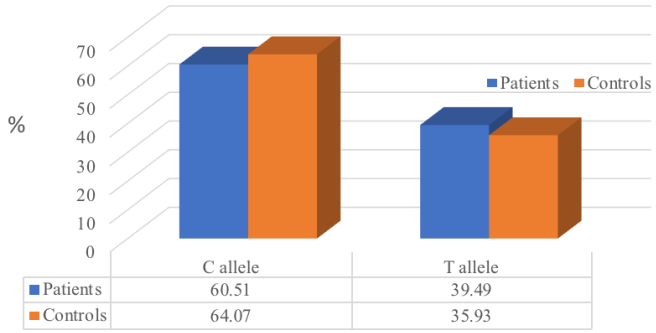

Figure 2. The frequency of alleles in the group of patients and in the control group

Discussion

The results of our study showed that there was no statistically significant difference in the frequency of genotypes and alleles of MTHFR gene C677T polymorphism between the patient group and in the control group. The analyzed polymorphism leads to the formation of a thermolabile form of an MTHFR enzyme, which results in its reduced activity. Reduced enzyme activity is associated with higher homocysteine levels (7). It has been shown that high level of homocysteine leads to the impaired vascularization of the chorionic villi, which results in placental abruption, preeclampsia, and early spontaneous abortion (10, 11).

So far, results regarding the influence of MTHFR C677T gene polymorphism on the occurrence of RAS remain inconclusive. Meta analysis of Nelen et al. has shown that there is a higher frequency of TT homozygotes and a higher level of total homocysteine (tHcy) in the group of women with recurrent spontaneous abortions (12). On the other hand, Creus et al. (5) and Vilas Boas et al. (6) did not confirm the association of homocysteine, folate and vitamin B12 levels, as well as MTHFR C677T polymorphism, with the occurrence of recurrent spontaneous abortions. Pihush et al. (13) analyzed the prevalence of five thrombophilic gene mutations in patients with RSA. One of the analyzed polymorphic variants was MTHFR C677T. In this study they could not confirm the association of MTHFR polymorphism with occurrence of spontaneous abortion. Also, in the study Papoutsakis et al. in which the

association of C677T polymorphism and the concentration of tHcy was investigated, in terms of gender, MTHFR genotype was not a strong predictor of Hcy values in the group of young women, independently of their folate status (14).

In this study we were not able to determine the folate and homocysteine level in our patients. Currently, women who plan their pregnancy are advised to take folic acid supplementation. It is possible that pathogenic effect of the analyzed polymorphism is modulated by the maternal folate status. It is also possible that the difference in the results comes from the difference between the patients in a gestation week when spontaneous abortion occurred, differences among the patient populations, as well as a small number of patients included in the studies.

Conclusion

The results of our study do not indicate that the C677T polymorphism of the MTHFR gene represents a risk factor of recurrent spontaneous abortion in women in Serbia. Further studies are needed on a larger sample.

Also, the examination of a larger number of polymorphisms of the MTHFR gene, as well as other gene polymorphisms whose products are involved in the metabolism of homocysteine, will provide better understanding of spontaneous abortion pathophysiology.

References

1. Hubacek JA, Rynekrova J, Kasparova D, Adamkova V, Holmes MV, Fait T. Association of MTHFR genetic variants C677T and A1298C on predisposition to spontaneous abortion in Slavonic population. Clin Chim Acta. 2015; 440:104-107.
2. Yalcintepe S, Ozdemir O, Hacivelioglu SO, Akurut C, Koc E, Uludag A et al. Multiple Inherited Thrombophilic Gene Polymorphisms in Spontaneous Abortions in Turkish Population. Int J Mol Cell Med. 2015; 4(2):120-127.
3. Mtiraoui N, Zammiti W, Ghazouani L, Braham NJ, Saidi S, Finan RR et al. Methylenetetrahydrofolate reductase C677T and A1209C polymorphism and changes in homocysteine concentrations in women with idiopathic recurrent pregnancy losses. Reproduction. 2006; 131:395-401.
4. Liu S. Studies on the etiology of recurrent spontaneous abortion. J. China Modern Med. 2013; 20:20.
5. Creus M, Deulofeu R, Penarrubia J, Carmona F, Balasch J. Plasma homocysteine and vitamin B12 serum levels, red blood cell folate concentrations, C677T methylenetetrahydropholate reductase gene mutation and risk of recurrent miscarriage: a case control study in Spain. Clin Chem Lab Med. 2013; 51(3):693-699.
6. Boas WV, Goncalves RO, Costa OL, Goncalves MS. Metabolism and gene polymorphisms of the folate pathway in Brazilian women with history of recurrent abortion. Rev Bras Ginecol Obstet. 2015; 37(2):71-76.
7. Ghaznavi H, Soheili Z, Samiei S, Soltanpour MS. Association of Methylenetetrahydrofolate Reductase C677T Polymorphism with Hyperhomocysteinemia and Deep Vein Thrombosis in the Iranian Population. Vasc Specialist Int. 2015; 31(4):109-14.
8. Nelen WL, Bulten J, Steegers EA, Blom HJ, Hanselaar AG, Eskes TK. Maternal homocysteine and chorionic vascularization in recurrent early pregnancy loss. Human Reproduction. 2000;15(4):954-960.
9. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. NucleicAcids Res.1988;16(3):1215.
10. Aubard Y, Darodes N, Cantaloube M. Hyperhomocysteinemia and pregnancy – review of our present understanding and therapeutic implications. European journal of obstetrics, gynecology and reproductive biology. 2000; 93:157-165.
11. Steegers Theunissen RP, Van Iersel CA, Peer PG, Nelen WL, Steegers EA.Hyperhomocysteinemia pregnancy complications, and the timing of investigation. Obstetrics and gynaecology. 2004;104:336-343.
12. Nelen WL, Blom HJ, Steegers EAden Heijer M, Eskes TK. Hyperhomocysteinemia and recurrent early pregnancy loss: a meta analysis. Fertility and sterility. 2000; 74:1196-1199.
13. Pihusch R, Buchholz T, Lahse P, Rübsamen H, Rogenhofer N, Hasbargen U et al.Thrombophilic gene mutations and recurrent spontaneous abortion: prothrombin mutation increases the risk in the first trimester. Am J Reprod Immunol. 2001; 46(2):124-131.
14. Papoutsakis C, Yiannakouris N, Manios Y, Papaconstantinou E, Magkos F, Schulpis KH et al.The effect of MTHFR (C677T) genotype on plasma homocysteine concentrations in healthy children is influenced by gender. Eur J Clin Nutr. 2006; 60(2):155-162.