Defect of methylenetetrahydrofolate reductase in a patient with ten habitual misscarriages: a case report

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Summary

This is a case report of a 47-year-old patient that came to our Clinic due to bleeding during the 23rd week of twin pregnancy after in vitro fertilization-intracervical insemination/embryo transfer (IVF-ICI/ET) treatment. Prior to this pregnancy, this patient had had ten spontaneous miscarriages, eight of which following IVF-ICI/ET, and two following spontaneous conception, all in the eighth week of pregnancy. After several miscarriages by the age of 43, the patient was suggested to be tested for thrombophilia; it was then discovered that she had the methylenetetrahydrofolate reductase (MTHFR) gene defect, in the homozygous Tobiano (TT) form. Thus she was treated with cardiolipin and folic acid before pregnancy, and continued with folic acid after the pregnancy had been diagnosed. Fraxiparine 0.4 ml subcutaneous (s.c.) should be introduced from the second month of pregnancy until one day before delivery. It is a useful treatment for the patients with MTHFR defect, as it prevents miscarriage and promotes successful pregnancy.

Key words: MTHFR defect; Recurrent miscarriage; Treatment.

Introduction

Interest in research of 5,10-MTHFR 677C→T polymorphism has been constantly increasing worldwide. The enzyme MTHFR has a central role in the folate cycle and metabolism of homocysteine. This enzyme is a catalyst of 5,10-MTHFR to 5-MTHFR, resulting in accumulation of active form of folate which is necessary for remethylation of homocysteine to methionine. It is 5,10-MTHFR that determines whether folate will be used for metilation or for nucleotide synthesis.

The 5,10-MTHFR gene is placed at the end of the short arm of chromosome 1 (1p 36.3) [1]. The C677T allel is characterized by a point mutation at the position 677 of the MTHFR gene, which alters cytosine into thymine. Three genotypes with changed bases have been described: homozygous CC-type, heterozygous CT-type, homozygous TT-type. This mutation of the MTHFR gene is thermo-labile and MTHFR activity is reduced about 70% in TT-genotype. The prevalence in Austria of CC-genotype is about 40%, of CT-genotype is 47%, and of TT-genotype is 13%. Such ratios of genotype frequency is approximately the same in other European and worldwide studies. MTHFR is essential for homocysteine metabolism [2, 3].

Mutation of MTHFR may induce hyperhomocysteinemia, associated with low level of folic acid, which may lead to mental retardation, skeletal anomalies, early vascular disorders, thrombosis, and may be the risk factor for repeated miscarriages. Hyperhomocysteinemia increases the risk for neural tube defects, loss of fetus, placental abruption, and placental infarction [4].

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Polymorphism of C677T is more frequent in homozygosis and heterozygosis, among subjects with spina bifida. This polymorphism is associated with spina bifida in 1.75% of TT-genotype, and in 1.16% of CT-genotype. In Italy, this polymorphism is much more frequent compared to other populations with dominating mutated TT homozygote in 15-25%. In C677T homozygote the risk of neural tube defect is 5.9% in women who have not been taking vitamin and folate supplements (both during periconceptional and pregnancy) and in 1.2% who have been taking supplements. Recommended daily dose of folate during pregnancy is 0.4 mg, while otherwise it is 0.2 mg. In MTHFR 677 TT genotype, average level of homocysteine is significantly higher than in MTHFR 677 CT and MTHFR 677 CC genotype [5]. Increased level of homocysteine and MTHFR 677 TT genotype was established in a research of Korean patients with unexplained recurrent spontaneous miscarriages [5].

Spontaneous miscarriage is defined as a spontaneous termination of two or more consecutive pregnancies before 20th week of pregnancy. In the majority of cases, it is extremely difficult to determine a causative factor, because up to 15-33% recurrent miscarriages are idiopathic. Efficient utero-placental circulation, which may be affected by hemostasis disorders, is necessary for successful outcome of pregnancy.

Maternal thrombophilias (factor V Leiden mutation, MTHFR defect, factor II mutation, protein C or S deficiency) are important disorders in obstetrics [6]. The exact mechanism between hyperhomocysteinemia and pregnancy loss is yet unknown. The studies demonstrated borderline increase of homozygous MTHFR defects in women with fetal loss and increase of relative risk for pregnancy loss in women with this defect. It is still unclear whether folate supplementation with folate decreases the risk of pregnancy loss [7-10].

MTHFR mutation is currently believed to be a risk fac-

tor for habitual miscarriage, although there are some differences in the available studies. The majority of studies imply that the incidence of homozygous form is more frequent in women with three or more repeated miscarriages, while few studies demonstrated no association between loss of fetus and MTHFR mutation [7-10].

The authors found a MTHFR defect in a patient with habitual miscarriage and decided to present it, since there are conflicting data in literature regarding the significance of MTHFR defects in repeated miscarriages.

Case Report

A 47-year-old patient came to our Clinic due to bleeding during the 23rd week of gestation (ng) of a twin pregnancy after the result of IVF-ICI/ET treatment. Diagnosis at admission included: *Grav ml V 1/2, St. post IVF-ICI/ET, Gravida vetusta, Gemelli, Ab. Imminens, MTHFR, St. post ab. habitualis N X*.

Status at admission included: cervix was 1.5 cm and there was trace of blood on the examination glove. The last period occurred on March 29, 2010, and the expected date of delivery was January 6, 2011. Complete lab tests were performed and all the results were within normal range, except for hemoglobin level which was decreased, so a treatment with iron and folic acid was introduced. Cervical and vaginal smears were normal, and urine and urine culture had no pathogens. The patient was also examined by a specialist for internal disorders (blood count, coagulation factors, biochemical analysis, electrocardiography, and chest examination) and a good general condition of this patient was confirmed.

Ultrasonic examination detected healthy, twin pregnancy, with sufficient quantity of amniotic fluid in both fetuses, eutrophic growth of both fetuses, and placenta placed on the posterior wall of uterus, with retro-placental hematoma with 19 mm in diameter. D-dimmer was 1.22 mg/L FEU, and CRP = 4.9 mg/L, leukocyte = 10.1, INR = 1.15.

The patient immediately underwent focolytic intravenous (i.v.) and anticoagulant therapies with heparin, and hematologist prescribed 0.4 ml of Fraxiparine subcutaneously (s.c), once in 24 hours. The D-dimmer was checked at 7-14 days. The hematologist insisted that D-dimmer value should not exceed 2.5 mg/L, otherwise the patient had to be urgently referred back to a hematologist, considering the homozygous TT defect of MTHFR (677 TT).

Only tonsillectomy and appendectomy were refered. At the age of 15, she began her menstruation cycles that continued to be regular. The patient is allergic to Penicillin.

The patient suffered from primary sterility, although the test results of both partners were normal. After these results, an intrauterine insemination preceded by clomiphene stimulation was unsuccessfully performed at the age of 36. A year later another IVF attempt equally failed. Afterwards eight procedures were performed in the following four years, but despite positive results, miscarriages resulted each time. The patient then refused to continue with further treatments.

At the age of 43 she consulted with a geneticist and was diagnosed with a 50% genetic mutation of MTHFR (disturbed folate metabolism), i.e. that she was a homozygote TT677 at the gene for MTHFR. The doctor proposed treatment with cardiolipin and folic acid. At the age of 45 and 46 years she got pregnant spontaneously, but the pregnancies were again interrupted at 8 ng by spontaneous miscarriage. Pathohistology showed the genetic error in chorion villi.

At the age of 46 she underwent the ninth attempt of IVF-ICI/ET and two embryos were transferred (two ET), both were accepted, and the pregnancy proceded. Treatment with folic acid and fraxiparine (0.4 ml s.c at 24 hours) commenced from the second month of pregnancy, with regular monitoring of D-dimmer and INR at 7-14 day intervals. In consideration of her obstetric history, she was hospitalized at 23rd ng due to bleeding. The aim was to maintain such a pregnancy as long as possible.

Results

Despite intensive treatment and monitoring, bleeding started at 26th ng and color Doppler imaging showed placenta previa marginalis. The patient was advised strict bed rest with intensive intravenous tocolysis with prolonged hospitalization. From this moment bleeding was alternate until the 29th ng when a strong abdominal pain occurred followed by abundant bleeding, and a cesarean section was urgently performed. The first fetus had placental abruption, while both fetuses had cephalic presentation; the first one was female, weighed 960 grammes (g), with an Apgar score (Ap score) of 4/5. The second fetus was male, weighed 1,050 g, with an Ap score of 6/7. Both babies were urgently transported to the Institute for Neonatology in Belgrade. The female newborn death occurred during the transfer, notwithstanding two resuscitation attempts. The male fetus survived, and is alive and healthy at the moment (weight 2,500 g).

Discussion

Approximately 15% of pregnancies end in miscarriage, while 0.5-1% couples have recurrent miscarriages. Habitual abortion is defined as a spontaneous miscarriage of two or more consecutive pregnancies, before the 20th week of pregnancy [11].

Causes of habitual abortion in most cases remain unknown. As described by the authors, all miscarriages occurred during the first trimester. The histopathological findings indicated a problem in the development of chorion villi. It is well known that MTHFR is an important enzyme in folate metabolism. MTHFR gene mutation causes a reduction in activity of this enzyme, resulting in increased plasma levels of homocysteine, which is a risk factor for the occurrence of thrombosis. Expressed MTHFR defects with hyperhomocysteinemia and homocysteinuria may result in peripheral neuropathy, mental retardation, thrombosis, and heart attack. Mild MTHFR defects are more prevalent in the general population, and represent a risk for disorders of the arteries.Nelen *et al.*, reported that MTHFR C677T mutation increased plasma homocystein level, resulting in two- and three-fold increase of recurrent spontaneous miscarriages [12]. The studies indicated a substantial increase of plasma homocysteine in patients with MTHFR C677T homozygote TT type, which was significantly associated with miscarriages, as was the case in the patient presented in this case report (she had had ten consecutive abortions). Data

regarding the association of MTHFR genetic mutation and the recurrent spontaneous miscarriages are still controversial.

Some researchers consider that MTHFR genetic mutation represents a risk factor for idiopathic spontaneous miscarriages. Other researchers demonstrated high influence of homozygous type in recurrent miscarriages (as it was the case with the presented patient), while others did not recognize the connection between MTHFR mutation and fetal loss. Feyzi *et al*., claim that even heterozygous MTHFR defects might result in spontaneous miscarriage, whether alone or in combination with other factors [13]. In the present case, the patient had homozygous MTHFR defect, TT type, and since both she and her husband were perfectly healthy, ten consecutive abortions might be attributed to MTHFR defect. Treatment with cardiolipin, folic acid, and fraxiparine assisted the patient to carry the pregnancy until 29th ng, at which she had to deliver by cesarean section, due to placenta abruption.

Conclusion

In healthy patients, who are not diagnosed with any acute, chronic, or systemic disorder, and who have recurrent idiopathic abortions, it is necessary to test MTHFR gene defect. If they test positive for the defect, treatment should include cardiolipin and folic acid before pregnancy, and continued with folic acid after the pregnancy has been diagnosed. Fraxiparine 0.4 ml s.c. should be introduced from the second month of pregnancy until one day before delivery.

This current case report presents a useful treatment for the patients with MTHFR defect, as it prevents miscarriage and promotes successful pregnancy.

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