

Is screening for hereditary thrombophilia indicated in first early pregnancy loss?

Dita KASPAROVA¹, Michal VRABLIK², Tomas FAIT¹

¹ Department of Obstetrics and Gynecology, 1st Faculty of Medicine, Charles University and General Teaching Hospital Prague, Czech Republic

² 3rd Department of Internal Medicine, 1st Faculty of Medicine, Charles University and General Teaching Hospital Prague, Czech Republic

Correspondence to: Tomas Fait, MD., PhD.
Dept. of Obstetrics and Gynecology, General Teaching Hospital
Apolinarska 18, Prague 2, 128 53, Czech Republic.
FAX: +420 224 922 545; E-MAIL: tfait@seznam.cz

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Abstract

OBJECTIVE: The aim of the study was to evaluate the importance of screening for thrombophilic mutations after the first early pregnancy loss.

SETTING: Thrombophilic mutations were examined in a sample of 100 women with at least one miscarriage. DNA was isolated from venous blood sample. We used methods of microarray, fragmentation analysis, High Resolution Melting and PCR-ARMS with following gel electrophoresis and visualisation. Chi-square test and in cases of low expected frequencies Yates correction were used to compare relative frequencies of individual mutations. The comparison of averages was performed by t-test.

RESULTS: We detected prevalence of factor V and II mutation of 9% and 3%, respectively. Single MTHFR mutation was found in 59% and double heterozygous MTHFR mutation in 23% of cases. No mutation was present in only 6% of the study group. Heterozygous mutations of factor V occurred 1.8 times more frequently in our study group compared to the general Czech women population. Also, the frequency of factor II mutation was 1.5–3 times higher. No carrier of these mutations had overt coagulation disorder, history of thromboembolic disease or that of habitual abortions.

CONCLUSIONS: The frequency of thrombophilic mutations in the group of women with early pregnancy loss is 1.5–3 times higher than in the general population.

Abbreviations:

DNA - deoxyribonucleic acid
FVL - mutation of factor V Leiden
VTE - venous thromboembolism
MTHFR - methylenetetrahydrofolate reductase

INTRODUCTION

Miscarriages occur in 15–20% of pregnancies. In the context of persisting maternity postponing, every pregnancy loss represents increasing psychological stress. Added to that, no targeted examination of aetiology is performed in cases of the first and second spontaneous abortions. Approximately 0.4–1.0% of women has personal history of 3 and more abortions (habitual abortions).

The aetiology of miscarriage may be genetic, anatomic, haematologic, endocrine, infectious, immunologic, environmental, psychological or idiopathic. Placental thrombosis may be one of the pathophysiological mechanisms in part of the women with the history of recurrent pregnancy loss. Good placentation and following sufficient placental circulation depends on the balance of procoagulation and anticoagulation factors. Inherited thrombophilia may impair this balance and, thus, occurrence of thrombophilic mutations has been associated with miscarriages (Blumenfeld & Brenner 1995; Brenner *et al.* 1999).

High prevalence of early pregnancy loss, the trend of maternity postponing with following claim of successful conception and an increasing availability of commercial genetic tests of thrombophilic mutations brought up the question whether to examine the presence of thrombophilic mutations in all women after the first spontaneous abortion. The question of preconception or broad screening for of thrombophilic mutations as risk factors of thromboembolic disease in hormonal contraception users or in pregnancy also remains open.

MATERIALS AND METHODS

The study included 100 patients, who were referred to our tertiary clinic with the diagnosis of missed abortion in first trimester. The patients with verified diagnosis of missed abortion were invited to participate in the study and signed informed consent. They filled in a detailed personal medical history questionnaire. Thorough physical examination as well as laboratory examination of blood count, biochemical and coagulation parameters were performed. The participants also underwent ultrasound examination. Surgical revision of uterine cavity was performed and acquired material was subjected to a cytogenetic and histological examination.

Exclusion criteria were inborn and acquired anatomic abnormalities of the uterus, primary endocrinopathy (such as thyreopathy, diabetes mellitus, and hyperprolactinaemia), known immunologic or hematologic disease, febrile infections during the first trimester, nicotine or drug abuse and/or abnormal results of cytogenetic examination of aborted tissue.

DNA was isolated from leukocytes of peripheral venous blood by standard salting-out procedure. Isolated DNA was analysed using the methods of molecular genetic analysis for the four most common inherited

coagulopathies. We used the methods of microarray, fragmentation analysis, High Resolution Melting and PCR-ARMS with following gel electrophoresis and visualisation.

For relative frequency comparison chi-square test and in case of low expected frequencies Yates correction were used in statistic analysis. The comparison of averages was performed by t-test. All tests were two-sided.

RESULTS

The average age was 33.7 ± 4.23 years, the oldest woman was 44 years old, and the youngest one was 23 years old. Total number of spontaneous abortions during the first trimester including anamnestic pregnancy losses was 136, the average number of abortions was 1.36 ± 0.8 per woman. 75 women (75%) aborted for the first time, 17 (17%) for the second time and 8 (8%) of women had the history of 3 or more abortions and, thus, fulfilled the criteria of the diagnosis of habitual abortions. Total number of live births, according to patients' medical history, was 91. In all born alive babies, 4 (4.4%) were hypotrophic, 3 (3.3%) were hypertrophic and 82 (92.3%) were eutrophic. The number of stillbirths was 7 (Table 1).

Factor V (Leiden) mutation (FVL) in heterozygous constitution was detected in 9 (9%) women. Their history contained no data of thromboembolic disease. No woman had laboratory abnormality in coagulation parameters. 8 women had a history of uncomplicated hormonal contraception use. 4 women had a history of an in-term delivery of a eutrophic fetus. All patients with this diagnosis had their first abortion.

Heterozygous mutation of factor II was detected in 3 (3%) women. No women with this diagnosis had a history of habitual abortions, positive personal or family history of VTE or abnormal blood coagulation parameters. One woman in this group had a history of stillbirth but that of a successful pregnancy as well. This patient was concurrently the carrier of homozygous MTHFR A1298C mutation.

The most frequently detected mutations were within the MTHFR gene. 59 women were heterozygotes for a single mutation and 24 were compound heterozygotes. We identified 28 heterozygous and 11 homozygous carriers of MTHFR C677T mutation and 10 heterozygotes and 10 homozygotes for the MTHFR A1298C mutation. There were 2 distinct combinations of thrombophilic mutations detected in our study. The most frequent (23 women, 23%) were concurrent MTHFR mutations C677T and A1298C (compound MTHFR heterozygous). We identified 1 carrier of a combination of heterozygous factor II mutation and homozygous MTHFR A1298C mutation.

All 8 women with the diagnosis of habitual abortions had been diagnosed with hereditary thrombophilia: 2 had heterozygous mutation of MTHFR C677T, 2 had its homozygous form, 1 had heterozygous and 1 homo-

zygous MTHFR A1298C mutation. The last 2 patients had concurrent mutations of MTHFR in heterozygous constitutions (compound MTHFR mutations). Also in all 7 women with the history of stillbirth hereditary thrombophilia was confirmed. There were 2 heterozygous and 2 homozygous MTHFR C677T mutation carriers, 1 heterozygote for MTHFR A1299C mutation, 1 compound heterozygote for MTHFR C677T and MTHFR A1298C mutation and 1 patient with concurrent presence of factor II mutation and MTHFR C677T mutation in homozygous constellation.

None of the MTHFR mutation carriers had positive personal or family history of VTE. 6 (6%) patients had no thrombophilic mutations detected; all of these subjects did not have the history of habitual abortions, stillbirth or VTE.

DISCUSSION

Hereditary thrombophilias represent a group of genetically determined coagulopathies that increase the risk of thromboembolic disease. While their role in pathogenesis of venous thromboembolism has been well described, their relationship to arterial thrombosis remains unclear. However, hereditary thrombophilias may play an important role during formation of

uteroplacental circulation and also in later pregnancy pathologies. Hereditary thrombophilias may cause thrombus formation in uteroplacental and intervillous space and, thus, lead to intrauterine growth retardation, intrauterine fetus death and other complications (Brenner *et al.* 2003). Factor V Leiden mutation (1691GA) is caused by adenine to guanine substitution in the DNA sequence encoding for coagulation factor V. This leads to an exchange of arginin for glutamin at position 506 of the aminoacid sequence of factor V. This results in greater resistance of factor V against cleavage by activated protein C. Thus, this mutation of factor V is 90% cause of resistance to activated protein C (APC resistance) (Girling & de Swiet 1998). As a result a significant increase of circulating prothrombin level up to 150–200% of normal can be detected. The prevalence of heterozygous factor V Leiden mutation in the Czech population is 5%, the frequency of homozygous factor V Leiden mutation carriers is 1/5000 inhabitants (Koudová *et al.* 2010).

Ridker *et al.* (1998) documented 2.33 times higher prevalence of this coagulopathy in patients who were diagnosed with habitual abortions (Table 2). In our study population we detected 9% prevalence of this mutation in heterozygous form. This is 1.8 times higher than in the general population (Koudová *et al.* 2010).

Tab. 1. Results of study.

Diagnosed thrombophilic mutations in file	Number of thrombophilic mutations in file	Early pregnancy loss			Stillbirth
		1	2	3 and more	
Thrombophilic single mutations					
Factor V Leiden heterozygous mutation	9	9	0	0	0
Factor II prothrombin heterozygous mutation	2	2	0	0	0
MTHFR C677T heterozygous mutation	28	20	6	2	2
MTHFR C677T homozygous mutation	11	8	1	2	2
MTHFR A1298C heterozygous mutation	10	8	1	1	1
MTHFR A1298C homozygous mutation	10	7	2	1	0
Compound thrombophilic mutations					
Prothrombin heterozygous mutation and MTHFR A1298C homozygous mutation	1	0	1	0	1
Double MTHFR heterozygous mutation	23	17	4	2	1
None of thrombophilic mutation					
	6	4	2	0	0
	total	75	17	8	7

Tab. 2. Factor Leiden in cohorts of women with pregnancy loss (PL).

Author	PL		Controls		OR	95% CI	p-value
Ridker <i>et al.</i> (1998)	9/113	(8%)	16/437	(3.7%)	2.3	1.0–5.2	0.05
Grandone <i>et al.</i> (1997)	7/43	(16%)	5/118	(4%)	4.4	1.3–14.7	0.01
Brenner <i>et al.</i> (1999)	24/76	(32%)	11/106	(10%)	4.0	1.8–8.8	0.001
Wramsby <i>et al.</i> (2000)	13/84	(15.5%)	2/69	(2.9%)	7.2	1.5–34.0	0.008

Regardless of ethnic differences many studies have confirmed higher prevalence of factor V mutations in women with the history of recurrent miscarriages (Brenner *et al.* 1997; Grandone *et al.* 1997; Meinardi *et al.* 1993; Younis *et al.* 2000). On the contrary, Linqvist (1999) study did not confirm this finding and suggested the necessity of presence of other, so far unknown, thrombophilias or local placental factors. The frequency of prothrombin mutations in Czech general population varies between 1–2% (Koudová *et al.* 2010). In our study we found 3 (3%) women with factor II mutation, thus, the prevalence 1.5–3 times higher compared to the general population.

According to Poort (1996) the mutation of prothrombin gene is associated with higher risk of deep venous thrombosis. In heterozygous carriers of this mutation the risk of venous thrombosis increases of 2–3 times. Sarig (2002) has described no association of single mutations of factor II and MTHFR C677T with increased risk of recurrent miscarriage. However, more studies have documented this association (Brenner & Blumenfeld 1997; Seremak-Mrozikiewicz *et al.* 2010). Both the mutation of factor V Leiden and the mutation of factor II have been linked to higher risk of intrauterine fetal death. [10] Eleven of 67 (16%) patients with late miscarriage and 13 of 232 (6%) patients in the control group had either the mutation of factor V or the mutation of factor II (Brenner *et al.* 2003).

The mutations of the gene for methylenetetrahydrofolate reductase – MTHFR C677T, MTHFR A1298C – are associated with increased homocysteine level, especially in case of vitamins B6, B12 or folic acid deficiencies. The level of homocysteine decreases during pregnancy by approximately 50%. Hyperhomocysteinemia was detected in 26% of patients with placental abruption, in 11% of patients with intrauterine fetal death and in 38% of patients with intrauterine growth restriction (Brenner & Kupferminc 2003). The frequency of heterozygous MTHFR C677T mutation in the Czech Republic is 34%, the frequency of heterozygous MTHFR A1298C mutation is 33% (Koudová *et al.* 2010).

A number of studies confirms the importance of MTHFR mutations for the risk of early pregnancy loss that even increases in case of compound heterozygosity (Martinelli *et al.* 2000; Preston *et al.* 1996) The prevalence of MTHFR mutations is high especially in patients with recurrent abortions. [18] Combinations of inherited thrombophilic mutations increase the risk of habitual abortions and thrombosis (Grandone *et al.* 2002; Ridker *et al.* 1998).

Twenty four carriers of more than one mutation were detected in our study group. The prevalence of compound MTHFR heterozygotes in Europe is 15% (Koudová *et al.* 2010). It was 2.3 times higher in our file. Compound MTHFR heterozygosity was detected in two of 8 women with the diagnosis of habitual abortions and in one of the 7 patients with a history of stillbirths. The study performed by Sarig detected at least one

thrombophilic mutation in 96 out of 145 (66%) patients with the history of early pregnancy loss compared to control group, in which thrombophilia was identified in 41 out of 145 (28%) patients. The combination of thrombophilic mutations was detected in 31 out of 145 (21%) patients with recurrent pregnancy loss compared to the control group with only 8 mutation carriers in 145 patients (5.5%) (Sarig *et al.* 2002).

CONCLUSION

Inherited thrombophilic mutations (except MTHFR mutation) and combination of more thrombophilic mutations increase the risk of venous thromboembolism. They all have a negative influence on female reproduction ability – especially if associated with early pregnancy losses.

We proved higher prevalence of factor II and factor V mutations in women with early pregnancy loss compared to the population data.

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