

DOI: <http://dx.doi.org/10.18203/2320-1770.ijrcog20180857>

Original Research Article

Thrombophilia gene mutations in relation to recurrent miscarriage

Tawfik Abdelsalam¹, Tarek Karkour¹, Magdy Elbordiny²,
Dina Shalaby¹, Ziad S. Abouzeid^{1*}

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

²Department of Clinical Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Received: 01 January 2018

Accepted: 31 January 2018

***Correspondence:**

Dr. Ziad S. Abouzeid,

E-mail: zsabouzeid@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Recurrent pregnancy loss is multifactorial involving clinical and biological risk factors. Evidence addressed the association of inherited thrombophilia with recurrent pregnancy loss 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. The aim of this study was to investigate the prevalence of thrombophilia associated gene mutations (factor V Leiden, prothrombin gene G20210A and methylene-tetrahydrofolate reductase MTHFR C677T) in relation to recurrent miscarriage.

Methods: Case control study conducted on 200 women recruited from Elshatby Maternity Hospital clinics. The cases group included 100 women with history of three or more unexplained consecutive pregnancy losses, while 100 healthy age matched women with no history of recurrent miscarriages served as controls. Blood samples were collected from all women enrolled in the study for DNA extraction and genotype analysis. Factor V, prothrombin and MTHFR gene mutations were assayed based on polymerase chain reaction (PCR) and reverse-hybridization.

Results: The prevalence of Factor V Leiden and prothrombin gene G20210A mutations did not differ significantly between cases and controls. However, MTHFR C667T mutations and the total prevalence of the three gene mutations were significantly increased in the patients group compared to controls ($p=0.001$, $p=0.003$ respectively). The prevalence of combined thrombophilia of Factor V Leiden and MTHFR C677T was significantly increased in the patients group compared to controls ($p=0.032$). Regarding homozygosity of each of the gene mutations, no homozygosity was detected in controls and heterozygotes were significantly increased in the patients group compared to homozygotes.

Conclusions: MTHFR mutations and the total prevalence of the three gene mutations were significantly increased in the patients group compared to controls. There was a significant increase in the prevalence of combined thrombophilia (Factor V Leiden and MTHFR C677T) in the patients group compared to controls without involvement of prothrombin gene.

Keywords: Factor V Leiden, MTHFR, Prothrombin gene mutation, Recurrent miscarriage, Thrombophilia

INTRODUCTION

Early pregnancy loss is a common occurrence among reproductive age women. It is defined as the termination of pregnancy before 20 weeks gestation or with a fetal weight < 500 gm.¹ Recurrent pregnancy loss (RPL) is

defined as 3 consecutive pregnancy losses prior to 20 weeks of gestation. Its incidence is approximately 1 in 300 pregnancies.²

Evidence addressed the association of inherited thrombophilia with RPL. The most common genetic

markers include factor V Leiden mutation and prothrombin gene (G20210A) mutation. Other genetic indicators include methylene-tetrahydrofolate reductase C677T (MTHFR) mutations, activated protein C resistance, protein C and protein S deficiencies, and antithrombin III deficiency.³ A thrombotic pathogenesis for pregnancy loss proposes that one or two copies of these variants could enhance the existing hypercoagulable state in pregnant women and that impaired fetal circulation or thrombotic vasculopathy in the placenta could result in placenta mediated pregnancy complications and fetal loss.⁴

Association between these heritable thrombophilia variants and other serious pregnancy complications such as fetal growth restriction, placental abruption, preeclampsia, eclampsia, prematurity and intrauterine fetal death has also been documented.⁵

Inherited thrombophilia refers to inborn conditions, usually hereditary, that increase the tendency to develop venous thrombosis (VTE). The most frequent causes of an inherited hypercoagulable state are factor V Leiden mutation and prothrombin gene mutation, which together comprise 50% to 60% of cases in Caucasian populations. Defects in protein S, protein C and antithrombin account for most of the remaining cases. Homozygosity for methylene-tetrahydrofolate reductase (MTHFR) polymorphisms (C677T, 1298C) is a relatively common cause of mildly elevated plasma homocysteine levels (hyperhomocysteinemia) and increased risk of thrombosis.^{6,7}

The objective of this study was to investigate the prevalence of thrombophilia associated gene mutations (factor V Leiden, prothrombin gene G20210A, as well as, methylene-tetrahydrofolate reductase MTHFR C677T) in relation to recurrent miscarriage.

METHODS

Case control study conducted on 200 women attending the Gynecology or family planning clinic at Elshatby Maternity University Hospital, in the period between October 2016 and July 2017. The cases group (Group I) consisted of 100 women with a history of three or more unexplained consecutive miscarriages, whereas, Group II included 100 healthy age matched women with uncomplicated pregnancies and no history of recurrent miscarriages. Any patient with known history of induced abortions, current infection, systemic disease and/or structural uterine anomalies was excluded from the study.

All patients signed an informed consent form to declare their agreement to be enrolled in the study, as agreed upon by the research ethics committee.

All selected patients were subjected to complete history taking, complete general and gynecological examination. To exclude causes of recurrent miscarriage other than

thrombophilias, a complete work-up including fasting blood glucose, serum FSH, LH, prolactin and thyroid function tests, anticardiolipin antibodies and lupus anticoagulant. Hysterosalpingography and/or hysteroscopy, karyotyping and transvaginal ultrasound were performed to all cases.

Blood samples were collected from all women enrolled in the study for DNA extraction and genotype analysis. Factor V (FV), prothrombin (PTH) and MTHFR gene mutations were assayed based on polymerase chain reaction (PCR) and reverse-hybridization. The frequency of homozygous and heterozygous gene mutations, as well as, the co-expression of mutations was determined.

The assays covered three mutations

- FV G1691A (Factor V Leiden Kit Roche ®)
- PTH G20210A (Prothrombin Kit Roche ®)
- MTHFR C677T (DiaPlexQ™ MTHFR Genotyping Kit ®)

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean±standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for Chi-square was conducted using Fisher's Exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agostino test. If it reveals normal data distribution, parametric tests were applied. If the data were abnormally distributed, non-parametric tests were used for normally distributed data. For normally distributed data, comparison between the two studied groups were done using independent *t*-test, while for abnormally distributed data, comparison was done using Mann Whitney test. Significance of the obtained results was judged at the 5% level.

RESULTS

Patients age in group I ranged between 23-36 years with a mean age of 28.65±9.82 years, whereas, in group II the range was 24-37 years with a mean age of 27.61±8.65 years. There was no significant difference in maternal age between the two studied groups ($p > 0.05$) (Table 1).

The total number of miscarriages in group I was 368 of which 231 were first trimester (62.7%) and 137 were second trimester (37.3%). In group II, the number of miscarriages was 20 of which 11 were first trimester (55%) and 9 were second trimester (45%). There was a statistically significant difference between groups as

regards the total number of miscarriages (p=0.001) (Table 2).

Table 1: Comparison between the studied groups as regards maternal age.

Age (years)	Group I	Group II
	Study group	Control group
Range	23-36	24-37
Mean	28.65	27.61
S.D.	9.82	8.65
T test	0.85	
P significance value	0.42	

Table 2: Distribution of miscarriages in the first and second trimester in the studied groups.

	Patients	Controls	P value
	n =100	n =100	
Total number of miscarriages	368	20	0.001*
First trimester	231 (62.7%)	11 (55%)	0.211
Second trimester	137 (37.3%)	9 (45%)	0.107

*Statistically significant at p ≤0.05

Thrombophilia polymorphisms in the studied groups

The prevalence of thrombophilia polymorphisms was assessed in the two studied groups. Considering each gene mutation individually, factor V Leiden and prothrombin gene G20210A mutations did not differ significantly between groups, whereas, MTHFR C677T mutations were significantly increased in patients compared to controls (p = 0.001).

Moreover, the total prevalence of the three gene mutations was significantly increased in patients (61%) compared to controls (21%) (p = 0.003) (Table 3).

Table 3: Prevalence of thrombophilia polymorphisms in the studied groups.

	Group I		Group II		P value of mean
	Study group		Control group		
	No.	%	No.	%	
Factor V G1691A (Leiden)	13	13.0	10	10.0	0.562
Prothrombin gene G20210A	6	6.0	2	2.0	0.412
MTHFR C677T	42	42.0	8	8.0	0.001*
Total	61	61.0	20	20.0	0.003*

*Statistically significant at p ≤0.05

Homozygosity, heterozygosity and the presence of combined thrombophilia in the studied groups

The number of homozygous and heterozygous individuals were assessed for each of the gene mutations studied and compared between groups. In the study group, there were only 2 homozygous individuals for Factor V Leiden and 3 cases of homozygosity for MHTFR C677T. No homozygosity was detected for prothrombin gene G20210A mutation. In controls, no homozygosity was detected for any of the gene mutations studied. There was no significant difference between groups as regards homozygosity and heterozygosity.

In the study group, 10 cases had combined Factor V Leiden and MHTFR C677T gene mutations, whereas, none of the controls had combined thrombophilia, which was statistically significant (p= 0.032). No patients had combined thrombophilia involving prothrombin gene G20210A mutation in the study group (Table 4).

Table 4: Analysis of thrombophilia gene mutations in the studied groups.

	Study group		Control group		P value
	No.	%	No.	%	
Factor V Leiden					
Heterozygous	11	84.6	10	100	0.187
Homozygous	2	15.4	0	0.0	
Prothrombin gene mutation					
Heterozygous	6	100.0	2	100.0	-
Homozygous	0	0	0	0.0	
MTHFR C677T mutation					
Heterozygous	39	92.8	8	100	0.426
Homozygous	3	7.1	0	0.0	
Combined mutations					
Factor V Leiden and MTHFR	10	16.4	0	0.0	0.032*

Table 5: Analysis of homozygous and heterozygous thrombophilia gene mutations in Group I.

	Heterozygous		Homozygous		Total	
	No.	%	No.	%	No.	%
Factor V G1691A (Leiden)	11	84.6	2	15.4	13	13
Prothrombin gene G20210A	6	100.0	0	0.0	6	6
MTHFR C677T	39	92.8	3	7.1	42	42
X ²	12.65					
P	0.013*					

χ²: Chi square test; *Statistically significant at p ≤0.05

Analysis of homozygous and heterozygous thrombophilia gene mutations in Group I

The number of homozygous and heterozygous individuals were assessed for each of the gene mutations studied and compared in the patients group with history of repeated miscarriages (Group I). Heterozygotes were significantly increased compared to homozygotes for each of the three gene mutations studied ($P = 0.013$). Moreover, there was a significant association between heterozygosity and the prothrombin gene mutation as all patients (100%) who were positive for the latter gene mutation were heterozygotes (Table 5).

DISCUSSION

Recurrent pregnancy loss is multifactorial involving clinical and biological risk factors. Evidence addressed the association of inherited thrombophilia with recurrent pregnancy loss, focusing on tests for three genetic variants; factor V Leiden, prothrombin G20210A and methylene-tetrahydrofolate reductase (MTHFR).³ However, the relation between thrombophilia associated gene mutations and adverse obstetric outcome is controversial and data in the literature are inconsistent because of study heterogeneity, potential publication bias and sequential testing.⁸

In the present study, MTHFR C667T mutations were significantly increased in the patients group compared to controls. Several studies reported increasing evidence for a pathogenetic role of MTHFR gene polymorphism C677T in recurrent pregnancy loss, in particular, early loss.⁹⁻¹¹ On the other hand, other authors found a negative association stating that MTHFR polymorphisms do not carry any risk in pregnancy.^{12,13} The different inclusion criteria and the different ethnic backgrounds of the selected patients may have contributed to the contradictory results.

In the current study, the total prevalence of the three gene mutations namely, factor V Leiden, prothrombin gene G20210A and MTHFR C677T were significantly increased in the patients group compared to controls. This is in accordance with some previous studies as those reported by Bradley et al, Aksoy et al and Raziel et al.¹³⁻¹⁵ However, other large prospective studies reported contradictory results stating that hypercoagulable thrombophilic gene mutations are not increased in women with recurrent miscarriage.^{8,16,17}

In fact, case control and cohort studies reflect methodological diversity and clinical heterogeneity. Studies have been conducted in different countries, using different study designs and in routine care settings and high-risk referral centers. Study limitations have included inadequately described and/or heterogeneous case and control groups and cohorts, insufficient information to adequately assess potential biases, and missing or

incomplete information on important covariates as maternal age and number and timing of losses.

In the present study, there was a significant increase in the number of cases with combined thrombophilia in the patients group compared to controls. Combined thrombophilia included Factor V Leiden and MTHFR C677T only and none of the cases involved prothrombin gene G20210A mutation. The same results were reported by previous studies that identified combined thrombophilic defects in women with recurrent pregnancy loss, both early and late.^{10,18,19} The study by Rozano-Gorelick et al reported that combined thrombophilia exists when inherited and /or acquired prothrombotic factors are pooled and every combination carries a different risk of thrombosis.¹⁸ Furthermore, Sarig et al proposed a scoring system for women with thrombophilia based on four major categories: obstetric history, previous thromboembolic events, family history of thrombosis or gestational vascular complications and type of thrombophilia.²⁰

Finally, the number of homozygous and heterozygous individuals were assessed for each of the gene mutations studied. No homozygosity was detected in controls and heterozygotes were significantly increased in the patients group compared to homozygotes.

A study by Couto et al reported a low prevalence of homozygotes for factor V Leiden and stated that the prothrombotic tendency during pregnancy and the risk of thromboembolic events is increased with antithrombin deficiency and homozygous factor V Leiden as single traits. In fact, the reported prevalence in the general population of factor V Leiden and prothrombin gene homozygotes is less than 1% with 2-4% risk of venous thromboembolism (VTE) per pregnancy increasing to around 17% in women with a previous history of VTE.^{21,22}

CONCLUSION

MTHFR C667T mutations and the total prevalence of factor V Leiden, prothrombin gene and MTHFR gene mutations are significantly increased in patients having recurrent miscarriages. Furthermore, the prevalence of combined thrombophilia (Factor V Leiden and MTHFR C677T) is increased in these patients. However, this combined thrombophilia does not involve prothrombin gene G20210A mutations.

The incidence of heterozygosity for each of the three gene mutations in recurrent miscarriage patients is significantly higher than homozygosity, while none of the controls had a homozygous gene mutation.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Wilcox AJ, Weinberg CR, O'Connor JF. Incidence of early loss of pregnancy. *N Engl J Med.* 2000;319(4):189-94.
2. Christian OB, Steffensen R, Nielsen HS, Varming K. Multifactorial etiology of recurrent miscarriage and its scientific and clinical implications. *Gynecol Obstet Invest.* 2008;66:257-67
3. Kupfermanc MJ. Thrombophilia and pregnancy. *Reprod Biol Endocrinol.* 2003;1:111.
4. Rodger MA, Paidas M, McLintock C. Inherited thrombophilia and pregnancy complications revisited. *Obstet Gynecol.* 2008;112:320.
5. Greer IA. The challenge of thrombophilia in maternal-fetal medicine. *N Engl J Med.* 2000;342:424.
6. Yamada H, Kato EH, Kobashi G, Ebina Y. Recurrent pregnancy loss: etiology of thrombophilia. *Semin Thromb Hemost.* 2001;27(2):121- 9.
7. Said JM, Higgins JR, Moses EK. Inherited thrombophilia polymorphisms in pregnancy outcomes in nulliparous women. *Obstet Gynecol.* 2010;115:5.
8. Dizon-Townson D, Miller C, Sibai B. The relationship of factor V Leiden mutation and pregnancy outcomes for mother and fetus. *Obstet Gynecol.* 2005;106:517-24.
9. Bouvier S, Cochery-Nouvellon E, Lavigne-Lissalde G. Comparative incidence of pregnancy outcomes in thrombophilia positive women from the NOH-APS observational study. *Blood.* 2014;123:414.
10. Di Mico P, D' Uva M. Recurrent pregnancy loss and thrombophilia. *Open Atherosclerosis Thrombosis J.* 2009;2:33-5.
11. Lissak A, Sharon A, Fruchter O, Kassel A. Polymorphism for mutation of cytosine to thymine location 677 in the methylene-tetrahydrofolate reductase gene is associated with recurrent early pregnancy loss. *Am J Obstet Gynecol.* 1999;181:126-30.
12. 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;52:60-6
13. Ren A, Wang J. MTHFR C677T polymorphism and the risk of unexplained recurrent pregnancy loss: a meta analysis. *Fertil Steril.* 2006;86:1716.
14. Aksoy M, Tek I, Karabulut H, Berker B, Soylemez F. The role of thrombophilia related to Factor V and Factor II G20210A mutations in recurrent abortions. *J Pak Med Assoc.* 2005;55:104-8.
15. Raziel A, Kornberg Y, Friedler S. Hypercoagulable thrombophilic defects and hyperhomocysteinemia in patients with recurrent pregnancy loss. *Am J Reprod Immunol.* 2001;45:65-71.
16. Roque H, Paidas MJ, Funai EF. Maternal thrombophilias are not associated with early pregnancy loss. *Thromb Haemost.* 2004;91:290.
17. Clark P, Walker ID, Govan L. The GOAL study: a prospective examination of the impact of factor V Leiden and ABO blood groups on haemorrhagic and thrombotic pregnancy outcomes. *Br J Haematol.* 2008;140:236.
18. Rozano-Gorelick A, Papadakis E, Brenner B. Combined thrombophilia and obstetric complications. *The Open Atherosclerosis Thrombosis J.* 2009;2:38-41.
19. Coulam CB, Jeyendran RS, Fishel LA, Roussev R. Multiple thrombophilic gene mutations rather than specific gene mutations are risk factors for recurrent miscarriage. *Am J Reprod Immunol.* 2006;55:360-8.
20. Sarig G, Vidergor G, Brenner B. Assessment and management of high risk pregnancies in women with thrombophilia. *Blood Rev.* 2009;23(4):143-7.
21. Couto E, Nomura ML, Barini R, Pinto Silva JL. Pregnancy associated venous thromboembolism in combined heterozygous factor V Leiden and prothrombin G20210A mutations. *Sao Paulo Med J.* 2005;123(6):286-8.
22. Lockwood CHJ, Bauer KA, Leung LK, Ramin SM,. Inherited thrombophilias in pregnancy. *UpToDate.* 2011. Available at <http://cursoenarm.net/UPTODATE/contents/mobipreview.htm?27/37/28241?source=HISTORY>

Cite this article as: Abdelsalam T, Karkour T, Elbordiny M, Shalaby D, Abouzeid ZS. Thrombophilia gene mutations in relation to recurrent miscarriage. *Int J Reprod Contracept Obstet Gynecol* 2018;7:796-800.