СПИСАНИЕ НА МАКЕДОНСКОТО ЛЕКАРСКО ДРУШТВО
Мак. мед. преглед, 2019; 73(1)

JOURNAL OF THE MACEDONIAN MEDICAL ASSOCIATION
Mac. Med. Preview, 2019; 73(1)

UDK: 61+061.231=866=20 CODEN: MKMPA3 ISSN: 0025-1097

Македонски Медицински Преглед

Macedonian Medical Review

Основано 1946
Founded 1946

www.mld.mk

1/19

Мак Мед Преглед

MMP

Списание на Македонското лекарско друштво

Journal of the Macedonian Medical Association

Наслов на Редакцијата и издавачот / Address of the Editorial Office and Administration:
1000 Skopje, Dame Gruev 3, Gradski yid blok 2
tel. 02/3162 577
www.mld.org.mk / mld@unet.com.mk
Жиро сметка / Bank Account
300000000211884 - Komercijalna banka Skopje

Печати: Бранко Гапо графичко производство - Скопје

Македонски медицински преглед се печати три пати годишно. Претплатата за списанието изнесува 10 евра за лекари, 50 евра за установа, странство 80 евра.

Основано 1946 Founded 1946

Содржина/Contents

I. Оригинални трудови/ Original Articles

Мак Мед Прегледˇ 2019; 73(1): 19-23

ММР

Original article

INHERITED THROMBOPHILIA AND IMPLICATIONS IN PREGNANCY LOSS

НАСЛЕДНА ТРОМБОФИЛИЈА И ИМПЛИКАЦИИ ВО ГУБИТОКОТ НА БРЕМЕНОСТА

Elena Petkovikj[1], Rada Grubovic Rastvorceva[1] and Rozalinda Isjanovska[2]

[1]Institute of Transfusion Medicine - Skopje, [2]Institute of Epidemiology and Biostatistics with Medical Informatics, Medical Faculty, Ss. Cyril and Methodius University – Skopje, Republic of North Macedonia

Abstract

Introduction. Thrombophilia is a hypercoagulable condition with predisposition to thromboembolism. Recently, the inheritedthrombophilicmutations of the Factor V Leiden (FVL) G1691A gene, the methylentetrahydrofolate reductase (MTHFR) C677T gene and Factor II gene Prothrombin G20210A have been implicated in pregnancy loss. The **aim** of the study wasto evaluate the clinical characteristics and to examine the representation of thrombophilic mutations in women with pregnancy loss and healthy controls.
Methods. In a retrospective-prospective case-control study we evaluated 79women, divided in two groups. The study group included 43 women with history of pregnancy loss (missed abortion, blighted ovum, miscarriage in the first or second trimester,foetusmortus in utero). The control group included 36 women, age matched, who gave birth to at least one healthy baby without obstetric complications. Presence of gene mutations forprothrombin G20210A, FVL and MTHFR C677T was examined in both groups. Sociodemographic data, data from personal, family and obstetric anamnesis was collected with standard questionnaire.
Results. The average age of the study group was lower than that of the control group (median 30.7, range 20-41 *versus* median 32.8, range 23-44, respectively). Prothrombin G20210A heterozygous was found in 23.5% of the study group *vs.* 5.5% of the control group; FVL heterozygous was found in 23.5% of the study group *vs*. 2.8% of the control group; MTHFR homozygous was found in 48.8% of the study group *vs*. 5.5.% of the control group witha significant statistical difference ($p<0.05$).
Conclusion. The presence of thrombophilic mutations may predispose to pregnancy loss.

Keywords: thrombophilia, factor V Leiden, MTHFR, prothrombin, pregnancy loss

Апстракт

Вовед. Тромбофилија е состојбана хиперкоагулабилност со предиспозиција за тромбоемболизам. Неодамна, наследните тромбофилни мутации на генотза Factor V Leiden (FVL) G1691A, метилентетрахидрофолатредуктаза (MTHFR) C677T и Фактор II Протромбин G20210A се вмешани во губиток на бременоста.
Цел. Да се евалуираат клиничките карактеристики да се испита застапеноста на мутациите за тромбофилијакај жените со губиток на бременост и контролна група.
Методи. Во ретроспективно-проспективна студија на случај и контрола се евалуирани 79 жени, поделени во две групи. Во испитуваната група (ИГ) се вклучени 43 жени со историја нагубиток на бременоста (missed abortus, blighted ovum, спонтан абортус во прво или второ тримесечје, мртов плод). Во контролната група (КГ) се вклучени 36 жени на иста возраст, што веќе родиле, без акушерски компликации барем едно здраво дете. Кај жените е испитано присуство на мутации на генот за протромбин (G20210A), FVLиMTHFR C677T. Социо-демографски податоци, податоци од лична, акушерска и семејна анамнеза беа прибрани со стандарден прашалник.
Резултати. Просечната возраст на ИГ беше пониска од онаа на КГ (просек 30.7, опсег 20-41 наспроти просек 32.8, опсег 23-44, соодветно). Протромбин G20210A хетерозигот бил пронајден кај 23.5% од ИГ наспроти 5.5% од КГ, FVL хетерозигот бил пронајден кај 23.5% од ИГ наспроти 2.8% од КГ, MTHFR хомозигот бил пронајден кај 48.8% од ИГ наспроти 5.5% од КГ со сигнификантна статистичка разлика за $p <0.05$.
Заклучок. Присуството на тромбофилични мутации може да доведе до губиток на бременоста.

Клучнизборови: тромбофилија, фактор V Leiden, MTHFR, протромбин, губиток на бременост

Correspondence to: Elena Petkovikj, Institute of Transfusion Medicine -Skopje, Republic of North Macedonia E-mail: elena.petkovic@yahoo.com

Introduction

Thrombophilia occurs when the normal balance of the coagulation system is impaired. It can be inherited, i.e. a genetically determined predisposition for thromboembolism with a common occurrence at the age of 45-50 years, with tendency of frequent relapse and thrombotic incidents in unusual places [1]. The most common causes ofinherited thrombophilia are: deficiency of natural antagonist III antagonist III (ATIII), protein C (PC) deficiency, protein C (PS) deficiency, and factor V Leiden G1691A (FVL), factor II prothrombin G2010A and methylenetetrahydrofolate reductase C677T (MTHFR). The most common cause of acquired thrombophilia is the presence of antiphospholipid antibodies (APA) and lupus anticoagulant (LA).

The gene mutation for factor V Leiden G1691A is inherited in an autosomal dominant fashion. FVL is characterized by a weak anticoagulant response to activated PC, which is a natural anticoagulant protein that breaks down and inactivates Va and VIIIa, thereby preventing the further creation of thrombin.Leiden mutation produces PC resistance resulting in a prothrombotic condition that leads to a thrombosis risk [2-6]. This mutation is very common in the general population with a prevalence of 2-15%. The highest heterozygosity rate is found in Europe, while the mutation is very rare among Asian, African and Indigenous Australians [7].

Gene mutation for factor II Prothrombin G2010A is associated with an increased risk of thrombosis, complications and an unfavorable outcome of pregnancy [4-6,8]. The prevalence of heterozygotes is 2-6.5% in the European population [7].

Gene mutation for methylenetetrahydrofolate reductase C677T (MTHFR) results in decreased synthesis of 5-methyltetrahydrofolate, the primary methyl donor in the conversion of homocysteine to methionine. This results in increasing the plasma homocysteine concentrations, which then induces platelet aggregation by promoting endothelial oxidative damage and is a risk factor for thrombosis, atherosclerosis, recurrent pregnancy loss and fetal neural tube defects [4,6,9]. Mild and moderate hyperhomocysteinemia is autosomal dominant inherited present in up to 20% of the white European population [10].

Miscarriage is defined as the spontaneous loss of a fetus before it reaches viability and occurs in up to 15% of clinically recognized pregnancies[11].In the first trimester, the terms miscarriage, spontaneous abortion, and early pregnancy loss are used interchangeably, and there is no consensus on this terminology in the literature. Approximately 1 in every 10 pregnancies ends in early death of the embryo or the fetus (that is, before 20 weeks of gestation), and 1 in every 200 pregnancies ends in late fetal loss. Recurrent pregnancy loss (RPL) refers to three or more consecutive losses and occurs in 1% of couples trying to conceive [12]. The cause of RPL is not apparent and clarification of the cause is difficult due to the heterogeneity of the condition.Many different abnormalities including fetal chromosomal inversions or translocations, anatomic abnormalities of the maternal uterus, endocrinological abnormalities, and autoimmune disorders can result in recurrent fetal loss, but 50% of the causes for RPL remain unexplained [13]. Inadequate or abnormal placental vasculature may result in several complications that have potentially serious or even lethal consequences for the mother and her unborn child. These complications include preeclampsia, placental abruption, intrauterine growth retardation, miscarriage and stillbirth [7].

Recently, the inherited mutation of the Factor V Leiden G1691A gene, the MTHFR C677T gene, Factor II gene Prothrombin G20210A has beenimplicated in the loss of early pregnancy and*invitro* fertilization (IVF) failure by disrupting the initial vascularization process occurs during implantation, which is necessary for successful pregnancy.

The aimof this study was to evaluate the clinical characteristics and examinethe representation of thrombophilic mutations in women with pregnancy loss and healthy controls.

Materials and methods

This is retrospective-prospective case-control study conducted at the Institute of Transfusion Medicine – Skopje (ITM-Skopje). The study included 79 women divided in two groups. The data from the performed examinations and analyses in the outpatient clinic at ITM-Skopje in the period 2015-2018 were processed for the women with diagnoses according to the inclusion criteria.

Inclusion criteria:

- Women aged 18-45,
- Women with history of adverse pregnancy outcome: missed abortion, blighted ovum, miscarriage in the first or second trimester, fetus mortus in utero.

Exclusion criteria:

- Women who had a previous history of venous thromboembolism,
- Women with pre-existing causes of secondary thrombophilia: autoimmune disorders (such as systemic lupus erythematosus, rheumatoid arthritis, Hashimoto thyroiditis) positive AFA, positive LA, extreme obesity, dyslipidemia, nephrotic syndrome,
- Women who refused to participate in the study or gaveup at some point in the study.

In the study group, 43 women with a history of adverse pregnancy outcome (missed abortion, blighted ovum, miscarriage in the first or second trimester, foetusmortus) were included.

The control group included 36 women, age-matched, who gave birth to at least one healthy child without obstetric complications.

Presence of gene mutations for factor IIProthrombin G20210A, factor VLeidenG1691A and methylentetrahydrofolate reductase (MTHFR C677T) was examined in both groups. Sociodemographic data, data from personal, family and obstetric anamnesis werecollected with a standard questionnaire. Blood samples from the included women were examined in the Molecular Biology Laboratory at ITM - Skopje, with previously given informed consent before taking blood samples and using the results in the preparation of the study, approved by the Ethics Committee at the Medical Faculty - Skopje.

Method used to examine factor II factor G20210A, factor V Leiden G1691A and MTHFR C677T - molecular detection of point mutations using the Operon kit. It took 2ml venous blood taken with a vacutainer in K2EDTA.

Statistical analysis was performed with the statistical package STATISTICA 7.1; SPSS 13.0. Numerical series were analyzed with measures of central tendency and measures of dispersion of data. The Student's t-test (t) was used for testing the significance of difference between groups. A p-value less than 0.05 was considered as statistically significant.

Results

The representation of nationality in the studygroup and the control group was homogenous and according to the national structure in the Republic of Macedonia wasapproximately the same. Thirty-one women (72.1%) in the study group and twenty-five women (69.5%) in the control group were Macedonians, 20.9% of the women in the study group and 19.4% of the controls were Albanians, and 7% of the women in the studied group and 11.1% of the controls were of other ethnicity (Table 1).

The average age of the study group was lower than that of the control group (median 30.7, range 20-41 *versus* median 32.8, range 23-44 respectively), but the difference did not reach statistical significance (p>0.05).

Table 1. Ethnicity

Ethnicity	Patients N (%)	Controls N (%)
Macedonians	31 (72.1%)	25 (69.5%)
Albanians	9 (20.9%)	7 (19.4%)
Other	3 (7%)	4 (11.1%)
Total	43	36

The 43 women in the study group had a total of 125 pregnancies (mean 2.9±1.5) and the 36 women in the control group had a total of 77 pregnancies (mean 2.1±0.5) and the difference was statistically significant for p<0.05 (t-test=2.871773, p=0.005270). The average number of spontaneous abortions in the study group was 1.7±1.6 *versus* the control group 0.1±0.2; this difference reached a statistical significance for p<0.05 (t - test = 6.429524, p = 0.000000) (Table 2).

Table 2. Clinical features of pregnancy loss patients and controls

Age	Median	N	Std.Dev.	p
Patients	30.7	43	4.559317	>0.05
Controls	32.8	36	4.674398	
Number of pregnancies	**Median**	**N**	**Std.Dev.**	**p**
Patients	2.9	43	1.540160	>0.05
Controls	2.1	36	0.487136	
Number of spontaneous abortions	**Median**	**N**	**Std.Dev.**	**p**
Patients	1.7	43	1.559808	>0.05
Controls	0.1	36	0.232311	

Thirteen women (36.1%) in the control group and two patients (4.7%) in the study group had no thrombophilia. The percentage difference wasstatistically significant between the study and the control group for p <0.05 (Difference test, p=0.0008). F II prothrombin G20210A heterozygous was recorded in 10 patients (23.5%) of the study group *versus* 2 women (5.5%) of the control group and this difference reached a statistical significance for p<0.05 (Difference test, p=0.0269). Ten women (23.5%) with Factor V Leiden heterozygous were recorded in the study group and one woman (2.8%) in the control group. The percentage difference was statistically significant for p<0.05 (Difference test, p=0.0084). There was no statistical significance (p>0.05) between the two groups regarding the MTHFR C677T heterozygous genotype. Two women (4.7%) from the study

Table 3. Prevalence of thrombophilic mutation in patients and controls

Thrombophilic mutations	Patients		Controls		*P* value
	N	%	N	%	
Without mutation	2	4.7	13	36.1	p=0.0008
F II G20210A heterozygous	10	23.5	2	5.5	p=0.0269
FV Leidenheterozygous	10	23.5	1	2.8	p=0.0084
MTHFR C677T heterozygous	15	34.9	20	55.6	p>0.05
FV Leidenhomozygous	2	4.7	-	-	-
MTHFR C677T homozygous	21	48.8	2	5.5	p=0.0000
Total	43	100.0	36	100.0	

group had F V Leiden homozygous mutation and none of the healthy controls.MTHFR C677T homozygous mutation was recorded in 21 women (48.8%) of the study group and in 2 women (5.5%) of the control group. The percentage difference between the studyand the control group showed a high statistical significance($p < 0.05$) (Difference test, $p=0.0000$) (Table 3).

Discussion

Although many case-control studies have reported association between recurrent pregnancy loss (RPL) and inherited thrombophilia, the results are heterogeneous and the nature of this association has not been clarified, yet [14].

A statistical significance ($p<0.05$) was found between the groups regarding the absence of thrombophilic mutations. The differences between the two groups in our study showed a statistical significance for prothrombin G20210A heterozygous, Factor V Leiden heterozygous and MTHFR C677T homozygous, which are consistent with the literaturedata. In our study the most frequent mutation in the control groupwasfor MTHFR heterozigous, which is similar with the studies from other authors.

Kovac *et al*. in their study found that thrombophilia was considerably more common in women with pregnancy-associated complications in comparison with women with normal pregnancies, with 38% of the women with RFL and 54.5% of women with fetus mortus in utero had thrombophilia [15].

Jusic *et al.* investigated the association of FVL, prothrombin G20210A and MTHFR C677T in women with RPL. The results showed that FVL and MTHFR C677T were significantly associated with RPL [16].

Kovacheva *et al.* investigated the three gene mutations in 156 women with fetal loss in different trimester of pregnancy and 80 matched controls. They found that FVL was associated with a significantly increased risk for RFL in the second and third trimester; F II G20210A or MTHFR C677T was more common in a group of women with fetal loss in the first trimester compared to the controls [17].

Howard Carp *et al.* in a case-control study compared 108 women with recurrent miscarriage with 82 fertile parous control women without miscarriages and concluded that thrombophilia was not associated with recurrent pregnancy loss [18].

Martinelli *et al.* conducted a case-control study and included women with first unexplained late fetal loss and compared them with women with normal pregnancies. 16% of the study group had either FVL or prothrombin mutation, which was associated with an approximate tripling of the risk of late fetal loss, and 13% were homozygous for MTHFR [19].

Ozdemir *et al.* investigated the prevalence of 12 thrombophilic gene mutations in RPL couples. They found that homozygosity of MTHFR C677T genes in women with RPL, and heterozygosity of FVL in both parents play a crucial role and should be considered as a risk factor in RPL [20].

The findings from TREATS study have shown that thrombophilia is associated with increased risks of VTE and adverse pregnancy outcomes in women with thrombophilia during pregnancy. A selective screening based on prior VTE history is more cost-effective than universal screening [21].

Conclusion

In conclusion, our findings confirmed that the thrombophilic mutations: F II Prothrombin G20210A heterozygous, FVL heterozygous and MTHFR C677T homozygous are associated with a pregnancy loss and it can be implicated in the pathogenesis of the adverse pregnancy outcomes. This study demonstrates the potential to improve patient care through the use of anticoagulant drugs which are effective in the prevention of complications of pregnancy in thrombophilia carriers. However, further studies of the risk-benefit ratio of anticoagulant treatment are needed.

Conflict of interest statement. None declared.

References

1. Kutteh WH, Triplett DA. Thrombophilias and recurrent pregnancy loss. *SeminReprod Med* 2006; 24(1): 54-66.
2. Dahlback B. Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the factor V gene. *Haemostasis* 1994; 24: 139-151.
3. Kujovich JL. Factor V Leiden thrombophilia. *Genetics in medicine* 2011; 13: 1-16.
4. Lockwood CJ. Inherited thrombophilias in pregnant patients: detection and treatment paradigm. *Obstet Gynecol* 2002; 99(2): 333-341.
5. Vucić N, Frleta M, Petrović D, Ostojić V. Thrombophilia, preeclampsia and other pregnancy complications. *Acta Med Croatica* 2009; 63(4): 297-305.
6. Robertson L, Wu O, Langhorne P, *et al*. Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) Study. Thrombophilia in pregnancy: a systematic review. *Br J Haematol* 2006; 132(2): 171-196.
7. Walker ID. Thrombophilia in pregnancy. *Journal of Clinical Pathology* 2000; 53: 573-580.
8. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996; 88: 3698-3703.
9. Kluijtmans LA, van denHeuvel LP, Boers GH, *et al*. Molecular genetic analysis in mild hyperhomocystinemia: a common mutation in the methylene-tetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet* 1996; 58: 35-41.
10. Wilcken B, Bamforth F, Li Z, *et al*. Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000

newborns from 16 areas worldwide. *Journal of Medical Genetics* 2003; 40: 619-625.
11. Creagh MD, Malia RG, Cooper SM, *et al*. Screening for lupus anticoagulant and anticardiolipin antibodies in women with fetal loss. *J ClinPathol* 1991; 44(1): 45-47.
12. Stirrat GM. Recurrent miscarriage. I Definition and epidemiology. *Lancet* 1990; 336: 673-675.
13. Practice Committee of American Society for Reproductive Medicine Definitions of infertility and recurrent pregnancy loss: a committee opinion. *FertilSteril* 2013; 99: 63.
14. El Hachem H, Crepaux V, May-Panloup P, *et al*. Precurrent pregnancy loss. *Int J Womens Health* 2017; 9: 331-345.
15. Kovac M, Mitic G, Mikovic Z, *et al*. Thrombophilia in women with pregnancy-associated complications: fetal loss and pregnancy-related venous thromboembolism. *Gynecol Obstet Invest* 2010; 69(4): 233-238.
16. Jusic A, Balic D, Avdic A, *et al*. The association of factor V G1961A (factor V Leiden), prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G polymorphisms with recurrent pregnancy loss in Bosnian women. *Med Glas (Zenica)* 2018; 15(2): 158-163.
17. Kovaceva K, Ivanov P, Konova E, *et al*. Genetic thrombophilic defects (Factor V Leiden, prothrombin G20210A, MTHFR C677T) in women with recurrent fetal loss. *AkushGinekol (Sofija)* 2007; 46(7): 10-16.
18. Carp H, Salomon O, Seidman D, *et al*. Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. *Human Reproduction* 2002; 17(6): 1633-1637.
19. Martinelli I, Taioli E, Cetin I, *et al*. Mutations in coagulation factors in women with unexplained late fetal loss. *N Engl J Med* 2000; 343(14): 1015-1018.
20. Ozdemir O, Yenicesu GI, Silan F, *et al*. Recurrent pregnancy loss and its relation to combined parental thrombophilic gene mutations. *Genet Test Mol Biomarkers* 2012; 16(4): 279-286.
21. Wu O, Robertson L, Twaddle S, *et al*. Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. *Health Technol Assess* 2006; 10(11): 1-110.