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Original scientific article

THE IMPACT OF HEREDITARY THROMBOPHILIAS IN RECURRENT PREGNANCY LOSS

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Introduction: Recurrent pregnancy loss (RPL) is defined as two or more consecutive pregnancy loss which occurs before the 20th weeks of pregnancies for the last menstrual period. Hereditary cause of thrombophilic gene mutations and polymorphism may play an essential role in RPLs.

Material and Method: 291 women with a history of two or more consecutive abortions as a study group and 61 women without the history of miscarriages as a control group were included in a study. In this study we analysed the effects of Factor II Prothrombin mutation ,FV Leiden mutation, MTHFR C677T, MTHFT A1298C, PAI-1, β-fibrinogen, Factor XIIIA (V34L) and Glycoprotein IIIa (L33P) polymorphisms on RPL by using pyrosequencing. Chi-square and multiple regression analysis were used for statistical analysis.

Results: FII prothrombin mutation, FV Leiden mutation, MTHFR C677T, MTHFR A1298C, PAI1 and Beta fibrinogen were found statistically significant in the chi-square test. Heterozygous FV G1691A (OR:8.092, CI: 1.280-51.165), homozygous MTHFR A1298C (OR:17.621, CI: 3.644 - 85.203), Heterozygous MTHFR C677T (OR: 2.921 CI: 0.811-10.515), Homozygous MTHFR C677T (OR: 3.619 CI: 1.647-7.954), heterozygous MTHFR A1298C (OR: 5.989, CI: 2.574-13.934), homozygous PAI1 (OR: 8.756, CI: 2.805 -27.334), heterozygous PAI1 (OR: 7.114, CI: 3.145- 16.096) homozygous

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FibrinogenG455A (4.085, CI: 1.438-11.610) were found statistically significant in logistic regression analysis for RPL($p<0.05$).

Discussion: This study indicated that there is a significant association between thrombophilias and RPL. Therefore, it is important to detect thrombophilic mutations in RPL.

Key words: Hereditary, thrombophilia, pregnancy loss, pyrosequence

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as two or more consecutive pregnancy loss, based on immunologic, endocrinologic, anatomic, genetic, infectious, and thrombophilic causes (CHATZIDIMITRIOU *et al.*, 2017) which occurs before the 20th weeks of pregnancies for the last menstrual period (BENDER ATIK *et al.*, 2018; PRACTICE *et al*., 2012). Although, the prevalence of RPL was 1-2% of all pregnancies (FORD *et al.*, 2015), the etiology of about half of the pregnancy losses in RPL's was not illuminated, yet.

It is thought that hereditary and acquired thrombophilias have been playing a significant role in recurrent pregnancy losses. Previous studies were mainly based on acquired causes than hereditary thrombophilias. However, last studies indicated that there might be a significant correlation between hereditary thrombophilias and RPL (DOSSENBACH-GLANINGER e*t al.*, 2013; KOCHER *et al.*, 2007; PEREZA *et al.*, 2017; REY *et al.*, 2003). Therefore, it is essential to detect that the impact of hereditary thrombophilias on RPL. During the pregnancy, the level of Factor 2, 5, 7, 8, 9, 10, fibrinogen, and D- dimer are increased, while the level of PAI-1, protein C, and protein S are decreased. It is known that due to the tissue factors that are released from trophoblastic cells, the placenta, itself, may cause to increase in coagulation (ORMESHER *et al.*, 2016).

Previous studies were primarily focused on FV Leiden mutations, Factor II Prothrombin mutations, and MTHFR C677T, MTHFR A1298C (MCNAMEE *et al.*, 2012). In the literature, there are limited studies focused on PAI-1, β-fibrinogen, Factor XIIIA (V34L), and Glycoprotein IIIa (L33P). Previous studies were carried out on conventional molecular methods, however, in this study, we analyze the effects of FV Leiden mutation, Factor II Prothrombin mutation and MTHFR C677T, MTHFT A1298C, PAI-1, β-fibrinogen, Factor XIIIA (V34L), and Glycoprotein IIIa (L33P) polymorphisms on RPL by using pyrosequence, one of the next-generation sequencing technologies.

Mutations and polymorphisms on thrombophilic genes that affect coagulation factors may cause an increase in coagulation. However, the outcomes of the studies on the effects of polymorphisms and mutations on RPL are contradictory (KOCHER *et al.*, 2007; MAHMUTBEGOVIĆ *et al.*, 2017; REY *et al*., 2003). When defining RPL, there is a controversy issues on how many pregnancy losses can assessed as a threshold value or not. In this study we aimed to assess thrombophilic mutations in both 2or more pregnancy losses, 3 or more pregnancy losses and also compared within the groups and a control group.

MATERIALS AND METHODS

Patient selection

330 patients referred to Pamukkale University Medical Genetic Department with the complaint of two or more consecutive pregnancy losses. All patients signed informed consent

were received. Their medical background and family history were questioned. All patients' and spouses' pedigrees were drawn. All patients were questioned for the history of infections, drugs, consanguineous marriage, exposure of radiation and heavy metal, contraceptive methods such as intrauterine devices, history of medical operation in pregnancies.

Inclusion and Exclusion Criteria

Study group includes women with no immunologic, anatomic, infectious, endocrine disease. Patients who have a different etiology from thrombophilias were excluded from the studies. G-banding was performed on all patients and spouses. Inv (9) (p11q12) were detected in 3 women and t (11;22) was detected in a woman. These four patients were excluded from the studies. Overall 291 of 330 RPL patients were included in a study. Healthy 63 women, between the ages 23 to 53, were selected as a control group.

Assesment Criteria

In this study, we evaluated the members of the study and control group for FII prothrombin and FV Leiden mutations, MTHFR C677T, MTHFR A1298C, PAI-1, β-fibrinogen, Factor XIIIA (V34L), and Glycoprotein IIIa (L33P) polymorphisms. All polymorphism and mutations were assessed according to allele types: wild, heterozygous, and homozygous.

Molecular analysis

Blood samples of patients were collected into EDTA tubes. DNA was extracted by QIAGEN DNA Mini kit following manufacturer's protocols. For genotyping 353 individuals, we used a thrombophilia pyrosequence analyzing kit (LABGEN) according to the manufacturer's instructions. The PCR reactions were performed in 20 µl reaction mixture including the specific primers and 5 µl genomic DNA under the following conditions: 95 °C for 15 min followed by 32 cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 80 s and a final extension at 72 °C for 15 min and sequenced by QIAGEN®, Pyromark Q24 System. Factor II Prothrombin (G20210A) and Factor V Leiden (G1691A) mutations, MTHFR (C677T, A1298C), PAI-1, β-fibrinogen (G455A), Factor XIIIA (V34L), and Glycoprotein IIIa (L33P) polymorphisms were analyzed with PyroMark Q24 Advanced Software.

Statistical Analysis

Statistical analyses were carried out by using SPSS software version 25.0 (Chicago, Illinois, USA). Descriptive statistics and chi-square methods were performed. Continuous variables were expressed as median and standard deviation, while categorical variables were expressed as percentages. Pearson chi-square or Fisher exact tests were used. If necessary, Yates corrections were used. Variables that were found statistical significance in the chi-square test were included in multiple logistic regression analysis models. Multiple logistic regression models were performed both enter and stepwise (backward, conditional) method. Both tests' p-value is less than 0.05 was considered as statistically significant.

RESULTS

The mean age of the study group was 29.57 ± 5.651 and their spouse was 32.88 ± 5.607. The age of the subjects ranged from 18 to 48, on the other hand, the age of their spouse ranged from 23 to 53. Marriages in 43 couples were consanguineous, whereas 248 couples were non-consanguineous. 23 women have a history of becoming pregnant by using in vitro fertilization technique. Most of the patients had a pregnancy 2 or 3 times. 164 patients had no history of live birth. 15 of all pregnancies were terminated voluntarily. In 184 patients had a history of 2 miscarriages, however, 82 had a history of 3 miscarriages. Detailed demographic variables were shown in Table 1.

Table 1. Demographic and descriptive data of patients

	Study group (n=291)	2 recurrent pregnancy loss (n=184)	3 or more pregnancy loss (n=107)
Woman age (ort+SD)	29.57 ± 5.651	29.05 ± 5.412	32.27 ± 5.418
Male age (ort+SD)	32.88 ± 5.607	30.47 ± 5.961	33.93 ± 5.793
Consanguineus marriage(n)	43 (%14.8)	27 (%14.7)	16 (%15)
IVF pregnancy(n)	23 (%7.9)	13 (%7.1)	10 (%9.3)
Number of Previous pregnancies (n)			
2	105(%36.1)	105(%57.1)	-
3	102(%35.1)	61(%33.2)	41(%38.3)
4	55(%18.9)	17(%9.2)	38(%35.5)
5 or higher	29(%9.9)	1(%0.5)	28(%26.2)
Number of abortus (n)			
2	184(%63.2)	184(%100)	-
3	82(%28.2)	-	82(%76.6)
4 or higher	25(%8.6)	-	25(%23.4)
Number of voluntary C/S (n)			
Absent	276(%94.8)	173(%94)	103(%96.3)
Present	15(%5.2)	11(%6)	4(%3.7)
Livebirth (n)			
Absent	164(%56.4)	111(%60.3)	73(%49.5)
Present	127(%43.6)	73(%39.7)	54(%50.5)

The study group, 2 or more pregnancy losses, were compared to the control group by using a chi-square test. Whilst FV Leiden, FII prothrombin, MTHFR A1298C, MTHFR C677T, PAI-1, and beta-fibrinogen polymorphisms were found statistically significant ($p<0.05$), Glycoprotein IIIa (V33L) and Factor XIIIA (V34L) were not ($p>0.05$) (Table 2). On the other hand, our logistic regression model was able to clarify a significant degree of the effects of thrombophilic mutations and polymorphisms on RPL (88.4%). The value of Hosmer and Lemeshov test was 0.351, Cox & Snell R square was 0.259 and Nagelkerke R square was 0.428. Carriers of Heterozygous FV Leiden mutations had an 8 times higher risk from wild-type allele carriers (OR: 8.092 CI for 1.280-51.165, p:0.026). Whilst the carriers of heterozygous MTHFR A1298C polymorphism had about 6 times higher risk from wild-type allele carrier (OR: 5.989,

95% CI: 2.574-13.934, $p<0.001$), carriers of homozygous MTHFR A1298C polymorphism had about 17 times higher risk from wild-type allele carriers for RPL (OR: 17.621, 95% CI for 3.644-85.203, $p<0.001$). While the carriers of heterozygous PAI-1 polymorphism had about 7 times the higher risk from wild-type allele carrier (OR: 7.114, 95% CI: 3.145-16.096, $p<0.01$,) carriers of homozygous PAI-1 polymorphism had about 8 times higher risk from wild-type allele carriers for RPL (OR: 8.160, 95% CI: 2.442-27.272, $p=0.001$). Carriers of homozygous Fibrinogen G455A polymorphism had about 8 times the higher risk from wild-type allele carriers for RPL (OR:4.085 95% CI 1.438-11.610, $p=0.008$). No, statistically significant relations were detected between the Fibrinogen(G455A) homozygous allele, glycoprotein 3a (V33L) homozygous, and heterozygous allele (Table 3).

Table 2. Genotypes and allele frequencies for coagulation factor mutations and polymorphisms in the study and control groups.

Mutations and polymorphisms	Genotype	2 pregnancy losses	3 or more pregnancy losses	Study group	Control group	P values*
FV Leiden G1691A	AA	-	-	-	-	p=0.040
	GA	27(14.7%)	9(8.4%)	36(12.4%)	2 (3.2%)	
	GG	157(85.3%)	98(91.6%)	255(87.6%)	60(96.8%)	
FIIProtrombin G20210A	AA	-	-	-	-	p = 0.018
	GA	16(8.7%)	6(5.6%)	22(7.6%)	-	
	GG	168(91.3%)	101(94.4%)	269(92.4%)	62(100%)	
MTHFR C677T	TT	22(12%)	8(7.5%)	30(10.3%)	4(6.5%)	P = 0.008
	CT	62(33.7%)	51(47.7%)	113(38.8%)	13(22.1%)	
	CC	100(54.3%)	48(44.9%)	148(50.9%)	45(72.6%)	
MTHFR A1298C	CC	18(9.8%)	20(18.7%)	38(13.1%)	2(1.6%)	P < 0.001
	AC	84(45.7%)	36(33.6%)	120(41.2%)	9(4.8%)	
	AA	82(44.6%)	51(47.7%)	133(45.7%)	51(92.6%)	
PAI-1	4G/4G	45(24.5%)	28(26.2%)	73(25.1%)	4(6.5%)	P < 0.001
	4G/5G	89(48.4%)	37(34.5%)	126(43.3%)	10(16.1%)	
	5G/5G	50(27.2%)	42(39.3%)	92(31.6%)	48(77.4%)	
F13 Val34Ile	Ile/Ile	4(2.2%)	1(0.9%)	5(1.7%)	6(9.7%)	p>0.05
	Val/Ile	38(20.7%)	14(13.1%)	52(17.9%)	-	
	Ile/Ile	142(86.1%)	92(86%)	234(80.4%)	56(90.3%)	
ITGB3 C98T	TT	1(0.5%)	-	1(0.3%)	1(1.6%)	p>0.05
	CT	33(17.9%)	23(21.5%)	56(19.2%)	8(12.9%)	
	CC	150(81.5%)	84(78.5%)	234(80.4%)	53(85.5%)	
Fibrinogen G455A	AA	6(3.3%)	2(1.9%)	8(2.7%)	1(1.6%)	p=0.002
	GA	47(25.5%)	30(28%)	77(26.5%)	5(8.1%)	
	GG	131(71.2%)	75(70.1%)	206(70.8%)	56(90.3%)	

*All p values were by using chi-square test and based on the comparison between the study group and the control group.

Table 3. Multiple logistic regression analysis results of thrombophilic mutations and polymorphisms

Mutations and polymorphisms	OR	Confidence interval		P value
		Lower	Upper	
FV G1691A G/A	8.092	1.280	51.165	0.026
MTHFR A1298C A/C	5.989	2.574	13.934	<0.001
MTHFR A1298C C/C	17.621	3.644	85.203	<0.001
MTHFR C677T C/T	2.921	0.811	10.515	0.01
MTHFR C677T T/T	3.619	1.647	7.954	0.001
PAI-1 4G/5G 4G/4G	1	-	-	-
PAI-1 4G/5G 4G/5G	7.114	3.145	16.096	<0.001
PAI-1 4G/5G 5G/5G	8.756	2.805	27.334	<0.001
Fibrinogen G455A G/G	1	-	-	-
Fibrinogen G455A G/A	1.689	0.125	22.871	0.693
Fibrinogen G455A A/A	4.085	1.438	11.610	0.008
ITGB3 C98T C/T	0.149	0.006	3.584	0.241
ITGB3 C98T T/T	1.984	0.771	5.111	0.156

*All values are calculated by using Enter method.

The study group and control group were also evaluated for two additional factors: consanguineous marriage and IVF pregnancy. Between the Consanguineous marriage and RPL were found to have a statistical significance ($p<0.05$), however, there was no statistical significance between the IVF pregnancy and RPL for the chi-square test ($p>0.05$).

DISCUSSION

In this study, we have found a significant association between the FV-Leiden, FII Protrombine mutations, MTHFR A1298C, MTHFR C677T, PAI-1 and Fibrinogen G455A polymorphisms and RPL. However, we did not found same statistically significant association between the ITGB3C98T, and F13 Val34Ile polymorphisms.

FV Leiden and FII Prothrombin mutations are the mutations that both are accepted as the most associated with hereditary thrombophilias. The studies on hereditary thrombophilias and RPL have been mainly focused on these two mutations, so far. FV Leiden mutation is a point

mutation c.1691G>A (p. Arg506Gln) such as FII Prothrombin mutation c.20210G>A (KASHIF, *et al* 2015, STEVENS *et al*. 2016). Studies revealed that FV Leiden heterozygous mutation carriers have a 5-10 times higher risk for venous thrombosis from the population (ROSENDAAL *et al*. 1995). A study carried out by Chatzidimitriou *et al*. indicated that heterozygous FV allele carriers have a 7-fold increased risk for wild-type allele carriers (OR: 6.88, 95% CI: 0.82-57.62, p<0.05) (CHATZIDIMITRIOU *et al.*, 2017). Another study showed that FV Leiden mutant allele carriers have a 6-12-fold higher risk for the population (WOLF *et al*. 2003). A study performed on 5000 pregnant women in Europe revealed that FV Leiden mutant allele carriers have a 5 to 11-fold higher risk for pregnancy loss and stillbirth from wild-type allele carriers (KOCHER *et al*. 2007). A meta-analysis carried out by Rey et al indicated that FV mutation carriers have an about 8-fold risk to those who do not carry mutations for RPL (OR: 7.83, %95 CI:2.83-21.67, p<0.05) (REY *et al*., 2003). In our study, we found that about 9-fold increased risk for heterozygous mutation carriers (Table 3). On the other hand, in a Bosnian study, there is no statistically significant difference between mutant allele carriers and wild-type allele carriers (p:0.759) (MAHMUTBEGOVIĆ *et al*, 2017.).Two studies which are performed in Turkey and Greece, have been indicated that there isn't any statistical significance relevance between FII mutations and RPL (p>0.05) (ALTINTAS *et al* 2007). In the literature, there is contradictory study outcomes have been available on the effects of FII prothrombin mutations on RPL (REY *et al*. 2003). We found statistically significant outcomes in the chi-square test. However, we couldn't find the same association in logistic regression analysis outcomes.

Methylenetetrahydrofolate (MTHFR) enzyme is responsible for DNA methylation and has a significant role in folic acid metabolism and is encoded by the MTHFR gene. Homocysteine level is increased in the blood in a deficiency of the Methylenetetrahydrofolate enzyme. This may cause a tendency to blood coagulation. MTHFR genes have two common polymorphic variants: MTHFR A1298C, MTHFR C677T. The prevalence of MTHFR A1298C polymorphic variant in the population is estimated to be 1-5%. On the other hand, the prevalence of the MTHFR C677T variant is thought to be 25-40% in the community (LEVIN *et al*., 2016; YANG *et al*., 2016). One of the studies has been indicated that the risk of RPL increases up to 5 times in the compound heterozygous carriers compared to wild-allele carriers. Furthermore, some study has been shown that compound heterozygous carriers have been faced with a risk of RPL up to a 7-fold increase in wild-type allele carriers (OR:7.147, 95% CI for 2.313-22.084)(XU *et al*., 2018.). A meta-analysis which is performed by Pereza *et al* put forward that the carriers of MTHFR mutations have been a 3-7 times increased risk from wild-type allele carriers (PEREZA *et al*., 2017). In a study which is performed by Torabi et al in Iran, MTHFR A1298C mutant allele carriers have a 7 times higher risk from wild-type allele carriers on the subject of RPL (OR: 7.147, 95% CI for OR: 2.313−22.084) (TORABI *et al*. 2012). In our study, compatible with the previous studies in the literature, revealed that there is an increased risk of MTHFR C677T homozygous mutation carriers, which is about 3.6-fold higher than MTHFR C677T heterozygous mutation carriers. There is about 18-fold increased risk for MTHFR A1298C homozygous mutation carriers and about 6-fold for heterozygous carriers (Table 3).

PAI-1 is produced by platelet or endothelial cells and responsible for the inhibition of fibrinolysis. On the other hand, FXIII is responsible for the arrangement of blood coagulation. Furthermore, FXIII plays a crucial role in maintaining the continuity of fibrinolysis and

regulating blood flow to the placental bed by affecting fibrin cross-links. PAI-1 variants can be mainly categorized under the following three titles for the variety of polymorphisms: PAI 4G/ 4G, PAI 4G/5G, PAI 5G/5G (DOSSENBACH-GLANINGER *et al.*, 2013; TICCONI *et al.*, 2011). The most common form of the FXIII polymorphic variant is the point mutation involving the valine isoleucine transformation at the codon 34 of exon 2 (V34L). This variant is accumulated in the placenta, therefore the increased level of the cross-links and disrupts the placental activity. Thus, it is thought that it may lead to RPL (JEDDI-TEHRANI *et al*. 2010). However, Fibrinogen is the major coagulation factor in the blood. Both primary hemostasis and secondary hemostasis are affected by the changes in fibrinogen level ((WOOD, 2004). The change in G455A in the 5'UTR region of beta-fibrinogen results in an increase in plasma fibrinogen levels. Increased beta fibrinogen levels may lead to placental thrombosis and arterial complications (LI *et al* 2015). Gp3a is an antigenic protein encoded by ITGB3 which is located on the 17th chromosome. It is known that c.98C>T point mutation in the ITGB3 gene may cause a reduction of placental perfusion and spiral artery thrombosis. Therefore, it may cause RPL (CURTIS et al 2014; FAZELNIA *et al* 2016).

A meta-analysis carried out by Li et al revealed that PAI polymorphic variants are associated with RPL, particularly they stressed that PAI 4G/4G variants have a higher associated risk for RPL in Caucasian people (SHI *et al*., 2016). One of the Chinese studies indicated that PAI 4G/4G variants have a higher risk for RPL than PAI 5G/5G allele carriers (OR:4.8, 95% CI 2.23-10.35)(GUAN *et al* 2005). Another study put forward that 4G/4G allele carriers have a much higher risk than 5G/5G allele carriers (OR: 3.52, CI: 0.90-13.72, p: 0.070)(ELMAHGOUB *et al*. 2014). In our study, we have found that both PAI 4G/4G and PAI 4G/5G allele carriers have a much higher risk than 5G/5G allele carriers (Table 3).

An Iranian study revealed that there is a statistically significant association between the beta-fibrinogen mutant allele and recurrent pregnancy loss (OR: 5.024, 95% CI: 2.206–11.439). However, there is no statistically significant association between the ITGB3 gene mutant allele and recurrent pregnancy loss (OR: 0.266, 95% CI: 0.128–0.552) (JEDDI-TEHRANI *et al*. 2011). These outcomes were compatible with our study. Similarly, Fazelnia *et al* suggested that there is no statistically significant association between ITGB3 mutant allele (c.98C>T) carriers and recurrent pregnancy loss. Torabi *et al* found that there is a statistically significant association between beta fibrinogen mutant allele (G455A) carriers and recurrent pregnancy loss. However, it has not been shown that there is a statistically significant association between the prevalence of ITGB3 mutant allele carriers and RPL. Furthermore, a meta-analysis conducted by Jung et al found that there is no statistically significant difference between FXIII mutant allele carriers and wild-type allele carriers in the European and South American populations (JUNG *et al*. 2017). In our study, we found that there is a statistically significant relationship between the homozygous beta-fibrinogen polymorphic variant and RPL, however, we have not been found that there is a statistically significant relationship between the FXIII and ITGB3 variant.

CONCLUSION

This study indicates that there is a significant association between thrombophilias and RPL. Therefore, it is important to detect thrombophilic mutations in RPL. Our study includes sufficient number of patients for the retrospective cohort study, however, it is conducted as a

single-center study. In the future, further large-multicentric studies will continue to shed light on the association between the RPL and thrombophilic gene mutations and polymorphisms.

Ethics approval and consent to participate

The patients' samples were obtained with the informed consent of all participants. The Pamukkale University review board of the Ethics committee for non-invasive Clinical Research approved, code 60116787-020/113806.

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REFERENCES

ALTINTAS, ABDULLAH, SEMIR PASA, NURTEN AKDENIZ (2007): Factor V Leiden and G20210A prothrombin mutations in patients with recurrent pregnancy loss : data from the southeast of Turkey, Annals of Hematology, *86*(10), 727–731..

BENDER ATIK, R., O. B.CHRISTIANSEN, J,ELSON, A M.,KOLTE, S. LEWIS, S. MIDDELDORP, W. NELEN, B. PERAMO, S,QUENBY, N.VERMEULEN, M. GODDIJN (2018): ESHRE guideline: recurrent pregnancy loss. *H*uman Reproduction Open, *2018*(2), 1–12.

CHATZIDIMITRIOU, M., D.CHATZIDIMITRIOU, M.MAVRIDOU, C.ANETAKIS, F. CHATZOPOULOU, T.LIALIARIS, S.MITKA (2017): Thrombophilic gene polymorphisms and recurrent pregnancy loss in Greek women. International Journal of Laboratory Hematology, *39*(6), 590–595.

CURTIS, B. R., J. G. MCFARLAND (2014): Human platelet antigens - 2013. *Vox Sanguinis*, *106*(2), 93–102.

DOSSENBACH-GLANINGER, ASTRID, MICK VAN TROTSENBURG, CHRISTIAN OBERKANINS, JOHANNA ATAMANIUK (2013): Risk for Early Pregnancy Loss by Factor XIII Val34Leu : The Impact of Fibrinogen Concentration, *J*ournal of Clinical Laboratory Analysis, *27*(6), 444–449.

ELMAHGOUB, IMAN RIFAAT, REHAM ABDELALEEM AFIFY, ASMAA AHMED ABDEL AAL, VE WALID SAYED EL-SHERBINY (2014). Prevalence of Coagulation Factor XIII and Plasminogen Activator Inhibitor-1 Gene Polymorphisms among Egyptian Women Suffering from Unexplained Primary Recurrent Miscarriage. Journal of Reproductive Immunology *103* (Haziran): 18–22.

FAZELNIA, S., T. FARAZMANDFAR, S. M. B. HASHEMI (2016): Significant correlation of angiotensin converting enzyme and glycoprotein IIIa genes polymorphisms with unexplained recurrent pregnancy loss in north of Iran, *I*nternational Journal of Reproductive BioMedicine, *14*(5), 323–328.

FORD, H. B. AND D. J SCHUST (2009): Recurrent Pregnancy Loss : Etiology , Diagnosis , and Therapy, Reviews in Obstetrics and Gynecology, *42*(4), 37–38.

GUAN, L., X. DU, J. WANG, L.GAO, R. WANG, H. LI, S. WANG (2005): Association of genetic polymorphisms in plasminogen activator inhibitor-1 gene and 5,10-methylenetetrahydrofolate reductase gene with recurrent early spontaneous abortion. *Z*honghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese journal of medical genetics *22* (3): 330–33.

JEDDI-TEHRANI, M., R. TORABI, AFSANEH MOHAMMADZADEH, S. AREFI, M. KERAMATIPOUR, H. ZERAATI, A. HASAN ZARNANI, M. M. AKHONDI (2010): Investigating Association of Three Polymorphisms of Coagulation Factor XIII and Recurrent Pregnancy Loss, *A*merican Journal of Reproductive Immunology, *64*(3), 212–217

.JEDDI-TEHRANI, MAHMOOD, RAHELEH TORABI, AMIR H ZARNANI, AFSANEH MOHAMMADZADEH, SHAGHAYEGH EMAMI, SAEED ZAREI (2011): Analysis of Plasminogen Activator Inhibitor-1 , Integrin Beta3 , Beta Fibrinogen , and Methylenetetrahydrofolate Reductase Polymorphisms in Iranian Women with Recurrent Pregnancy, American

Journal of Reproductive Immunology, *66*(2), 149–156.

JUNG, JAE HYUN, JAE-HOON KIM, GWAN GYUSONG, SUNG JAE CHOI (2017): Association of the F13A1 Val34Leu polymorphism and recurrent pregnancy loss: A meta-analysis. European Journal of Obstetrics and Gynecology. *215*, 234–240. h

KASHIF, S., M. ALI KASHIF, A. SAEED (2015): The Association of Factor V Leiden Mutation with Recurrent Pregnancy Loss. *J*PMA. The Journal of the Pakistan Medical Association *65* (11): 1169–72.

KOCHER, O., C. CIROVIC, E. MALYNN, C. M ROWLAND, L. A BARE, B. A YOUNG, J. G HENSLEE (2007): Obstetric Complications in Patients With Hereditary Thrombophilia Identified Using the LCx Microparticle Enzyme Immunoassay A Controlled Study of 5 , 000 Patients, *A*merican Journal of Clinical Pathology, *127*(1), 68–75. h

LEVIN, BROOKE LEVENSELLER, AND E. VARGA (2016): MTHFR : Addressing Genetic Counseling Dilemmas Using Evidence-Based Literature. Journal of Genetic Counseling, 901–11. https://doi.org/10.1007/s10897-016-9956-7.

LI, J., H.WU, Y.CHEN, H. WU, H. XU, L.LI (2015): Genetic association between FXIII and β -fibrinogen genes and women with recurrent spontaneous abortion : a meta- analysis. *J*ournal of Assisted Reproduction and Genetics, *32*(5), 817–825.

MAHMUTBEGOVIĆ, E., D. MARJANOVIĆ, E.MEDJEDOVIĆ, N. MAHMUTBEGOVIĆ, S. DOGAN (2017): 677C > T polymorphisms in Bosnian women with pregnancy loss, **Bosnian Journal of Basic Medical Sciences**, *17*(4):309-314.

MCNAMEE, K., E. PREGNANCY, F. DAWOOD (2012): Best Practice & Research Clinical Obstetrics and Gynaecology Thrombophilia and early pregnancy loss. *B*est Practice & Research Clinical Obstetrics & Gynaecology *26* (1): 91–102. https://doi.org/10.1016/j.bpobgyn.2011.10.002.

ORMESHER, L., L. SIMCOX, C. TOWER, I. A GREER (2016): Management of inherited thrombophilia in pregnancy. Women's Health, *12*(4), 433–441. PEREZA, N. AND D. PH. (2017): Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion. Fertility and Sterility, vol*107*, no1, 150-159.

PEREZA, N., S.OSTOJIĆ, M., KAPOVIĆ, B.PETERLIN (2017): Systematic review and meta-analysis of genetic association studies in idiopathic recurrent spontaneous abortion. Fertility and Sterility, *107*(1), 150-159.e2.

PRACTICE, T., & R.MEDICINE (2012):. Evaluation and treatment of recurrent pregnancy loss : a committee opinion. Fertility and Sterility, *98*(5), 1103–1111.

REY, E., S. R KAHN, M. DAVID, I.SHRIER (2003): Thrombophilic disorders and fetal loss : a meta-analysis, Lancet, *361*(9361), 901–908.

ROSENDAAL, F R, T KOSTER, J P VANDENBROUCKE, P H REITSMA (1995):High Risk of Thrombosis in Patients Homozygous for Factor V Leiden (Activated Protein C Resistance). Blood *85* (6): 1504–8.

SHI, X., X. XIE, Y. JIA, S. LI (2016): Maternal genetic polymorphisms and unexplained recurrent miscarriage: a systematic review and meta-analysis". Clinical Genetics *91*(2).

STEVENS, S. M., S. C. WOLLER, K. A. BAUER, R.KASTHURI, M. CUSHMAN, M. STREIFF, W. LIM, J. D. DOUKETIS (2016): Guidance for the evaluation and treatment of hereditary and acquired thrombophilia. *J*ournal of Thrombosis and Thrombolysis *41* (1): 154–64. https://doi.org/10.1007/s11239-015-1316-1.

TICCONI, C., AND P. GRAVINA (2011):"Beta-Fibrinogen G – 455A Polymorphisms, Gynecologic and Obstetric Investigation, *71*(3), 198–201.

TORABI, R., S. ZAREI, H. ZERAATI, A. H. ZARNANI, M. M. AKHONDI, R. HADAVI (2012): Combination of Thrombophilic Gene Polymorphisms as a Cause of Increased the Risk of Recurrent Pregnancy Loss, Journal of Reproduction and Infertility, *13*(2), 89–94.

WOOD, M. (2004). Review Article Plasma Fibrinogen. *A*nn Clin Biochem 2004;, 430–440.

WOLF, C. E, H. HAUBELT, H. U. PAUER, B. HINNEY, C. KROME-CESAR, T. J LEGLER, P. HELLSTERN, G. EMONS, B. ZOLL, M. KÖHLER (2003): Recurrent Pregnancy Loss and Its Relation to FV Leiden, FII G20210A and Polymorphisms of

Plasminogen Activator and Plasminogen Activator Inhibitor. *P*athophysiology of Haemostasis and Thrombosis *33* (3): 134–37. https://doi.org/10.1159/000077821.

XU, Y. Y. BAN, L.RAN, Y. YU, S. ZHAI (2018): Relationship between unexplained recurrent pregnancy loss and. Fertility and Sterility, Fertility and Sterility, *111*(3), 597–603.

YANG, Y., Y. LUO, J, YUAN, Y.TANG, L.XIONG, M. M. XU, X. D. RAO, H. LIU (2016): Association between maternal, fetal and paternal MTHFR gene C677T and A1298C polymorphisms and risk of recurrent pregnancy loss: a comprehensive evaluation. Archives of Gynecology and Obstetrics, *293*(6), 1197–1211.

UTICAJ NASLEDNIH TROMBOFILIJA NA PONAVLJANJE GUBITKA TRUDNOĆE

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Izvod

Uvod: Rekurentni gubitak trudnoće (RPL) se definiše kao dva ili više uzastopnih gubitaka trudnoće koji se javljaju pre 20. nedelje trudnoće za poslednju menstruaciju. Nasledni uzrok trombofilnih genskih mutacija i polimorfizma mogu igrati suštinsku ulogu u RPL.

Materijal i metod: U studiju je uključena 291 žena sa istorijom dva ili više uzastopnih abortusa kao studijska grupa i 61 žena bez istorije pobačaja kao kontrolna grupa. U ovoj studiji analizirali smo efekte mutacije faktora II protrombina, mutacije FV Leidena, MTHFR C677T, MTHFT A1298C, PAI-1, b-fibrinogena, faktora KSIIIA (V34L) i glikoproteina IIIa (L33P) polimorfizama na RPL-e. Za statističku analizu korišćena je hi-kvadrat i analiza višestruke regresije.

Rezultati: FII mutacija protrombina, FV Leiden mutacija, MTHFR C677T, MTHFR A1298C, PAI1 i Beta fibrinogen su utvrđeni kao statistički značajni u hi-kvadrat testu. Heterozigot FV G1691A (OR:8.092, CI: 1.280-51.165), homozigot MTHFR A1298C (OR:17.621, CI: 3.644 - 85.203), Heterozigot MTHFR C677T:10. 3,619 CI: 1,647-7,954), heterozigot MTHFR A1298C (OR: 5,989, CI: 2,574-13,934), homozigot PAI1 (OR: 8,756, CI: 2,805 -2,805 -27,334 -27,334 heterozi1 1,334, heterozi1 1,334 1,334) homozigotni FibrinogenG455A (4.085, CI: 1.438-11.610) je utvrđeno kao statistički značajno u analizi logističke regresije za RPL ($p<0.05$).

Diskusija: Ova studija je pokazala da postoji značajna povezanost između trombofilije i RPL. Zbog toga je važno otkriti trombofilne mutacije u RPL.

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