TEKRARLAYAN DÜŞÜKLÜ HASTALARDA PIHTILAŞMA GENLERİ MUTASYONLARI

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ÖZET

Giriş: Tekrarlayan gebelik kaybının (RPL) çok farklı sebepleri olmakla birlikte bunlardan bir kısmıda pıhtılaşma sorunları ile ilgilidir. Bu noktada pıhtılaşma mekanizmasında rol alan protein ve enzimleri sentezleten FV LEIDEN, FVR 2, FXIII, β-FIBRINOGEN, PROTROMBIN, PAI 1, HPA 1, MT 677, MT 1298, ACE, APO E ve APO B genlerindeki belirtilen mutasyonlar mekanizmayı etkileyeceği için oldukça önemlidir. **Amaç:** Bu çalışmada tekrarlayan gebelik kaybı yaşayan hastalarda FV LEIDEN, FVR 2, FXIII, β-FIBRINOGEN, PROTROMBIN, PAI 1, HPA 1, MT 677, MT 1298, ACE, APO E ve APO B mutasyonlarını araştıran güncel makalelerin bulgularını birleştirip, kendi gözlemlerimizle mukayese ederek, mevcut veriler ışığında ortak bir sonuca ulaşmak hedeflendi. **Gereç ve yöntem:** Tekrarlayan düşüklü hastalarda pıhtılaşma faktörlerini belirleyen genlerdeki mutasyonları konu alan bilimsel makaleler tarandı, sonuçları özetlenerek tablo halinde sunuldu. Ayrıca pıhtılaşma ile ilgili mutasyonların diğer hastalıklarla olan ilişkileri de belirtildi. **Bulgular:** Tekrarlayan düşüklü hastalarla ilgili çalışmaların % 45?inde FV LEIDEN, %31'inde PROTROMBIN , % 89'unde FXIII , %29'unda β-FIBRINOGEN, % 75'inde PAI 1, % 44'ünde MT 677 ve % 33'ünde MT 1298 mutasyonuna rastlandı. **Sonuç:** FV LEIDEN, FVR 2, MT 677, FXIII ve β-FIBRINOGEN mutasyonları dünyanın farklı bölgelerinde tekrarlayan düşüklü hastalarda gözlenenler ile benzerlik göstermektedir. Bu bağlamda, RPL'li hastalarda RPL'nin nedenini açıklığa kavuşturmak için bu pıhtılaşma faktörlerini özellikle izlemek yararlı olabilir.

Anahtar Kelimeler: Habitüel Abortus, Trombofili, Factor V, Protrombin, Factor XIII, β-Fibrinojen

THROMBOPHILIC GENE MUTATIONS IN PATIENTS WITH RECURRENT PREGNANCY LOSS

ABSTRACT:

Background: Recurrent pregnancy loss (RPL) is caused by different factors, including genetics and thrombophilia. FV LEIDEN, FVR 2, FXIII, β-FIBRINOGEN , PROTROMBIN, PAI 1, HPA 1, MT 677, MT 1298, ACE, APO E, and APO B have been identified linking to hereditary thrombophilia.

Objective: In this study, the effect of 12 factors, FV LEIDEN, FVR 2, FXIII, β-FIBRINOGEN , PROTROMBIN, PAI 1, HPA 1, MT 677, MT 1298, ACE, APO E, and APO B are statistically evaluated in RPL patients data from recent articles.

Materials and methods: In patients with recurrent miscarriage, the polymorphisms in which clotting factor genes were statistically analyzed. In addition, were discussed coagulation genes which relation to other diseases.

Results: According to the studies related to this subject, FV LEIDEN (45%), PROTROMBIN (31%), FXIII (89%), β-FIBRINOGEN (29%), PAI 1 (75%), MT 677 (44%) and MT 1298 (33%) were related to the abortions.

Conclusion: Most commonly encounter mutations FV LEIDEN, FVR 2, MT 677, FXIII and β-FIBRINOGEN genes similarly between country of the world. In this context, it may be useful in patients with RPL to monitor the clotting factors in order to clarify the cause of RPL.

Keywords: Habitual Abortion, Trombophylia, Factor V, Protrombin, Factor XIII, β- Fibrinogen

INTRODUCTION

In developed countries one out of every ten pregnancies ends with early abortion (before 20th gestational week), one out of every two hundred pregnancies ends with late abortion (after 20th gestational week). The causes of of fetal loses may include; chrosomal aberration, non-chrosomal birth defects, antiphospholipid antibodies, diabetes mellitus, hypertensive illnesses, trauma, nonimmune hydrops, infections, placental (placental, fetal-maternal hemorrhage, cord problems, placental insufficiency, intrapartum asphyxia, placenta previa, twin-twin transfusion syndrome, chorioamnionitis), problematic birth, sepsis, acidosis, hypoxia, uterine rupture, postterm pregnancy, medicines). Although genetic and gynecological advances 10% of fetal losses are unexplained (1,2).

Despite the fact that first trimester fetal loses are to much causes, second and third trimester fetal losses are related to placental insufficiencies (3). Placental insufficiency and associated fetal losses have been reported in thrombophilia cases with antiphospholipid-antibody syndrome, antithrombin III, protein C and protein S deficiency (4,5,6). In recent years, a point mutation in the factor V gene (Guanine → Adenine change in nucleotide # 1691 : Factor V Leiden mutation) and the G20210A mutation in the prothrombin gene have also been associated with thrombophilia (7,8). Later, as a result of studies on the mutation of the Factor V Leiden was found to be associated with first and second trimester fetal losses.[9] Although the relationship between the third trimester loss and FVL mutation has been established in many studies, this issue has not yet been clarified.[6,10,11] It is not clear that the prothrombin (PT) gene mutation is associated with fetal losses. The studies in this subject shown different results (4). An effective uteroplacental circulation is an essential condition for successful pregnancy and this circulation can be affected by hemostatic disorders. Therefore, maternal thrombophilia (Factor V Leiden, MTHFR defect, Factor II mutations, Protein C, Protein S deficiencies) are obstetrically important pathologies (6). While the prevalence of F V Leiden mutation in healthy European populations was 0.47%, it have been detected 0.48% in whites including individuals living in North America and Australia (12). Adenine exchange of the guanine at nucleotide 20210 of the 3'-untranslated region of the prothrombin gene increases translation. Thus, more PT is synthesized from the liver. This leads to increased production of thrombin, which in turn increases the risk of thrombosis. Mutation is present in the white population at 1-5%. The mutation frequency increases from northern Europe to the south. The incidence of 1.7% in northern Europe is 3-5% in southern Europe and the Mediterranean (13). MTHFR which is another risk

factor in RPL is required for homocysteine metabolism. Genes associated with homocysteine metabolism and MTHFR gene mutations are among the causes of hyperhomocysteinemia. As a result of the C677T mutation in the MTHFR gene, the alanine which is in the catalytic domain of the MTHFR enzyme changed with valine. This mutation makes the enzyme thermolabile, reducing MTHFR activity in homozygotes and heterozygotes by 70% and 35%, respectively, in vitro conditions. Especially homocysteine levels in the period of the folate deficiency is increased in patients which has got C677T alleles in homozygous form. Plasma homocysteine levels are slightly icreased in individuals carrying this heterozygous form of the allele (14,15,16). The exact mechanism of the relationship between hyperhomocysteinemia and loss of pregnancy is not yet known, considerations about this mechanism are structural and neurological effects of the fetus, or increased thrombogenic potential in affected women and thrombosis. MTHFR deficiency is a cause of severe hyperhomocysteinemia and this mutation is accompanied by low folate levels, which can lead to mental retardation, skeletal anomalies, premature vascular disease, or thrombosis. High levels of homocysteine lead to increase the risk of neural tube defects, fetal loss, placental abruption and placental infarction. Research on women with fetal loss showed that the prevalence of homozygous MTHFR and fetal loss risk is sligthly increased compared to the control group (17). It is still not clear whether adding folate to diets reduces the risk of loss of pregnancy. MTHFR mutations are expressed as one of the risk factors in the etiopathopathogenesis of habitual abortions. In some studies, the frequency of homozygous variants was high among women with three or more recurrent loss pregnancies, whereas no correlation was found between MTHFR mutations and other pregnancy losses. In patients with idiopathic pregnancy losses, MTHFR mutation prevalences were found to be similar or lower than in the control group (18,19,20,21). Wramsby and colleagues conducted a study to analyze the prevalence of the G1691 mutation in the Factor V gene, the C677T mutation in the MTHFR gene, and the G20210A polymorphism in the Factor II gene in patients with primary habitual abortion. As a result of this study, the prevalence of Factor V Leiden mutation was higher in patients with primary habitual abortion and there was no difference between the control group and patients carrying the heterozygous MTHFR C677T and the Factor II G20210A mutation. According to these researchers results Factor V mutation causes high risks on patient with primary abortus than patients with secondary abortus (22,23).

1.1.The association of the coagulation factors with other diseases:

Factor V G1691A (Leiden): The G1619A mutation causing the formation of "Factor V Leiden" is one of the three cut-off points of the "Active Protein C APC)", which breaks down "Factor Va". For this reason APC resistance develops and Factor V Leiden is 10 times slower remains in circulation than Factor Va. While the risk of venous thrombosis is increased 3-5 times in mutation heterozygous carriers, the risk of thrombosis is 20 times higher in carriers carrying homozygous mutations. The prevalence of this mutation in Turkish society was reported as 5.2% (24,25,26).

Factor V H1299R (R2): This mutation causes a lighter APC resistance (27).

Prothrombin G20210A: Although the idea of hetererozygous mutation carriers cause thrombosis is controversial there is an increased risk for arterial and venous thrombosis in homozygous mutations, and the relationship with mutation fetal losses has also been reported. The prevalence of mutation in Turkish society is 2,6% (25,28,29).

Factor XIII V34L: Mutation causes increased Factor XIII activity (30).

Fibrinogen-455 G-A: It has been reported that there is an increased risk for ischemic stroke, particularly in those carrying mutations and in which fibrinogen levels are increased in heterozygous carriers of this promoter region mutation and this increase is more pronounced in Homozygotes (31,32).

PAI-1 4G-5G: Single nucleotide deletion / insertion (4G / 5G) mutation. The 4G deletion mutation results in "fibrinolytic activity deteriorating" due to an increase in the concentration of Plasminogen Activator Inhibitor 1 (PAI1 and increased tendency to thrombotic diseases (33,34).

GPIIIa L33P (HPA-1): Glycoprotein IIIa (GPIIIa) is the fibrinogen receptor located on the platelet membrane. The normal allele is referred to as A1 (a). The A2 (b) mutant allele has been reported to play a role in tendency to stroke, myocardial infarction and at an early age in acute coronary disease (35).

MTHFR C677T: At the 677 position in the methylenetetrahydrofolate reductase (MTHFR) gene, the enzyme that is synthesized after the AT exchange is more thermolabile and less active. This causes the folate concentration in the plasma to decrease and increase in the homocysteine concentration. It is known that the increase in homocysteine concentration predisposes to thromboembolism and atherosclerosis.[36,37]

MTHFR A1298C: It has been reported that mutations cause to increase plasma homocysteine level (36).

ApoB R3500Q: Apo B 100 is the main apolipoprotein of LDL and the ligand of the LDL receptor. Mutation delays the binding of LDL to the receptor The cleansing of LDL is reduced. As a result, susceptibility to atherosclerosis and cardiovascular diseases is increasing (38).

ApoE (E2, E3, E4): This mutation reduces the binding of LDL to the receptor in E4 allele carriers. As a result, the tendency to atherosclerosis and cardiovascular diseases increases. The normal allele is E3. The lowest amount of cholesterol is associated with the E2 allele (39,40).

ACE (I / D): Important in terms of susceptibility to hypertension. Persons with I / D polymorphism are more susceptible to hypertension.

MATERIALS AND METHODS

This study is based on comprehensive literature search. Research on the role of trombophilia genes involved in recurrent abortions has increased after 2000. The main reasons for this are spread of DNA Strip Technology and spesfic oligonucleotide probes for these genes have been synthesized for PCR. Researchers working in this issues choose normal people fort he control group and statisticaly compared the patients and control groups. If there is a difference “related gene mutation or polymorphism responsible for RPL” comment is made. The results of the studies summarized on the tables after related literature revieved (Table 1).

Table 1. A literature review comparing the frequency of coagulation factors between control and patient groups. (ND = no difference, DCG = There is a significant difference between the control group and the patient group, RF = There is no statistical difference between the control group and the patient groups, but the researcher stated that the region is "Risk Factor")

No	Reference	FV LEIDEN	FVR 2 PROTROMBIN	FXIII B FIBRINOG	PAI 1	HPA 1	MT 677	MT 1298	ACE	APO B	APO E
41	**Pihusch et al 2001**	ND	*	ND * ND	*	*	ND	*	*	*	*
42	**Carp et al 2002**	ND	*	ND * *	*	*	ND	*	*	*	*

43	**Finan et al 2002**	RF	*	RF	*	*	*	*	*	*	*	*	*
44	**Hohlagschwandtner et al 2003**	ND	*	ND	*	*	*	ND	ND	ND	*	ND	*
45	**Pauer et al 2003**	ND	*	ND	*	*	*	*	ND	*	*	*	*
46	**Li et al 2004**	*	*	*	*	*	*	*	ND (RF)	*	*	*	*
47	**Camilleri et al 2004**	DCG	*	*	*	ND	*	*	ND	*	*	*	*
48	**Guan et al 2005**	*	*	*	*	*	DCG	*	DCG	*	*	*	*
49	**Kobashi et al 2005**	*	*	*	*	*	*	*	*	*	*	*	*
50	**Jivraj et al 2006**	ND	*	ND	*	*	*	*	ND	*	*	*	*
51	**Coluam et al 2006**	ND	ND	ND	ND	ND	ND	ND	ND	ND	*	*	*
52	**Goodman et al 2006**	ND	ND	ND	DCG	ND	ND	ND	DCG	ND	*	*	*
53	**Mtiraoui et al 2006**	*	*	*	*	*	*	*	DCG	DCG	*	*	*
54	**Xu et al 2007**	ND	*	ND	*	*	*	*	DCG	*	*	*	*
55	**Androutsopoulos et al 2007**	RF	*	RF	*	*	*	*	RF	*	*	*	*
56	**Kovacheva et al 2007**	DCG	*	ND	*	*	*	*	ND	*	*	*	*
57	**Altintas et al 2007**	ND	*	ND	*	*	*	*	*	*	*	*	*
58	**Coulam et al 2008**	ND	ND	ND	DCG	ND	DCG	ND	ND	ND	*	*	*
5	**Kadir et al 2009**	*	*	*	R	R	*	*	*	*	*	*	*

9					F	F							
60	Torabi et al 2012	DCG	*	*	DCG	DCG	*	*	*	DCG	*	*	*
61	Tabibian et al 2016	*	*	*	DCG	*	*	*	*	*	*	*	*
62	Jaslow et al 2009	*	ND	*	*	*	*	*	*	*	*	*	*
63	Su et al 2013	*	*	*	*	*	DCG	*	*	*	DCG	*	*
64	Isazadeh et al 2017	*	*	*	DCG	*	*	*	*	*	*	*	*
65	Li et al 2015	*	*	*	*	*	DCG	*	*	*	*	*	*
66	Izuhara et al 2017	ND	*	*	*	*	*	*	*	*	*	*	*
67	Yenicesu et al 2010	DCG	*	DCG	DCG	*	DCG	*	DCG	*	*	*	DCG
68	Elmahgouba et al 2014	*	*	*	DCG	*	DCG	*	*	*	*	*	*
69	Sharma et al 2015	DCG	*	*	*	*	*	*	*	*	*	*	*
70	Fakhr-Eldeen et al 2017	DCG	*	DCG	*	*	*	*	ND	*	*	*	*
71	Lenz et al 2016	DCG	*	DCG	*	*	*	*	DCG	*	*	*	*

RESULTS

As shown in Table 1, 12 different thrombophilia polymorphisms related to recurrent abortions have not been thoroughly studied in all studies. However, the majority of panel tests are sufficient. FV LEIDEN one of the studied test parameters, was the subject of 20 different researches, there were no differences between control and patient groups at 11 of 20. But 9 of 20 were identified as statistically different and risk factors which corresponds to 45 per cent. 9 Different studies were

carried out on FXIII gene polimorphism. There was no difference on 1 of these studies. There was a difference on 8 of them. This value corresponds to 89%. 8 Different studies were carried out on PAI 1 gene polimorphism. There was no difference on 2 of these studies. There was a difference on 6 of them. This value corresponds to 75%. 18 Different studies were carried out on MT 677 gene polimorphism. There was no difference on 10 of these studies. There was a difference on 8 of them. This value corresponds to 44%. 16 Different studies were carried out on PROTROMBIN gene polimorphism. There was no difference on 11 of these studies. There was a difference on 5 of them. This value corresponds to 31%. 6 Different studies were carried out on MT 1298 gene polimorphism. There was no difference on 4 of these studies. There was a difference on 2 of them. This value corresponds to 33% and 7 different studies were carried out on β-FIBRINOGEN gene polimorphism. There was no difference on 5 of these studies. There was a difference on 2 of them. This value corresponds to 29% (Table 2).

Table 2. Summarized literature review. (ND = no difference, DCG = There is a significant difference between the control group and the patient group, RF = There is no statistical difference between the control group and the patient groups, but the researcher stated that the region is "Risk Factor")

	FV LEIDEN	**FVR 2**	**PROTROMBIN**	**FXIII**	**β-FIBRINOG**	**PAI 1**	**HPA 1**	**MT 677**	**MT 1298**	**ACE**	**APO B**	**APO E**
Number of works done	20	4	16	9	7	8	4	18	6	1	1	1
No difference (ND)	11	4	11	1	5	2	4	10	4	0	1	0
Risk Factor (RF)	2	0	2	1	1	0	0	2	0	0	0	0
Statistically different (DCG)	7	0	3	7	1	6	0	6	2	1	0	1
(ND)%	55%	100 %	69%	11 %	71 %	25 %	100 %	56%	67%	0%	100 %	0%
(RF + DCG) %	45%	0%	31%	89 %	29 %	75 %	0 %	44%	33%	100 %	0 %	100 %

DISCUSSION

According to recent research ultraplacental failure cause increases ratio of fetal loss in patients with unsufficient protein C, Protein S and antitrombine III.The fact that this phenomenon of thrombosis resulted from resistance of Active Protein C that result from mutation of Factor V Leiden has been reported (72).

Nelen et all showed that mutation of MTHFR induced to high bood level of homocystein and Recurren spontane aborthus frequencies was 2 - 3 times increased by hiperhomocysteinemia Protrombine 20210 G A mutation cause low tromboembolia risk as well as rarely see in population

than Factor V Leiden mutation (73). Poort and colleagues found prothrombin 20210 G → A mutation in 29 of 471 patients (6.2%) who had a thromboembolic attack for the first time. This ratio is 2.3% in the control group. and the prothrombin 20210 G → A mutation increases the risk of venous thrombosis by 2.8 fold (74). When the literature on the coagulation factors was carefully examined, Factor V Leiden frequency showed a significant difference between the control group and the patients group according to the findings of Camilleri and Kovacheva (47,56). According to the studies of Goodman et al (2006), Yenicesu et al (2010), Sharma et al (2015), Fakhr-Eldeen et al (2017), Lenz et al (2016) and Coulam et al (2008), Factor XIII showed significant differences between the control and patients groups as in the case of Elmahgouba et al (2014) (52,58,67,68,69,70,71). In addition, Kadir et al (2009) reported this as a risk factor. Guan et al (2005) and Coulam et al (2008) found that there was a significant difference between controls and patients groups on PAI 1 (48,59). Factor V R2 and β- Fibrinogen showed significant differences only in this study between control and patient groups. Prothrombin, HPA 1 and APO B, and there was no significant difference between control and patients groups according to as we observed in our patients. ACE and APO E have not been studied by other researchers. Goodman et al 2006, Guan et al 2005, Mtiraoui et al 2006, and Xu et al 2007 did not show any significant differences between the control and patients groups in their study of MT 677. But Androutsopoulos et al 2007 and Li et al 2004 reported these as risk factors. Factor V can be identified as high risk factors according to in our experiences (46,53,54,55). The events we observed in our clinic are consistent with the literature too.

CONCLUSION

If the patient has had miscarriage at 2 or more miscarriages, the clotting factors should be carefully observed. In conclusion, our results add evidence of the role of some clotting factors genotypes may the risk of miscarriages. In particular, they may help explain why some patients are at high risk for recurrent abortus, In the future, genotyping for this factors genetic markers may help identify subgroups of patients with recurrent abortuse who might benefit from various therapies. Consequently it is very important to investigate the clotting factors in the abortus that occur in the first trimester.

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