



CODEN [USA]: IAJPBB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCE**<http://doi.org/10.5281/zenodo.1001734>Available online at: <http://www.iajps.com>

Research Article

**SURVEY THE MUTATION OF FGB (BETA FIBRINOGEN) AND FV
(FACTOR V LEIDEN), FACTOR XIII AND FACTOR II
(PROTHROMBIN), IN PATIENTS WITH RECURRENT ABORTIONS
ALONG WITH NORMAL KARYOTYPE****Maryam Hosseini Moghadam¹, Pegah Larki², Jafar Panahi³, Niloofar Mojtahedifard⁴,
Abbas Shakoori^{5*}**¹Cardiogenetic Research Laboratory, Rajaie Cardiovascular Medical and Research Center, Iran
University of Medical Sciences, Tehran, Iran.²Shahid Beheshti University of Medical sciences, Medical Genetics, Tehran, Iran.³Ilam University of Medical Sciences, Ilam, Iran.⁴Islamic Azad University Varamin Pishva Branch, Tehran, Iran.⁵Department of Medical Genetics, Imam Khomeini hospital Complex, Tehran University of
Medical Sciences, Tehran, Iran.**Abstract:**

Some pregnancies are abnormal in human genetically and end with the spontaneous abortion, which is the most common problem of pregnancy. The recurrent abortions are often referred to as multifactorial disease that one of which is thrombosis. The thrombosis in placenta capillaries seems to disturb the blood circulation between the mother and the fetus and eventually lead to abortion. Recently, studies have shown that genetic basis for thrombophilia relates with recurrent abortion. The aim of this study is the survey of G1691A and G4070A mutations in the Factor V gene, -455G>A mutation in the gen of XIII factor, G103T mutation in Beta fibrinogen and A20210G mutation in the thrombin gene. The samples were collected from 60 patients referred to Tehran Imam Khomeini hospital .DNA was extracted from patients' blood samples by multiple PCR simultaneously containing different mutations were duplicated then the existence of mutation was evaluated by the strip technique. The genes mutation of G1691A in Factor V, G4070A in Factor V, G103T in Beta fibrinogen, -455G>A in the XIII factor and G20210A were identified 6.6%, 45%, 36%, 40% and 3.3% respectively. Studies on the other population showed that frequency of examined mutations varies with other communities. Anyway, more samples are required in order to obtain more accurate statistics related to the frequency of mutations.

Keywords: Factor V, Factor XIII, fibrinogen, prothrombin, spontaneous abortion, thrombosis, thrombophilia, strip technique.

Corresponding author:**Abbas Shakoori,**

Department of Medical Genetics,
Imam Khomeini Hospital Complex,
Tehran University of Medical Sciences,
Tehran, Iran.

Email: jahanj852@gmail.com

QR code



Please cite this article in press as Abbas Shakoori et al, Survey the Mutation of FGB (Beta Fibrinogen) and FV (Factor V Leiden), Factor XIII and Factor II (Prothrombin), in Patients with Recurrent Abortions along with Normal Karyotype, Indo Am. J. P. Sci, 2017; 4(10).

INTRODUCTION:

Spontaneous abortion is the spontaneous loss of pregnancy before embryo viability. Therefore, this term covers all the loss of pregnancy until the 24th week of pregnancy which occurs continuously or occasionally [1, 2 and 3]. Recurrent spontaneous abortion is often classified as a multifactorial diseases, in which various causes and factors play a role in its incident such as genetically, immunological, coagulation and thrombophilic, hormonal and anatomical [4]. Repeated abortion is classified into primary- secondary types. In the primary abortion happens several consecutive abortion and the secondary type begin successive abortion after a successful pregnancy [5, 6 and 7]. Immunological reactions play an important role in recurrent abortions. A current finding is concentrated on interaction between mother's immune system and embryonic antigens [8]. Blood coagulation factors during pregnancy play a significant role in occurrence of repeated fetal abortion and hereditary thrombophilia is known as an agent for the recurrent abortion. During pregnancy, it occurs pre-coagulation naturally for increasing coagulation pre-factor and decreasing the level of anticoagulation agents. Also, thrombosis in placenta capillaries is caused disorder in blood circulation between mother and fetus, ultimately leading to abortion [9, 10 and 11]. Thrombin regulates conversion of fibrinogen to fibrin which is the main component of blood clot. Thrombin also activates blood coagulation factors as V, VIII, XI and XIII factors (3 from 5). In addition to the central role in blood Pcoagulation cascade, thrombin leads to create inflammatory responses regulated by receptor, cell proliferation and apoptosis [12, 13 and 14]. Resistant of factor V to degradation as a genetic risk factor with other environment risk factors increases the likelihood of the venous blood clots. Some studies reported that over 95% APC resistance was due to mutation of Factor V Leiden [15, 16 and 17]. The Factor V Leiden causes increasing in abortion and end of pregnancy 2 to 3 times. Coagulation factor XIII is a proenzyme that has transglutaminase property. This factor acts on the last part of the coagulation cascade and fibrin clot without covalent bonds is converted to fibrin clot by the enzyme. Recurrent abortion is often referred to a multifactorial disease one of which is thrombosis. Thrombosis in placenta capillaries seems to cause disordering in the blood circulation between mother and fetus, finally lead to abortion. Recently, studies have known that existence of genetic basis in thrombophilic related to recurrent abortion [18, 19 and 20]. The goal of this study is the survey mutations of G1691A and G4070A in factor V gene,

-455G>A in factor XIII gene, G103T in Beta fibrinogen gene and A20210G in thrombin gene.

MATERIALS AND METHODS:

The present study is case series study and was performed for surveying mutations of Beta fibrinogen gene (FGB) and Factor V Leiden (FV), factor XIII and factor II (prothrombin) on the patients referred to genetic section of Imam Khomeini hospital with recurrent abortion along with normal karyotype. For this purpose, sampling was done from 60 patients with a range of 20 to 38 years old in 18 months. Each of the patients had at least one spontaneous abortion.

DNA Extraction from Blood Samples and Determining Concentration

DNA extraction was done using saturated salt method. It was used NanoDrop device for determining quantity and quality of extracted DNA. After the blanking device, DNA absorption was read just with 2 microliter from extracted DNA. Quantity and quality of extracted DNA was found by the data from NanoDrop device. Most of extracted samples had absorption over 500 ng/ul and must be diluted for using in genetic analysis such as PCR.

Multiplex PCR

Multiplex PCR are used to reproduce multiple regions of the genome simultaneously. In this method of PCR is used to various primers for proliferation of the genome different parts with various sizes in a single PCR mixture. In order to the size of proliferating parts is very close together, so it isn't possible to separate segments on the gel and to ensure the replication of segments by agarose gel. Therefore, polyacrylamide was used to confirm the replication of segments.

Strip Assay

Strip technique is a simple way for simultaneous checking several risk factors in a disease. It was used CVD Strip Assay for identification of several various mutations in different genes that are the risk factor of heart disease. ViennaLab CVD Strip Assay is capable to identify various mutations in the desired genes. ViennaLab CVD Strip Assay function is biotinized based on reverse hybridization of PCR products. Special probes of various variant and controls are hybrid with PCR product. It is used labeled oligonucleotides on Strip test and the results are detectable by the enzymatic color reactions with the naked eyes.

RESULTS:

In this research, mutation of Beta fibrinogen genes, factor XIII, factor II and mutation of factor V Leiden

in people with spontaneous recurrent abortion was investigated by Strip technique. After analyzing the results, these mutations were identified in various patients. Some patients only carry one and some patient had multiple mutations. In this project, special mutations in genes of factor V, factor XIII, prothrombin and Beta fibrinogen was surveyed. After

analyzing the results, mutations in people were 31 subjects in Factor V gene (27 mutation of G4070A and 4 mutation of G1691A), 2 mutation of G20210A in prothrombin, 22 mutation of G103T in Factor XIII and 22 mutation of -455G>A in Beta fibrinogen gene (Figure 1 - 2).

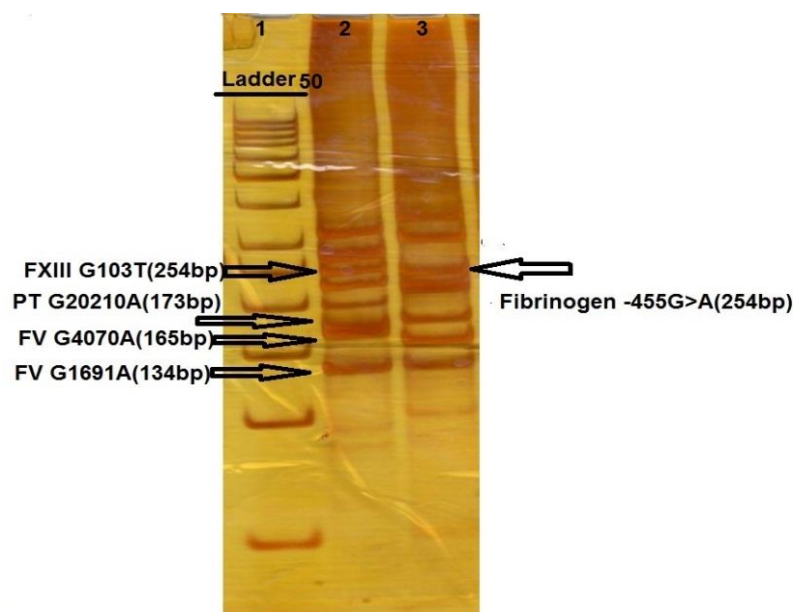


Fig 1: Confirmation of amplification by polyacrylamide gel. As indicated in the gel, various bands amplified for different segments are separated well. Lane 1: ladder 50 for identification of amplified segments. Lane 2: Strip T for surveying the mutation in the genes of FXIII, PT, FV. Lane 3: Strip A for checking the mutation in Beta fibrinogen.

Gene	Nucleotide mutations	Protein mutations	Alleles	Mutations in alleles	Frequency (%)
V Factor	G1691A	Arg506Gln	120	4	3/3
V Factor	A4070G	His1299Arg	120	27	2/25
XIII Factor	G103T	Val34Leu	120	26	2/16
Protromobin	G20210A	-----	120	2	1/6
Beta fibrinogen	-455G>A	-----	120	22	1/83

Fig 2: Frequency of mutations in Alleles

People with Mutation in a Gene

After the strip method, some of the patients had mutation in a gene. Of the total 60 patients, 28 patients carried mutation in one gene. Of the 28 patients who had mutation in a gene, 10 patients had mutation of V34L in the factor XIII gene, one of which was homozygote and the other was heterozygote. 5 patients had mutation of -455G>A in the Beta fibrinogen gene and 13 patients carried mutation of H1299R in the factor V gene.

People with Mutation in Several Genes

After reviewing the results, it was found that some patients carry a mutation in more than one gene. A disease with a distinct hereditary pattern may create due to a mutation in more than one gene; this phenomenon is called heterogeneity of the genetic location. In the current research, 23 out of 60 studied patients had mutation in several genes. Of these patients, 22 patients were mutated heterozygote in different genes and just 1 in mutant in factor XIII was homozygote, mutation in Beta fibrinogen was heterozygote.

People with Two Mutations in a Gene

The heterogeneity also occurs at the allele level and it was identified as mutation heterogeneity. There are people who have two different mutations in a genetic location, they are called compound heterozygote. In the research, after the tests, one of the patients who was compound heterozygote and had two mutations of G1691A and H1299R in factor V gene was identified.

DISCUSSION:

Studies that were done by Reznikoff *et al* in France in 2001, the frequency of factor V Leiden mutation was evaluated on 260 women with a history of spontaneous recurrent abortion. After analyzing, mutation of Factor V Leiden was detected in 10.38 % of the studied patients which didn't confirm to the present study [21]. Another study in 2002, the frequency of factor V Leiden was investigated by Sumreen Kashif in Pakistan and it was identified mutation of factor V Leiden in 5% (3 out of 56 patients)[22]. Reviews were done for identification of factor V Leiden mutation by Ekim *et al* in Turkey. The studied population included 90 people, 42 men and 48 women. Mutation of factor V Leiden had higher frequency in men than women; the frequency was 23.8% in men and 12.5% in women [23]. In the present research was detected mutation of factor V Leiden in 6.6% of patients, which differs from the frequency of mutations in other populations. All patients carrying the mutation of factor V Leiden were heterozygote. The allele frequency was 3.3%

for the allele of factor V Leiden that differs with allele frequency in population of France, Pakistan, Britain and Turkey. This difference is less with Pakistan and the reason of the difference with the other population included France may be due to the difference in the number of examined samples. Zaher K. Otrock *et al* was studied the frequency of A4050G polymorphism in Lebanon. The studied population consisted 73 patients with VTE1 and 125 normal people. A4070 polymorphism had 16.4% prevalence in the patients. Also, in the study of Yensso *et al* that was done in Turkey in 2010, the FXIII G103T heterozygote genotype was higher than the control group in the women with recurrent abortion significantly. In contrast, some studies have rejected the relationship between the factor and recurrent abortion including the study of Dosenbakh Glaninger *et al* in Australia in 2003, Baronoza *et al* in Brazil in 2008. A4070G polymorphism is related to increased risk of vascular thrombosis [24]. The frequency of A4070G polymorphism differs in various populations. The observed frequency of the polymorphism in patients with vascular thrombosis has been reported 7.8% to 18.5% in different population. The polymorphism frequency also has been reported 8.1% to 12.1% in normal people in various societies [25]. In this research, A4070G polymorphism was identified in 27 patients (45%) which all people had heterozygote mutation. The observed allele frequency was 0.225 for allele G in studied population is more than the allele frequency for the other societies. In the study that was done in Lids, the mutations of factor XIII gene was surveyed in 6 patients. All patients suffered from bleeding problems. In the study, all exogenous of factor XIII gene was sequenced and identified 10 various mutations in the patients. For the first in this study, the G103T mutation in exogenous 2 factors XIII was reported in one of the patients. It was detected the G103T mutation in 24 patients that 22 patients were heterozygote and 2 homozygote. 40% of the surveyed patients carried G103T mutation and the allele frequency was 0.433 for the mutant allele. The study was done in Turkey; the A20210 mutation was evaluated in 90 people who had no family relationship together. The studied population included 42 men and 48 women with a range of 17 to 78 years old. After analyzing, it was found that 2 people had heterozygote A20210 mutation. In fact, mutation of A20210 existed in 0.022 of the investigated subjects [23]. In a research, the frequency of A20210 mutation of prothrombin gene was evaluated in 110 people in Lebanon. The reviews showed that there are the mutations in 13.6% patients with history of spontaneous recurrent abortion and in 2.99% normal subjects that all people were

heterozygote. Based on the statistics obtained from the research was found that the A20210 mutation increases the risk of spontaneous abortion in women who are carrier of this mutation [26]. It was found A20210 mutation in 3.3% of the patients with spontaneous abortion. In fact, only 2 out of 60 patients had heterozygote mutation. The frequency of A20210 mutation observed in the studied population differed from the mutation frequency in other societies such as Turkey and Lebanon. In this research that 60 subjects was evaluated, it was identified nucleotide change in -455G>A mutation that all of them were heterozygote. The allele frequency observed for allele A was 1.83% in the studied population that was higher than allele frequency in Asian East countries and less than Lebanon, Greece and Lebanon.

CONCLUSION:

The results of the researches indicated that FVG4070A polymorphism has been the highest prevalence among the examined people. Also, the prevalence rate was different in the other mutations. Since the incidence of recurrent abortion has many destructive psychological and physical effects on the subjects, identification of agents that cause recurrent abortion is significant important to evaluation and presenting experimental tests for identifying the reason of the abortion and ultimately providing correct counseling to help couple involved. In order to the number of the samples in recent study to obtain more accurate statistic on the mutation frequency, it is suggested that more samples be evaluated in Iranian population.

REFERENCES:

1. Li TC, Makris M, Tomsu M, Tuckerman E, Laird S. Recurrent miscarriage: aetiology, management and prognosis. *Human reproduction update*. 2002; 8(5): 463-81.
2. Stephenson MD, Awartani KA, Robinson WP. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. *Human Reproduction*. 2002; 17(2): 446-51.
3. Regan L, Rai R. Epidemiology and the medical causes of miscarriage. *Best practice & research Clinical obstetrics & gynaecology*. 2000; 14(5): 839-54.
4. Carrington B, Sacks G, Regan L. Recurrent miscarriage: pathophysiology and outcome. *Current Opinion in Obstetrics and Gynecology*. 2005; 17(6): 591-97.
5. Nielsen HS. Secondary recurrent miscarriage and HY immunity. *Human reproduction update*. 2011; 17(4): 558-74.
6. Koopman LA, Kocow HD, Rybalov B, Boyson

- JE, Orange JS, Schatz F, Masch R, Lockwood CJ, Schachter AD, Park PJ, Strominger JL. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *Journal of Experimental Medicine*. 2003; 198(8): 1201-12.
7. Parazzini F, Boccolonev, Fedele L, Negri EVA, Vecchiam CLA, Acaia B, Risk Factors for Spontaneous Abortion. *International Journal of Epidemiology*. 1991; 20(1): 157-61.
8. Moffett-King A. Natural killer cells and pregnancy. *Nature reviews. Immunology*. 2002; 2(9): 656-63.
9. Baek KH, Lee EJ, Kim YS. Recurrent pregnancy loss: the key potential mechanisms. *Trends in Molecular Medicine*. 2007; 13(7): 310-17.
10. Kesmodel U, Wisborg K, Olsen SF, Brink Henriksen T, Jørgen Secher N. Moderate alcohol intake in pregnancy and the risk of spontaneous abortion. *Alcohol and Alcoholism*. 2002; 37(1): 87-92.
11. Regan L, Braude PR, Trembath PL. Influence of past reproductive performance on risk of Spontaneous abortion. *Bmj*. 1989; 299(6698): 541-45.
12. Ossovskaya VS, Bunnnett NW. Protease-activated receptors: contribution to physiology and disease. *Physiological reviews*. 2004; 84(2): 579-621.
13. Steinhoff M, Buddenkotte J, Shpacovitch V, Rattenholl A, Moormann C, Vergnolle N, Luger TA, Hollenberg MD. Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response. *Endocrine Reviews*. 2004; 26(1): 1-43.
14. Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. *BMJ*. 2000; 320(7251): 1708-12.
15. Greengard J, Sun X, Xu X, Fernandez J, Griffin J, Evatt B. Activated protein C resistance caused by Arg506Gln mutation in factor Va. *The Lancet*. 1994; 343(8909): 1361-62.
16. Van Cott EM, Khor B, Zehnder JL. Factor V Leiden. *American journal of hematology*. 2016; 91(1): 46-9.
17. Knudsen UB, Hansen V, Juul S, Secher NJ. Prognosis of a new pregnancy following previous spontaneous abortions. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 1991; 39(1): 31-6.
18. Komaromi I, Bagoly Z, Muszbek L. Factor XIII: novel structural and functional aspects. *Journal of Thrombosis and Haemostasis*. 2011; 9(1): 9-20.
19. Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Human Reproduction*. 1999; 14(11): 2868-71.

20. Martinelli I, Taioli E, Bucciarelli P, Akhavan S, Mannucci PM. Interaction between the G20210A mutation of the prothrombin gene and oral contraceptive use in deep vein thrombosis. *Arteriosclerosis, thrombosis, and vascular biology*. 1999; 19(3): 700-3.
21. Reznikoff-Etiévant MF, Cayol V, Carbonne B, Robert A, Coulet F, Milliez J. Factor V Leiden and G20210A prothrombin mutations are risk factors for very early recurrent miscarriage. *British Journal of Obstetrics and Gynaecology*. 2001; 108(12): 1251-54.
22. Kashif S, Kashif MA, Saeed A. The association of factor V Leiden mutation with recurrent pregnancy loss. *J Pak Med Assoc*. 2015; 65(1): 1169-72.
23. Ekim M, Ekim H, Yılmaz YK. The prevalence of Factor V Leiden, prothrombin G20210A, MTHFR C677T and MTHFR A1298C mutations in healthy Turkish population. *Hippokratia*. 2015; 19(4): 309-13.
24. Otrrock ZK, Taher AT, Shamseddeen WA, Zaatari G, Bazarbachi A, Mahfouz RA. Factor V HR2 haplotype: a risk factor for venous thromboembolism in individuals with absence of Factor V Leiden. *Annals of Hematology*. 2008; 87(12): 1013-16.
26. Finan RR, Tamim H, Ameen G, Sharida HE, Rashid M, Almawi WY. Prevalence of factor V G1691A (factor V-Leiden) and prothrombin G20210A gene mutations in a recurrent miscarriage population. *American journal of hematology*. 2002; 71(4): 300-5.