ORIGINAL ARTICLE

The association of factor V leiden mutation with recurrent pregnancy loss

Sumreen Kashif,[1] Muhammad Ali Kashif,[2] Anjum Saeed[3]

Abstract

Objective: To determine the association of factor V Leiden mutation with recurrent pregnancy loss.

Methods: The case-control study was conducted at the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from January to June 2012, and comprised women of 18 to 45 years of age who had a history of recurrent pregnancy loss, and controls with no history of pregnancy loss. All the subjects belonged to Punjabi ethnic group. Three ml blood was taken from cases and controls and deoxyribonucleic acid was extracted. In order to identify Factor V Leiden mutation, polymerase chain reaction method was utilised combined with the amplification refractory mutation system. Data was analysed using SPSS 17.

Results: Of the 112 subjects, 56(50%) were in each of the two groups. The presence of factor V Leiden mutation among the cases was 3(5.4%) while it was absent among the controls. The mutation was significantly associated with recurrent pregnancy loss (p=0.017).Recurrent pregnancy loss was higher in cases than controls (p=0.001).

Conclusion: Factor V Leiden mutation was significantly associated with recurrent pregnancy loss. It should be considered one of the causes of recurrent pregnancy loss.

Keywords: Factor V Leiden mutation, Recurrent pregnancy loss, PCR (Polymerase chain reaction). (JPMA 65: 1169; 2015)

Introduction

Recurrent pregnancy loss (RPL) is a significant health problem, affecting 5% females of reproductive age. Women at reproductive age face significant economical, emotional and social problems due to RPL.[1] Pakistan has an estimated abortion rate of 29 per 1000 pregnancies.[2] Spontaneous abortion is defined as loss of a pregnancy before the completion of 20 gestational weeks from the previous menstrual period or foetal weight of <500g according to the recommendation of the World Health Organisation (WHO).[3]

Pregnancy is a normal physiological hypercoagulable state which may be exaggerated by underlying thrombophilia. This condition may cause the complications during pregnancy.[4] Recently thrombophilia has been suggested as a possible cause of RPL. Although many causes have been established but more than 50% of cases remain unexplained. Thrombophilia can be acquired or inherited. Hereditary thrombophilia is a group of genetic disorders of blood coagulation. The most common cause of hereditary thrombophilia is factor V Leiden (FVL) and prothrombin gene mutation.[5] Studies have reported prevalence of FVL mutation among women with recurrent miscarriage ranging from 3% to 42%.[6] FVL prevalence in Caucasian population is 4% to 7%.[7]

FVL mutation is autosomal dominant disorder in which the Glutamine to Arginine missense mutation occurs at nucleotide 1691 of the factor V gene.[8] The resulting arginine (Arg) at amino acid 506 is substituted with glutamine (Gln) and this factor V mutation induces the activated protein C resistance (APCR) and contributes to increased risk of thrombosis.[8] APC is a natural anticoagulant which inactivates the activated factor V by cleaving it at amino acid 506.[4]

Activated factor V stabilises the prothrombinase complex and enhances the prothrombin activation. FVL mutation is responsible for 95% of cases of APCR. The risk of venous thrombosis is increased 7 times in heterozygote and 80 times in homozygote carriers.[9] Recently several studies have suggested that FVL mutation, through the production of micro thrombosis on placental bed blood vessels, cause low placental perfusion, placental infarction, and is strongly associated with RPL and maternal and foetal complications.[10] It causes 2-6 fold increase in the risk of preeclamsia in FVL carriers. Fatal complications include pregnancy loss, abruption, and intrauterine growth retardation.[11] FVL mutation is detected accurately with molecular deoxyribonucleic acid (DNA) technique. Genomic DNA extracted and polymerase chain reaction (PCR) is used to amplify factor V gene. FVL has 44,521,323 base pair (b p) gene segment containing nucleotide 1691 which is where most common mutation occurs. The amplified product is subjected to electrophoresis directly on a polyacrylmide gel.

[1]Department of Pathology, Shaheed Mohtarma Benazir Bhutto Medical College, Lyari, Karachi, [2]Combine Military Hospital Cherat, [3]Department of Pathology, Frontier Medical College, Abbottabad.

Correspondence: Sumreen Kashif. Email: sumreen.kashif@yahoo.com

Many studies done worldwide have shown a significant correlation between FVL and RPL.[12] This approach may be helpful to solve this major health problem that involves up to 5% of women of reproductive age by an appropriate antithrombotic treatment.[13] There is lack of data in Pakistan regarding FVL frequency in recurrent abortions. The current study was planned to provide beneficial information regarding the frequency of FVL mutation in recurrent abortions in local population.

Patients and Methods

The case-control study was conducted at the Department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from January to June 2012, after getting approval from the institutional ethics committee. Informed consent was obtained from all the cases and the controls. Females of reproductive age group (18-45 years) referred to AFIP with history of two or more foetal losses were included as cases, while age-matched females with no history of foetal loss were taken as the controls.

Those excluded from among the cases were patients of recurrent abortions with previously diagnosed aetiology such as: less than two abortions, chromosomal abnormalities, anatomical causes, acquired thrombophilia and diagnosed case of diabetes mellitus. From among the controls, women with history of foetal loss were excluded. Convenience sampling was employed and sample size was calculated using matched case-control situation. Spontaneous abortion was defined as loss of a pregnancy before the completion of 20 gestational weeks from the previous menstrual period or foetal weight of <500g according to WHO recommendation.

A full and thorough clinical history was taken from patients, including their demographic details. Past and present history associated with any infection, medical disease, and any gynaecological problem, previous or present history of thrombophilia. Ethnically, women of only Punjabi origin from paternal and maternal sides were included. Personal history was also taken, including socioeconomic profile, and nutritional status. Nutritional status was elaborated by body mass index (BMI). A uniform questionnaire was used to collect information about age, parity, medical and obstetric history, residency, consanguineous marriage. Data was collected by direct interview between the researcher and each participant. Samples for FVL were collected for PCR from cases and controls. Three ml venous blood was collected from each participant into Ethylenediaminetetraacetic acid (EDTA) tube. DNA was extracted from the blood sample by kit method (cat. No. D 5001 Gentra, USA).In order to identify FVL mutation, PCR method was utilised combined with the Amplification refractory mutation system (ARMS).

Data was analysed using SPSS 17. Continuous variables like age of patients and duration of symptoms were expressed as mean ± standard deviation (SD), whereas categorical data was expressed in the form of frequency and percentage. Any association was analysed by Chi-square test. $P \leq 0.05$ was considered statistically significant.

Result

A total of 112 subjects were included; 56(50%) cases and controls each. FVL mutation was present in 3(5.4%) cases, while it was absent in all the controls (Figure-1). Frequency of FVL mutation was higher in cases compared to controls (p=0.243) FVL mutation was significantly associated with RPL (p=0.017); and RPL was higher in cases than controls (p=0.001) Cases of FVL mutation in 1st and 2nd trimester of pregnancy were noted separately (Figure-2).

Mean age of the cases was 28.55±4.69 years and that of controls was 28.61±4.38 years (p=0.950).

The cases had mean BMI of 22.86±2.95kg/m2 whereas controls had 22.50±2.44 (p=0.487).

Among the cases, 32(57.1%) had zero parity followed by parity 1 in 19(33.9%) and parity 2 or more in 5(8.9%). Among the controls parity 2 or more was in 51(91.1%) followed by parity 1 in 5(8.9%) (p<0.001).

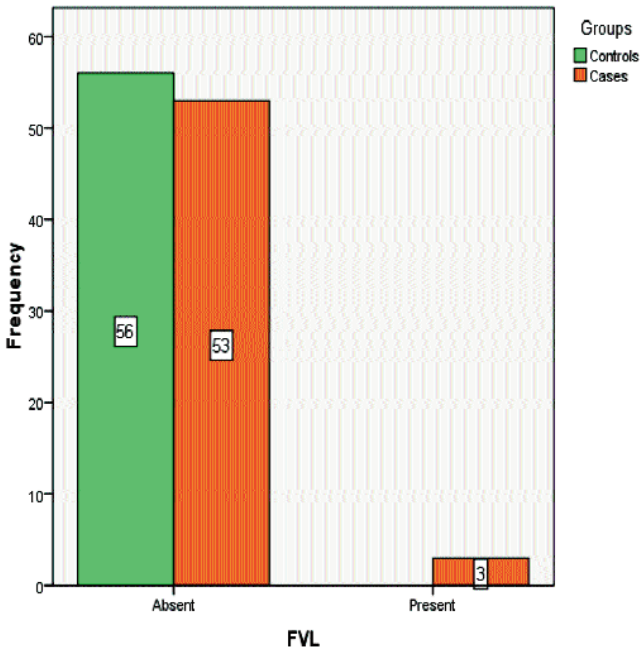

Figure-1: Comparison of Factor V Leiden (FVL) mutation between Cases and Controls.

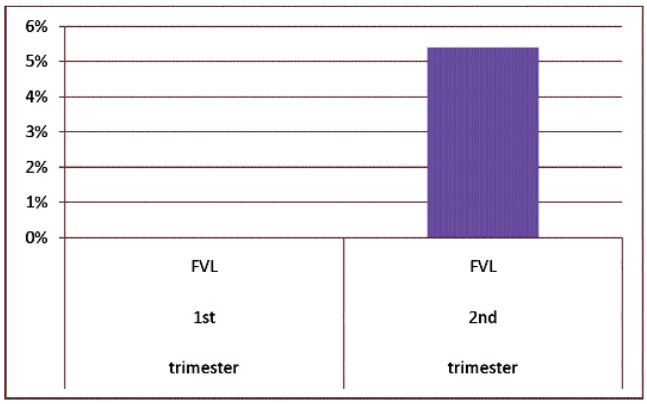

Figure-2: Comparing frequency of Factor V Leiden (FVL) in first and second trimester of Recurrent Pregnancy Loss (RPL).

All the cases (100%) had positive history of previous abortions, while all the controls had no history of previous abortions (0%) ($p<0.001$). Among the cases, 23(4.1%) had 2 previous abortions, followed by 21(37.5%) 3 abortions, and 7(12.5%) had 4 abortions. Mean RPL in first trimester was 1.35±2.65 and mean RPL in 2nd trimester was 1.46± 2.56.

Two (3.6%) cases had positive family history of abortions, while only 1(1.8%) control had such history ($p=1.000$).

Among the cases, 36(64.3%) belonged to middle socioeconomic class, followed by 17(30.4%) lower socioeconomic class, and 3(5.4%) upper socioeconomic class. Likewise, among the controls, 42(75%) were middle class, followed by 14(25%) lower class ($p=0.153$).

Among the cases, frequency of consanguinity was 24(42.9%), while among the controls it was 13(23.2%) ($p=0.044$).

Discussion

RPL occurs due to multiple aetiologies; genetic factor is considered one of those aetiologies. Advances in molecular genetics technology provide an accurate and reliable tool for precise study of the genetic abnormalities associated with recurrent abortions.

The role of some thrombophilia in foetal loss has been well-studied in different populations. On the other hand, the role of FVL mutation is still under debate. Therefore, it is of great importance to explore the association between FVL mutation and RPL. The prevalence of FVL mutation was tested and calculated in both case and control groups. The presence of FVL mutation in cases was 5.4% while it was absent in the control group. All cases of FVL mutation were found in second trimester RPL.

FVL mutation has been proposed as one of the leading factors associated with poor pregnancy outcomes. In 1999 a study[14] showed that pregnant women with serious complications have higher incidence of FVL mutation, predisposing them to higher risk of developing thrombosis compared to the control group. Similarly, one study[15] reported that Lebanese women with recurrent idiopathic abortions had a significant difference of the prevalence of FVL mutation in favour of the cases. Moreover, a study on Tunisian women[16] showed clearly the positive association between presence of FVL mutation and recurrent abortion. In Italy, a study[17] also demonstrated association between unexplained late foetal loss and FVL mutation.

Similar conclusion has been reached between FVL mutation and recurrent miscarriages among Greek women.[18] Moreover, association of FVL mutation with recurrent abortion was also reported among Israeli women in a study.[19] Another study[20] showed that there was a strong association between FVL mutation and foetal loss in Brazilian women.

Our study clearly demonstrated that the family history of abortions was insignificant.

There are several studies which show that there is no significant association between FVL mutation and thrombophilia. In 1996, a study[21] failed to show association between FVL mutation and recurrent foetal loss among European women participating in a European prospective cohort study on thrombophilia. Another study[22] reported similar findings involving 40 American women having recurrent foetal losses.

This discrepancy can be explained based on the genetic background variation in different populations. In addition, the difference in sample size between the various studies may be a good explanation for divergent conclusions. In our study, 5.4% women with RPL who carried the FVL mutation had heterozygous genotype; only 0% had homozygous, while all control subject had no FVL mutation.

In terms of limitations, the study had subjects only from one ethnic group at only one centre. Hence, long-term outcomes need to be explored by a large multi-centre study.

Despite the limitations

Further studies should be performed to explore the possibility of screening policy for persons who are at risk for genetic thrombophilia. Our data may be used for future investigative and surveillance studies.

Besides, awareness must be generated among medical

personnel with regard to FVL mutation and its role in different thromboembolic events.

Conclusion

FVL mutation is an important cause of thrombosis. Our results showed that FVL mutation is significantly associated with RPL.

Reference

1. Brenner B. Thrombophilia and pregnancy loss in first intended pregnancy. J Thromb Haemost 2005; 3: 2176-7.
2. Sathar ZA, Susheela S, Fikree FF. Estimating the incidence of abortion in Pakistan. Stud. Family Planning.2007; 38:11-22.
3. Rashid MU, Torres D, Rasheed F, Sultan F, Shakoori AR, Amin A, et al.Breast Cancer Res Treat. 2009; 116:211-3.
4. Jivraj S, Makris,Saravelos S, Li TC. Pregnancy outcome in women with factor V Leiden and recurrent miscarriage. BJOG 2009; 116: 995-8.
5. Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT. Evaluation of the Association between Hereditary Thrombophilias and Recurrent Pregnancy Loss: a meta-analysis.Arch Intern Med 2004; 164:558-63.
6. Kupferminc MJ. Thrombophilia and pregnancy. Reprod Biol Endocrinol. 2003;1:111.
7. Deitcher S R, Rodgers G M. Thrombosis and Antithrombotic Therapy. In: Greer J P, Foester j, Rodger G M, Paraskevas F,Glader B. and Arber D A, eds. Wintrobe's Hematology. 12thed. Philadelphia: Wolters klumer & Lipponcott, 2009; pp 1468-3.
8. Lindqvist PG, Svensson PJ, Marsaál K, Grennert L, Luterkort M, Dahlbäck B. Activated protein C resistance (FV:Q506) and pregnancy. Thromb Haemost. 1999 ;81:532-7.
9. Brummel-Ziedins K, Orfeo T, Jenny N S, Everse S J, Mann KG. Blood Coagulation and Fibrinolysis In: Greer J P, Foester j, Rodger G M, Paraskevas F,Glader B. and Arber D A, eds. Wintrobe'sHematology. 12thed. Philadelphia: Wolters klumer&Lipponcott,2009;pp 552.
10. Mohammad A M M, Monem S M F, Al-Habib G M. Prevalence of FVL mutation and its relation with recurrent spontaneous pregnancy loss in group of Syrian womens. Middle East Fertil Soc J 2007; 12: 179-182.
11. Jivraj S. Recurrent Miscarriage and Role of Genetic Thrombophilic mutations. Curr Wom Health Rev 2009;5:14-23.
12. Hussein A S, Darwish H, Shelbayeh K. Association between factor V Leiden mutation and poor pregnancy outcomes among Palestinian women. Thromb Res. 2010; 126:e78-82.
13. DuVa M ,Micco PD , Strina I, Placido GD. Recurrent Pregnancy Loss and Thrombophilia. JCMR 2010; 2: 18-22.
14. Kupferminc M J, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, et al. Increased frequency of genetic thrombophilia in women with complications of pregnancy. N Engl J Med 1999;340: 9-13.
15. Finan RR, Tamim H, Ameen G, Sharida HE, Rashid M, Almawi WY. Prevalence of factor V G1691A (factor V-Leiden) and prothrombinG20210A gene mutations in a recurrent miscarriage population. Am J Hematol 2002; 71: 300-5.
16. Mahjoub T, Mtiraoui N, Tamim H, Hizem S, Finan R R, Nsiri B, et al. Association between adverse pregnancy outcomes and maternal factor V G1691A (Leiden) and prothrombin G20210A genotypes in women with a history of recurrent idiopathic miscarriages. Am J Hematol. 2005; 80:12-9.
17. Martinelli I, Taioli E, Cetin I, Marinoni A, Gerosa S, Villa M V, et al. Mutations in coagulation factors in women with unexplained late fetal loss. N Engl J Med. 2000; 343:1015-8.
18. Foka Z J, Lambropoulos A F, Saravelos H, Karas G B, Karavida A, Agorastos T, et al. Factor V Leiden and Prothrombin G20210A mutation, but not methylentetrahydrofolatereductase C677T, are associated with recurrent miscarriages. Hum Reprod. 2000; 15:458-62.
19. Younis JS, Brenner B, Ohel G, Tal J, Lanir N, Ben-Ami M. Activated protein C resistance and factor V Leiden mutation can be associated with first as well as second- trimester recurrent pregnancy loss. Am J Reprod Immunol. 2000; 43:31-5.
20. Souza SS, Ferriani RA, Pontes AG, Zago MA, Franco RF. Factor V leiden and factor II G20210A mutations in patients with recurrent abortion. Hum Reprod. 1999; 14:2448-50.
21. Preston F E, Rosendaal F R, Walker I D, Briet E, Berntorp E, Conard J, et al. Increased fetal loss in women with heritable thrombophilia. Lancet 1996; 348: 913-6.
22. Dizon-Townson DS, Kinney S, Branch DW, Ward K. The factor V Leiden mutation is not a common cause of recurrent miscarriage. J Reprod Immunol. 1997; 34:217-23.