BJFS Bangladesh Journal of Fertility & Sterility

Bangladesh J Fertil Steril, 2022;Vol.2(1):49-51

Case Report

Heterozygote Factor V Leiden mutation in a Subject with Recurrent Pregnancy Loss: A Case Report

Sanjukta Chowdhury[1], Masuda Sultana[2], Surayea Bulbul[3], Ferdous Ara Banu[4], Nahreen Akhtar[5], Firoza Begum[6]

Abstract: Recurrent pregnancy loss (RPL) is usually defined as the loss of three or more consecutive pregnancies up to 24 weeks of gestation, which occurs in approximately 1-5% of reproductive-aged women. Recently, both inherited and acquired thrombophilia has drawn attention as one of the major causes of RPL. Inherited predisposition to thrombophilia is most often associated with Factor V Leiden mutation, prothrombin gene mutation, and methylenetetrahydrofolate reductase gene variants. Factor V Leiden is considered as one of the associated factors in RPL. It imposes abnormal blood clotting in placental micro vasculature that links with pregnancy loss. It is an autosomal dominant form of inherited thrombophilia, with variable penetrance, characterized by single nucleotide polymorphism (SNP) in Factor V gene, results in activated protein C resistance. The net effect is an increased cleavage of prothrombin to thrombin and excessive blood coagulation and venous thrombosis in different sites.

Keywords: Recurrent pregnancy loss, Hereditary thrombophilia, Factor V Leiden mutation

Introduction

Recurrent pregnancy loss is an emotionally traumatic experience for the couples and poses a strenuous clinical challenge to the High risk Pregnancy Specialists [1]. 1-5% of prospective couples' experience RPL [2].

Royal College of Obstetricians and Gynaecologists (RCOG, 2011), stated RPL as loss of three or more consecutive pregnancies from the time of conception up to 24 weeks' gestation[3]. Later American Society for Reproductive Medicine [4] (ASRM, 2012; GDG) and European Society for Human Reproduction and Embryology [5] (ESHRE, 2017) liberalized RPL by stating consecutive two or more clinical pregnancy losses and two or more consecutive or non-consecutive pregnancy losses respectively.

Embryonic chromosomal abnormalities, parental chromosomal rearrangement, maternal congenital or acquired uterine anomalies and cervical insufficiency are established causes of RPL[3,5]. In 50% cases of RPL, no cause is identified, even after extensive investigations and coined as unexplained recurrent pregnancy loss. Hereditary thrombophilia has been suggested as one putative etiology in the field of unexplained RPL [6].

Hereditary thrombophilia predisposes to vascular thrombosis in susceptible persons due to presence of mutated and functionally altered blood coagulation factors. During pregnancy, it may manifest as thrombosis in decidual vessels and inhibition of trophoblast differentiation causing spontaneous pregnancy loss, preeclampsia, fetal growth restriction, placental abruption, and stillbirth. Numerous polymorphisms are listed in hereditary thrombophilia, such as polymorphisms in Factor V, Factor II or methylene tetrahydrofolate reductase gene[7]. "Factor V Leiden" (A1691G; R506Q) is the ultimate result of replacement of Guanine (G) by Adenine (A) at nucleotide 1691 position of Factor V gene directing missense substitution of Arginine (R) to Glutamine (Q) at 506 – APC cleavage site and causes activated protein C (APC) resistance. Mutated Factor V inactivates at a 10-fold slower rate than normal[8]. This mutation can be presented as homozygous-AA or heterozygous-GA state with the clinical implication of more thrombosis in homozygous state[9]. Persons with mutated Factor V may present with venous thromboembolism and RPL [8].

Case summary

Mrs. X, primipara, with history of consecutive three second trimester pregnancy losses at 14, 16 and 14 weeks, attended

1. Junior Consultant (Obs & Gynae), FCPS Subspeciality course, Department of Fetomaternal medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU)
2. Junior Consultant (Obs & Gynae), FCPS Subspeciality course, Department of Fetomaternal medicine (BSMMU)
3. Junior Consultant (Obs & Gynae), FCPS Subspeciality course, Department of Fetomaternal medicine (BSMMU)
4. Junior Consultant (Obs & Gynae), FCPS Subspeciality course, Department of Fetomaternal medicine (BSMMU)
5. Chairman, Department of Fetomaternal medicine, BSMMU
6. Founding Chairman, Department of Fetomaternal medicine, BSMMU

Corresponding Author: Sanjukta Chowdhury, Junior Consultant (Obs & Gynae), Dept. of Fetomaternal Medicine, BSMMU. Email:sanjuktak58dmc@gmail.com

Date of Submission: 24-11-2021 | Date of Acceptance: 30-12-2021

the RPL clinic of Fetomaternal medicine Department, BSMMU for preconceptional counselling. Detailed socio-demographic information (age, educational status, monthly income), obstetric history, gestational age at which previous pregnancy losses occurred, menstrual history, history of consanguinity, history of diabetes mellitus, hypertension or thyroid disorder were recorded. Clinical examination was performed and BMI (27.6 kg/m2) was calculated. Fasting blood sugar, blood sugar 2 hours after 75-g glucose, transvaginal sonography, parental karyotyping, Lupus anticoagulant and Anticardiolipin antibody IgG and IgM were done to exclude established causes of recurrent pregnancy loss for this patient. After exclusion of all the factors, under informed written consent, patient was counselled to test for hereditary thrombophilia. With all aseptic precaution, 3 ml venous blood was collected from the patient into a EDTA test tube and was transferred to PCR Lab. of the Department of Microbiology & Immunology, BSMMU.

DNA extraction

Genomic DNA was extracted for SNP (single nucleotide polymorphism) assay by using specific nucleic acid isolation kit (QIAGEN) from the specimen according to manufacturer's instructions. Extracted DNA was stored at -20°C until PCR (Polymerase Chain Reaction) run.

DNA mutation procedure

Extracted pure genomic DNA was assayed to see hereditary thrombophilia panel mutation by "Thrombophilia Real-Time PCR kit multiplex (SNP Biotechnology R&D Ltd. Hacettepe Technopolis – Ankara/Turkey, Cat. No: 10R-20-09).

- Tubes containing 20 microlitre (µl) mastermixes and 5 µl (~10-100 ng) DNA were placed in validated 7500 Fast Dx Real-time PCR Instrument (Applied Biosystems).
- DNA was amplified and DNA polymerase cleaved the probe at 5′ end and separated reporter dye from quencher dye only after probe hybridization with target DNA.
- This cleavage resulted in fluorescent signal. An increase in fluorescent signal (CT) was proportional to the amount of specific PCR product.

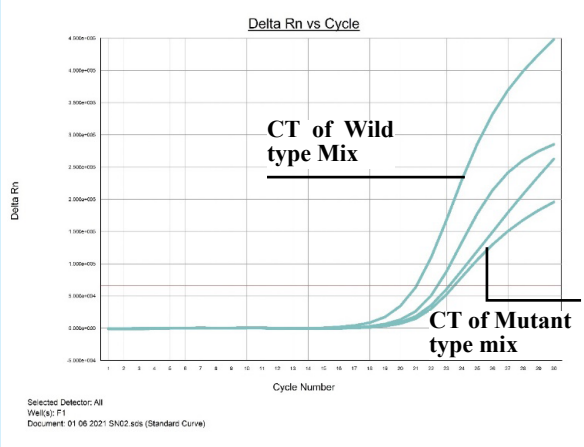
DNA mutation analysis

Data were analysed using software with HEX (JOE), TEXAS RED, CY5, and FAM dyes. Homozygous wild type sample was given amplification signal only with wild type mastermix and heterozygote sample was given amplification signal both with wild type and mutant mastermix. Factor V Leiden gene mutation (R506Q) was detected by specific primer. In our patient, Factor V heterozygote variant was identified.

Discussion

Here we present a case of a woman with a history of three recurrent pregnancy losses in second trimester that was complicated with the heterozygote factor V Leiden mutation genotypes. In several studies, the potential clinical significance of hereditary thrombophilia factors in recurrent pregnancy loss, such as the factor V Leiden mutation, prothrombin G20210A mutation, and methylenetetrahydrofolate reductase C677T and A1298C gene variants has been addressed [10-12].

Fig. 1: Curve of Factor V Leiden Heterozygote



Factor V Leiden mutation is predominantly present in Caucasians, and the prevalence varies between 2% to 15%. It has been demonstrated that the mutation is less common in Hispanics and African-Americans, and it's extremely rare in people of Asian descent. Presence of the FVL mutation considerably increases risk of developing abnormal blood clotting. Homozygous variants for the mutated allele present a 50–100 fold increasing risk of developing abnormal clots versus heterozygous variant present a quite low risk (5–10 fold). Combined association of another risk factors for venous thrombosis such as age, obesity, smoking, estrogen based hormonal contraception, or hormone replacement therapy, recent surgery, further enhance the risk of thrombosis[13]. Sarig, et al. (2002) described the characteristics of thrombophilia in one hundred forty-five patients with repeated pregnancy loss and 145 matched controls[14]. Prevalence of Factor V Leiden, Factor II, and Methylene-tetrahydrofolate reductase gene mutations, along with other thrombophilia were assessed. At least one thrombophilic defect was found in 66% of patients compared with 28% in control. Combined thrombophilic defects were documented in 21% of women with pregnancy loss compared with 5.5% of control. Factor V Leiden mutation was more common in case (25% vs. 7.6%) than other thrombophilic defects. Study conducted by Settin et al. (2011) on cases of Nile Delta region revealed a significant association of FVL GA heterozygous (GA) mutation with unexplained RPL (OR=21·38, P<0·0001)[15]. Parveen et al. (2012) studied factor V Leiden mutation on 1000 women with recurrent miscarriages and 500 healthy parous women of North India and found 5% heterozygous mutation of Factor V Leiden in the case and 2.4 % in controls (OR 2.14; 95 % CI 1.12-4.05)[16].

Kashif, et al. (2015) conducted a case-control study comprising 56 women in each case and control group[17]. Presence of factor V Leiden mutation among cases was 5.4% while it was absent among controls. The mutation was significantly associated with recurrent pregnancy loss (p=0.017). Rodger, et al. (2010) found that the odds ratio

of pregnancy loss appears to be 52% higher in women with factor V Leiden mutation than in pregnant women without the mutation[18].

Conclusion

We recommend to take into consideration the genetic markers of thrombophilia during pregnancy, especially for women with recurrent unexplained pregnancy losses. An appropriate clinical intervention comprising of antithrombotic therapy should be considered in women with unexplained recurrent pregnancy loss associated with hereditary thrombophilia to improve live birth rates.

Acknowledgments

We are deeply grateful to the Dept. of Microbiology and Immunology, BSMMU for helping us with the facilities of thrombophilia test.

How to Cite this Article: Chowdhury S, Sultana M, Bulbul S, et al. Heterozygote Factor V Leiden mutation in a Subject with Recurrent Pregnancy Loss: A Case Report. *Bangladesh J Fertil Steril*; 2022;1(1):49-51

References

1. Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. Bmj. 2000 Jun 24;320(7251):1708-12.
2. Bennett SA, Bagot CN, Arya R. Pregnancy loss and thrombophilia: the elusive link. British journal of haematology. 2012 Jun;157(5):529-42.
3. The investigation and treatment of couples with recurrent first-trimester and second-trimester miscarriage. RCOG: London, UK. 2011 Apr.
4. American society for reproductive medicine, 2012, Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril, 98, pp. 1103-11.
5. ESHRE Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, Nelen W, Peramo B, Quenby S, Vermeulen N. ESHRE guideline: recurrent pregnancy loss. Human reproduction open. 2018;2018(2):hoy004.
6. Babker AM, Gameel FE, Elzaki SG. Heterozygosity of maternal factor V G1691A (Leiden) and relationship with times of pregnancy loss among unexplained recurrent pregnancy loss women. Hematol Transfus Int J. 2018;6(5):208-10.
7. Kovalevsky G, Gracia CR, Berlin JA, Sammel MD, Barnhart KT. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta-analysis. Archives of internal medicine. 2004 Mar 8;164(5):558-63.
8. Kujovich JL. Factor v Leiden thrombophilia. Genetics in Medicine. 2011 Jan;13(1):1-6.
9. Ornstein DL, Cushman M. Factor V Leiden. Circulation. 2003 Apr 22;107(15):e94-7.
10. Martinelli I, Taioli E, Cetin I, Marinoni A, Gerosa S, Villa MV, et al. Mutations in coagulation factors in women with unexplained late fetal loss. N Engl J Med. 2000 Oct 5;343(14):1015-8.
11. Rai R, Shlebak A, Cohen H, Backos M, Holmes Z, Marriott K, et al. Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage. Human Reproduction. 2001 May 1, 2001;16(5):961-5.
12. 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 firstas well as second-trimester recurrent pregnancy loss. Am J Reprod Immunol. 2000 Jan;43(1):31-5.
13. Perez-Pujol S, Aras O, Escolar G. Factor V Leiden and inflammation. Thrombosis. 2012;2012.
14. Sarig G, Younis JS, Hoffman R, Lanir N, Blumenfeld Z, Brenner B. Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. Fertility and sterility. 2002 Feb 1;77(2):342-7.
15. Settin A, Alkasem RA, Ali E, ElBaz R, Mashaley AM. Factor V Leiden and prothrombin gene mutations in Egyptian cases with unexplained recurrent pregnancy loss. Hematology. 2011 Jan 1;16(1):59-63.
16. Parveen F, Shukla A, Agrawal S. Should factor V Leiden mutation and prothrombin gene polymorphism testing be done in women with recurrent miscarriage from North India?. Archives of gynecology and obstetrics. 2013 Feb;287(2):375-81.
17. Kashif S, Kashif MA, Saeed A. The association of factor V leiden mutation with recurrent pregnancy loss. J Pak Med Assoc. 2015 Nov 1;65(11):1169-72.
18. Rodger MA, Betancourt MT, Clark P, Lindqvist PG, Dizon-Townson D, Said J, Seligsohn U, Carrier M, Salomon O, Greer IA. The association of factor V leiden and prothrombin gene mutation and placenta-mediated pregnancy complications: a systematic review and meta-analysis of prospective cohort studies. Plos medicine. 2010 Jun 15;7(6):e1000292.