Inherited thrombophilia profile in patients with recurrent miscarriages: Experience from a tertiary care center in north India

Narender Kumar¹, Jasmina Ahluwalia¹, Reena Das¹, Meenakshi Rohilla², Sunil Bose¹, Hari Kishan¹, Neelam Varma¹
Departments of ¹Hematology and ²Obstetrics & Gynecology, Postgraduate Institute of Medical Education & Research, Chandigarh, India

The cause of recurrent miscarriage (RM) remains unexplained in approximately 30% to 50% cases. The association of inherited thrombotic factors and RM patients has not been documented from the northern part of India. A total of 40 patients had been investigated for inherited thrombophilia workup (protein C, protein S [PS], antithrombin III, and factor V Leiden [FVL] mutation) over a period of 10 years (2005 to 2014). RM patients were divided in to three groups. Group I (only 1st trimester loss), group II (only 2nd and 3rd trimester), and group III (mixed). Each group comprised of the following numbers of patients respectively: I, 24; II, 2; III, 14. Heterozygous FVL mutation was found in 10% (4/40) cases. PS deficiency was detected in 2.7% (1/37) cases. In the present study FVL and PS were seems to be associated with a subset of patients however further studies with larger numbers of patients are recommended for better evaluation.

Keywords: Abortion, habitual; Factor V Leiden; Protein S; Thrombophilia

Introduction

Recurrent miscarriage (RM) or recurrent pregnancy loss (RPL) is defined by American Society of Reproductive Medicine as two or more failed pregnancies [1]. Several etiological factors like endocrinologic problems, uterine structural, chromosomal anomalies and antiphospholipid antibody syndrome can be the cause in some of these cases. However, about 30% to 40% of the RPL cases are idiopathic [2]. The pregnancy itself is an acquired hypercoagulable state and fetal outcome depends upon on adequacy of placental circulation. Any compromise in placental circulation can result intrauterine growth restriction, placental abruption, preeclampsia, pregnancy loss, intrauterine fetal demise [3]. Hereditary thrombophilia comprise a number of conditions, such as antithrombin (AT) III deficiency, protein S (PS) and protein C (PC) deficiencies, factor V Leiden (FVL), prothrombin 20210A mutation, elevated factor VIII level, and mutation of gene encoding the enzyme methylenetetrahydrofolate reductase. There are a number of studies showing an increased risk of RPL in women with inherited thrombophilia [4-9]. However, it is still not much clear that heritable thrombophilia is responsible for RPL and routine testing for hereditary thrombophilia in such women should be advised or not? The purpose of this retrospective study was to find out the association between the common markers of inherited thrombophilia (FVL mutation, AT III, PC and PS functional activities) and RPL.
Obstetrics & Gynecology Science

Narender Kumar, et al. Inherited thrombophilia in miscarriages

Materials and methods

The retrospective data for thrombophilia work up (both clinical and laboratory records) was collected from the archives of Coagulation Laboratory of Hematology Department. A total of 40 female patients with history of RPLs had been investigated for inherited thrombophilia workup over a period of last 10 years (2005 to 2014). Among these patients 16 had 2 fetal loss and 24 had more than 2 fetal losses. These patients were divided to three groups according to period of miscarriages: group I (1st trimester abortion only), group II (2nd and 3rd trimester), and group III (mixed, both early and late abortions).

All these patients did not have any history of endocrine, anatomic, neoplastic, systemic, thrombotic disease. The women were not pregnant at the time of the workup, and none of the women were taking oral contraceptives. The laboratory work up for a thrombotic state was carried out at least after 6 weeks after the abortion.

1. Blood collection

Ten milliliter of blood was collected by venepuncture and aliquoted into 3.18% trisodium citrate (1:9 anticoagulant to blood) and ethylenediamine tetraacetic acid tubes.

2. Testing for inherited thrombophilia

PC and PS functional activity were measured by clot based assay using commercial kits (Diagnostica Stago, Asnieres, France). AT III was measured by chromogenic assays using commercial reagents (Diagnostica Stago). All assays were performed on Stago STA-R Evolution or Stago Compact (Diagnostica Stago) automated analyzers. Patients with abnormal results underwent repeat testing on fresh specimens after at least one month. Normal PC and PS functional activity level ranged between 63% to 123% and between 55% to 123% respectively while ATIII level ranged between 80% to 120%.

The Genomic DNA was isolated from peripheral blood leucocytes by standard phenol chloroform extraction method [10]. FVL mutation was identified by polymerase chain reaction amplification of a 220 bp fragment followed by digestion with MnI restriction enzyme. MnI digests the 220 bp fragment of normal factor V allele in three fragments of 37, 67, and 116 bp. The FVL allele is cleaved in only two fragments of 67 and 153 bp [11].

Results

RM patients were divided in to three groups on the basis of pregnancy losses. Group I (only 1st trimester loss), group II (only 2nd and 3rd trimester), and group III (mixed). Each group comprised of the following numbers of patients respectively: I, 24; II, 2; III, 14. The clinical details of one patient was not available. The laboratory profile of each group is summarized in Table 1. A total of 12.5% (5/40) patients had abnormality in inherited thrombophilia profile. Heterozygous FVL mutation was found in 10% (4/40) cases. Two of these cases were in group I (8.3%, 2/24), one in group II (50%, 1/2) and 1 in group III (7%, 1/14). No homozygous FVL mutation was found. The PS functional assay was performed in 37/40 patients of RM. PS deficiency was detected in 2.7% (1/37) cases and this was in group I (4.2%, 1/24). None of the cases had PC and AT III deficiency. Combined deficiency of inherited thrombophilic factors was not seen in any patient.

Discussion

The RPL is an enigmatic condition. Despite extensive investigations a large population of patients does not have any specific aetiology. The role of thrombophilia in the pathogenesis of
RPL has been studied in recent times. Both acquired and heritable causes of thrombophilia are found to be associated with RPL [4-9]. The present study was a retrospective data analysis in north Indian subset of patients with RPL. The frequency of FVL mutation in RM cases in different population and countries varies from 3% to 40% [4,5,7,8,12]. Among Indian population the frequency ranges from 2.3% to 5% [9,13,14]. In the current data, we found heterozyosity for FVL mutation in 10% of patients. No homozygous mutation was identified in this targeted population. Among these 50% of heterozygous were associated with only 1st trimester loss although within this group the value is 8.3%. Second and third trimester loss group had mutation in 50% of patients although the group population was very small (n=2) to make a clear association. The group with both early and late trimester loss had mutation in 7% of patients. Rey et al. [6] have carried out a meta-analysis and have shown almost same frequency in early and late pregnancy losses. Our data although have a small number of patients but showed this mutation in all the groups.

The inherited thrombphilias factors (PC, PS, and AT III) were significantly associated with RPL in the literature worldwide. Among this PS deficiency had the strongest association [4,6-8]. Among Indian literature Vora et al. [9] had 17.8% RPL patients with PS deficiency while Hansda and Roychowdhury [15] had 50% RPL patients. In our data, we had 2.7% of patients with PS deficiency. The group with only 1st trimester loss had 4.2% of patients with PS deficiency. The lower rates of PS deficiency in our population may be partly due to the stringent criteria of sampling the patients in the non-pregnant state. It is well documented that functional PS deficiency occurs in pregnancy. In cases testing positive for PS deficiency, it is recommended that repeat testing be performed well after the puerperium. Combinations of more than 1 thrombophilic risk factor were not seen in this cohort; however a larger sample size maybe needed to cement this observation. Since the data of Indian RPL patients on inheritable thrombophilic factors is sparse, the determination of exact prevalence need more studies. This preliminary study shows that cases with RPL may test positive for the heritable thrombophilia factors.

In conclusion, we have studied retrospective inherited thrombophilic data in patients with RPLs. A total of 12.5% patients had deranged inherited thrombophilia factors. A case control study with larger numbers is recommended for better evaluation. Documentation of abnormal thrombophilic factors and appropriate management is necessary for successful outcome of the pregnancy.

Conflict of interest
No potential conflict of interest relevant to this article was reported.

References
1. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil Steril 2008;89:1603.
2. Fawzy M, Shokeir T, El-Tatongy M, Warda O, El-Refaiey AA, Mosbah A. Treatment options and pregnancy outcome in women with idiopathic recurrent miscarriage: a randomized placebo-controlled study. Arch Gynecol Obstet 2008;278:33-8.
3. Salafia CM, Minior VK, Pezzullo JC, Popek EJ, Rosenkrantz TS, Vintzileos AM. Intrauterine growth restriction in infants of less than thirty-two weeks’ gestation: associated placental pathologic features. Am J Obstet Gynecol 1995;173:1049-57.
4. Preston FE, Rosendaal FR, Walker ID, Briet E, Berntorp E, Conard J, et al. Increased fetal loss in women with heritable thrombophilia. Lancet 1996;348:913-6.
5. Alonso A, Soto I, Urgelles MF, Corte JR, Rodriguez MJ, Pinto CR. Acquired and inherited thrombophilia in women with unexplained pregnancy loss. Am J Obstet Gynecol 2002;187:1337-42.
6. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet 2003;361:901-8.
7. 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. Fertil Steril 2002;77:342-7.
8. Vossen CY, Preston FE, Conard J, Fontcuberta J, Makris M, van der Meer FJ, et al. Hereditary thrombophilia and fetal loss: a prospective follow-up study. J Thromb Haemost 2004;2:592-6.
9. Vora S, Shetty S, Ghosh K. Thrombophilic dimension of recurrent fetal loss in Indian patients. Blood Coagul Fibrinolysis 2008;19:581-4.
10. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press; 1989.
11. Arruda VR, Annichino-Bizzacchi JM, Costa FF, Reitsma PH. Factor V Leiden (FVQ 506) is common in a Brazilian population. Am J Hematol 1995;49:242-3.
12. 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. Am J Hematol 2002;71:300-5.
13. 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? Arch Gynecol Obstet 2013;287:375-81.
14. Biswas A, Choudhry P, Mittal A, Meena A, Ranjan R, Choudhry VP, et al. Recurrent abortions in Asian Indians: no role of factor V Leiden Hong Kong/Cambridge mutation and MTHFR polymorphism. Clin Appl Thromb Hemost 2008;14:102-4.
15. Hansda J, Roychowdhury J. Study of thrombophilia in recurrent pregnancy loss. J Obstet Gynaecol India 2012;62:536-40.