

Inherited Thrombophilia and Early Recurrent Pregnancy Loss among Egyptian Women

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Abstract

Inherited thrombophilia has been implicated as a possible cause of recurrent pregnancy loss. Although numerous studies are available in literature, thrombophilia rate seems to vary from study to another. The aim of our study was to determine the frequency of FII Prothrombin (G20210A), Factor V Leiden (G1691A), as well as methyl tetrahydrofolate reductase (MTHFR C677T) polymorphisms, protein C, protein S and antithrombin III deficiency in a series of patients with unexplained RPL compared to control. Patients and Methods: 100 patients of unexplained RPL and 43 age-matched healthy controls were investigated for inherited thrombophilia. Results: MTHFR and Factor V Leiden were the commonest gene defects among cases studied (63%, 60% respectively) and control groups (41.9%, 41.9% respectively) ($p = 0.019$, $p = 0.046$ respectively). The least common deficiencies were protein S and protein C deficiency in cases (3%, 2% respectively) as well as in controls (1%, 0% respectively). 4 cases were homozygous for MTHFR and 2 cases homozygous for Factor V Leiden mutation. Odds ratio for MTHFR and Factor V mutation was 2.36 and 2.08 respectively (CI 95%). Combined defects were seen in cases and controls ($p < 0.05$). Conclusion: Our study found an association between MTHFR and Factor V Leiden mutations in patients with unexplained RPL among Egyptian women. Further studies are needed to define the management of genetic thrombophilia in cases of recurrent pregnancy loss.

Keywords

Thrombophilia, Recurrent Pregnancy Loss, Genetic Mutations, MTHFR

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1. Introduction

Recurrent pregnancy loss (RPL) represents a significant health problem with a rate of 5% among women in the reproductive age [1]. Inherited thrombophilia has been implicated as a possible cause [2] [3]. Although numerous studies are available in literature, thrombophilia rate seems to vary from study to another [4] [5].

Combined thrombophilia, namely inherited thrombophilia associated with acquired thrombophilia or more than one inherited thrombophilic defect, has also been identified as a cause of RPL, but its real frequency is not clear. Several studies in the latest years identified combined thrombophilic defects in women with both early and late RPL [6]-[9].

Factor V Leiden mutation was found to be the most common inherited thrombotic risk factor associated with RPL [10]. Its inheritance is autosomal dominant and its frequency in whites varies from 3% to 8% and 1 in 1000 are homozygous. It is rare in African Americans, Asians and Native Americans [11].

The incidence of genetic prothrombotic mutations in women with unexplained pregnancy loss was examined in various studies: some of these studies supported the association [5] [12] [13], while others detected no association [14]-[16].

A meta-analysis [13] of pooled data from 31 retrospective studies suggested that the association between inherited thrombophilia and fetal loss varies according to type of fetal loss and type of thrombophilia. The association between thrombophilia and late pregnancy loss has been consistently stronger than for early pregnancy loss. In this meta-analysis, factor V Leiden was associated with recurrent first-trimester fetal loss (OR 2.01, 95% CI 1.13 - 3.58), recurrent fetal loss after 22 weeks (OR 7.83, 95% CI 2.83 - 21.67) and non-recurrent fetal loss after 19 weeks (OR 3.26, 95% CI 1.82 - 5.83). Prothrombin gene mutation was associated with recurrent first-trimester fetal loss (OR 2.32, 95% CI 1.12 - 4.79), recurrent fetal loss before 25 weeks (OR 2.56, 95% CI 1.04 - 6.29) and late non-recurrent fetal loss (OR 2.3, 95% CI 1.09 - 4.87). Protein S deficiency was associated with recurrent fetal loss (OR 14, 95% CI 0.99 - 218) and non-recurrent fetal loss after 22 weeks (OR 7.39, 95% CI 1.28 - 42.83). Methylenetetrahydrofolate mutation and protein C and antithrombin deficiencies were not associated with fetal loss. However, since protein C and antithrombin III deficiencies are rare, the number of women included in the study was too small to show any difference in pregnancy outcome.

Another meta-analysis [2] of 16 case-control studies showed that patients with factor V Leiden or prothrombin gene mutation have double the risk of recurrent miscarriage when compared with women negative to thrombophilic mutations.

The ALIVE, SPIN and HepASA trials all studied the use of LMWH and aspirin and compared them with either aspirin alone or mere intensive pregnancy surveillance in patients with early recurrent pregnancy loss. All showed no significant difference in reduction of pregnancy loss or increase in live birth rate [17]-[19]. None of those trials studied the effect of therapy in the subgroup of women with thrombophilia. Randomized placebo-controlled trials are needed to study the use of anticoagulants in women with recurrent miscarriage and inherited thrombophilia [20].

With the identification of genetic risk factors, there has been synergistic amplification of thrombotic risk when one has an abnormal gene (e.g., factor V Leiden) plus environmental issues (e.g., pregnancy). Currently, a combination of risk factors (pregnancy, multiple inherited thrombotic defects) is associated with secondary hypercoagulable states and has a strong association with pregnancy complications [21]. Women with antithrombin III deficiency or more than one thrombophilic defect (including homozygous factor V Leiden, homozygous prothrombin G20210A and compound heterozygotes) or who have with additional risk factors should be referred to specialized centers for monitoring future pregnancies and for considering thrombo-prophylaxis during pregnancy [22].

The aim of our study was to determine the frequency of Factor V Leiden (G1691A), FII Prothrombin (G20210A) and methyl tetrahydrofolate reductase (MTHFR C677T) polymorphisms as well as protein C, protein S and antithrombin III levels in a series of patients with unexplained RPL compared to age matched, healthy controls with no history of medical disorders, thrombosis or miscarriage.

2. Patients and Methods

2.1. The Study Population

A case-control study was conducted on a total number of 143 participants: 100 patients and 43 age matched

controls.

Consent was taken from all participants and the Ethics Committee at Kasr Al-Ainy Hospital approved the Research work.

2.1.1. Patient Group

All patients were not pregnant, with history of 2 or more pregnancy losses ≤ 13 weeks. Patients were referred from the outpatient clinic of the Obstetrics and Gynecology Department at Kasr Al-Ainy Faculty of Medicine, Cairo University.

To exclude other causes of recurrent pregnancy loss, we looked for chromosomal alterations, endocrine, infectious and uterine factors.

All patients underwent karyotyping (both parents) to exclude chromosomal aberrations as balanced translocations. 3D Ultrasound was performed to exclude uterine congenital anomalies. Screening for diabetes, thyroid dysfunction, hyperprolactinemia, luteal insufficiency and PCOS were performed (Basal FSH, LH E2, luteal progesterone, FT3, FT4, TSH, Prolactin and androgen levels). Lupus anticoagulant and antiphospholipid antibodies were also excluded. Cases included had no history of thrombosis.

Patients were then tested for the following inherited thrombophilias: Factor V Leiden, Prothrombin as well as MTHFR (C677T) gene polymorphism, Protein S, C and antithrombin III deficiency.

2.1.2. Control Group

43 age-matched, healthy women with at least one uncomplicated full term pregnancy with no history of pregnancy loss, no known medical disorders or previous thrombosis were included in the control group. They were recruited from the gynecology outpatient clinic, Kasr Al-Ainy hospital, Cairo University.

2.2. Genotyping

The FV-PTH-MTHFR Strip assay is based on the reverse hybridization principle. The DNA was isolated from EDTA-anticoagulant blood samples using Vienna Lab Kit (Labordiagnostika GmbH kit-cat. #4-260, Vienna, Austria). Then factor V, prothrombin and MTHFR gene sequences were simultaneously *in vitro* amplified and biotin-labeled in a single (multiplex) amplification reaction. Finally, the amplification products were selectively hybridized to a test strip, which contains oligonucleotide probes (wild type and mutant type) immobilized as parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates. The assay covered 3 mutations: FV (G1691A), PTH (G20210A) and MTHFR (C677T).

2.3. Protein C, S and Antithrombin III

Functional activity of protein C and S and antithrombin III (Diagnostica Stago Kits, France) were assessed by immunoturbidometric assay using plasma samples, which were collected on tubes containing trisodium citrate. Normal ranges of protein C, protein S and antithrombin III activity were considered as 70% - 140%, 60% - 140% and 80% - 120% respectively.

2.4. Statistical Method

Data were statistically described in terms of mean \pm standard deviation (\pm SD), median and range, or frequencies (number of cases) and percentages when appropriate. Odds Ratio (OR) and the 95% confidence interval (95% CI) were calculated for the presence of mutation between cases and controls. Comparison of numerical variables between the study groups was done using Student t test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

3. Results

Mean age \pm SD for patients and controls was 28.2 ± 4 and 25.3 ± 6 respectively. Patients had a minimum of 2

and maximum of 8 miscarriages. Details about mutation frequencies and defects are shown in **Table 1** and **Table 2**.

88% of cases and 55.8% of controls had thrombophilia abnormalities. The evaluation of the analyzed thrombophilic conditions revealed that MTHFR and Factor V Leiden were the commonest gene defects among cases studied (63%, 60% respectively) and controls (41.9%, 41.9% respectively), ($p = 0.019$, $p = 0.046$ respectively). There were 4 cases with homozygous MTHFR and 2 cases with homozygous Factor V Leiden mutation. Homozygosity was otherwise absent in other cases and in all controls.

Heterozygous MTHFR was present in 59 (59%) cases vs. 18 (41.9) controls ($p = 0.04$), while Factor V Leiden heterozygosity in cases was 58% (58) and 41.9% (18) in controls ($p = 0.105$). Heterozygous Prothrombin mutation was found in 11 cases (11%) and 2 controls (4.7%) ($p = 0.34$). Odds ratio for MTHFR and Factor V mutation, was 2.36 and 2.08 respectively (CI 95%, 1.008 - 4.306, 1.14 - 4.94).

Antithrombin III deficiency was found in 7 cases (7%) and 2 (4.7%) controls, protein S in 3% of cases and 2.4% controls, and protein C in 2% of cases and 0% controls.

41% of cases and 27.9% of controls had combined gene mutations ($p < 0.05$) (**Table 3**).

Table 1. Frequencies of inherited thrombophilia among cases and controls.

	Cases (normal %)	Controls (normal %)	Total	p value
MTHFR	63 (63%)	18 (41.9%)	81 (56.6%)	0.019 (s)
FVL mutation	60 (60%)	18 (41.9%)	78 (54.5%)	0.046 (s)
FII G20210A	11 (11%)	2 (4.7%)	13 (9.1%)	0.344
Antithrombin III deficiency	7 (7%)	2 (4.7%)	9 (6.3%)	0.72
Ptn S deficiency	3 (3%)	1 (2.3%)	4 (2.8%)	1.00
Ptn C deficiency	2 (2%)	0	2 (1.4%)	1.00

Table 2. Genotype frequency of thrombophilias.

	Genotype	Cases, n (%)	Controls, n (%)	p value
MTHFR	GG (normal)	37 (37%)	25 (58.1%)	0.04
	GA	59 (59%)	18 (41.9%)	
	AA	4 (4%)	0	
FVLeiden	GG (normal)	40 (40%)	25 (58.2%)	0.105
	GA	58 (58%)	18 (41.9)	
	AA	2 (2%)	0	
FII G20210A	GG (normal)	89 (89%)	41 (95.3%)	0.34
	GA	11 (11%)	2 (4.7%)	
	AA	0	0	

Table 3. Number of combined mutated genes in the studied groups.

Number of mutated genes	Cases	Controls
2	36 (36%)	12 (27.9%)
3	5(5%)	0 (0%)

$p < 0.05$ by Pearson Chi-square test.

4. Discussion

Our study reports the frequency of inherited thrombophilia in a group of Egyptian women with RPL.

The cause of RPL is largely unclear. Epidemiological studies suggested that it might be multifactorial with involvement of environmental and genetic factors [23].

Inherited thrombophilia has been implicated as a possible cause [4]. Although numerous studies are available in literature, thrombophilia rate seems to vary from study to another due to different selection criteria of the patients [1].

Inherited thrombophilia may be due to a gene defect or deficiency of clotting inhibitors leading to a hypercoagulable tendency. Gene defects frequently associated with RPL are prothrombin A20210G and factor V Leiden [24]. Deficiency of clotting inhibitors, such as protein S, protein C and antithrombin III has been associated with RPL since 1996 [25].

In our study, 88% of cases had positive thrombophilic defects (47% single defect and 41% more than one defect). Similar figures were obtained in a study on patients from southern Italy. They found 78% of cases having one or more thrombophilic defect in cases of unexplained RPL [5]. Others found the association between intrauterine fetal death and thrombophilia to be 54.5%, and 38% for recurrent fetal loss [12].

We found MTHFR and Factor V Leiden to be the commonest gene defects among cases (63%, 60% respectively) and control groups (41.9%, 41.9% respectively).

Prothrombin gene G20210A mutation was found in 11% of cases and 2% of controls ($p = 0.344$). Factor V Leiden mutation is frequent in Caucasians and absent in many African populations. Available data show that this mutation might be of Middle East origin as it is frequent in Jordan, Syria, Lebanon and Israeli Arabs. In Northern Africa, it is common in Tunisia, but very low in Algeria and nearly absent in Morocco [3]. Our results are in agreement with the studies, which concluded the association between FV Leiden mutation and RPL [2] [11] [13] but are also in partial disagreement as regards the association of Prothrombin mutation and MTHFR with RPL [13].

In two large cohorts of unselected pregnant women, carriage of Factor V Leiden mutation was not associated with increased rate of pregnancy complications. Patients positive for the mutation did not differ from non-carriers in obstetric outcome including preeclampsia, small for gestational age, babies, pregnancy loss, abruption, oligohydramnios and gestational age at delivery [14] [15]. Some case-control studies did not show an association between thrombophilia and RPL [26]. Low prevalence of inherited thrombophilia in non-Caucasian populations was observed and testing for thrombophilia in Colombian population was unlikely to play a role in RPL [27]. Also no association was found between FVL, FII, MTHFR and RPL in a group of Turkish and Palestinian women [8] [28]. Neither MTHFR nor FVL mutations were associated with unexplained RPL in a group of Japanese women [29].

The least common deficiencies in our study were protein S and protein C deficiency in cases (3%, 2% respectively) as well as in controls (1%, 0% respectively) and the difference was not significant. This was not in concordance with other case-control studies, which found a significantly higher frequency of protein S deficiency in patients with RPL compared to controls [26] [30]. This difference among studies may be attributed to the difference in ethnic population, different definitions for adverse outcomes, combining thrombophilia or adverse outcomes or both into summary statistics, and the small size of most studies. The vast majority of data that link inherited thrombophilia to adverse obstetric events are derived from case-control studies [5] [12] [31]-[35]. When the study group was unselected pregnant women, no association was found between Factor V Leiden [15] [16] and Prothrombin [2] mutations and pregnancy complications. So the difference in the type of patients (case-control versus unselected cohort study) included in the study may also explain the discrepancy in the results.

12% of our cases were negative for thrombophilia. This suggests the presence of other factors that could be related to RPL that were not studied as environmental, occupational, psychological, epidemiological factors and immunological factors.

Many physicians treated thrombophilia patients with heparin or low molecular weight heparin during pregnancy hoping to improve their obstetric outcome. However, there is little evidence to guide the treatment of those women [36]. A recent randomized cohort study found no benefit from thromboprophylaxis in women with thrombophilia, regardless of whether they had prior obstetric complications [37]. Trials have been conducted on effect of LMWH treatment [17]-[19] but none of them studied the effect of therapy in the subgroup of women

with thrombophilia. Randomized placebo-controlled trials are needed to study the use of anticoagulants in women with recurrent miscarriage and inherited thrombophilia [20]. Heparin therapy during pregnancy may improve the live birth rate of women with second-trimester miscarriage associated with inherited thrombophilia [38].

Our study was not without limitations. The study was self-funded, which explains the small number especially the controls.

Strengths of the study were the meticulous choice of cases (after exclusion of other causes of recurrent pregnancy loss), and controls (age-matched, healthy with no medical diseases or history of thrombosis, no previous pregnancy losses and at least one full term delivery). Another strength was the analysis of 3 inherited thrombophilia as well as 3 deficiencies for every case.

5. Conclusion and Recommendations

We found MTHFR and FVL mutation to be significantly higher in cases than controls in a group of Egyptian women with unexplained early recurrent miscarriage. Further research is still needed in this field including larger numbers of cases as well as research involving feasible therapeutic intervention for this group of patients.

Disclosure

There was no conflict of interest, and the paper is not being considered by another journal. The paper was self-funded.

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