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Original Article

Prevalence of Thrombophilia in Patients with Adverse Pregnancy Outcome

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Abstract

Background: Thrombophilia is hereditary and acquired conditions that predispose the patients to thrombosis. Pregnancy is hypercoagulable state. The tendency to thrombosis develops rapidly in adverse conditions in pregnancy and has been linked to many aspects of pregnancy. It is recently shown that severe pregnancy complications such as severe preeclampsia intrauterine growth retardation abruptio placentae and stillbirth have been shown to be associated with thrombophilia. Recurrent miscarriage has also been associated with thrombophilia. Finally, thromboembolism in pregnancy as in the non-pregnant state is linked to thrombophilia.

Aim of the Study: This study aims to study the prevalence of acquired and inherited thrombophilia in women with adverse pregnancy outcome (APO) and their diagnosis with the help of biological markers. The objective was also to study the incidence of each type of thrombophilia in patients with APO.

Materials and Methods: A total of 69 patients with a history of APO in previous pregnancies were included in this study to understand the prevalence of hemophilia in them. Various thrombophilic studies were undertaken using the blood samples of the patients. They included anticardiolipin antibodies test, lupus anticoagulant (LAC) test, protein C assay, protein S assay, activated protein C-resistant test, antithrombin assay, homocysteine estimation, prothrombin gene mutation test, anti-*β*-2 glycoprotein antibodies assay, Proglobal C assay, and factor V Leiden (FVL) mutation test. Based on the test results, the type of thrombophilia was diagnosed, analyzed, and compared with other studies.

Observations and Results: A total of 69 patients with a history of APO were screened for inherited and acquired thrombophilia. The patients were enrolled over a period of 2 years from July 2006 to June 2008 from the OPD at the Department of Obstetrics and Gynaecology, AIIMS, New Delhi. Recurrent abortion in 32 (58.18%) patients was the most common APO in women screened for thrombophilia. Other AOPs observed were intrauterine death of fetus (IUD) in 17 (24.63%), intrauterine growth retardation (IUGR) in 11 (15.94%), and severe preeclampsia in 9 (13.04%). The mean gravidity of the patients was 3.7 ± 1.25. The mean gestation of the patients with recurrent abortion was 3.81 ± 0.86, while that for patients with IUD was 3.41 ± 1.12, with IUGR it was 4 ± 0.89 and preeclampsia it was 4.33 ± 0.87. The thrombophilic tests were positive in 33/69 (47.82%) patients in the study, and among them, there were 8/69 (10.14%) patients with inherited thrombophilia in the present study. Protein C deficiency in 4 (5.79%) patients, hyperhomocysteinemia in 2 (2.89%), antithrombin III deficiency in 1 (1.44%), and FVL mutation in 1 (1.44%) patients each. 25 patients tested positive for Anti-β-2 glycoprotein antibodies, nine patients for ACL, and two for LAC.

Conclusions: Thrombophilia is common among patients with APO. Inherited thrombophilia is less common than the acquired ones. Biomarkers such as protein C deficiency, hyperhomocysteinemia, antithrombin III deficiency, and FVL mutation help to diagnose inherited type and treat these patients early. Among the acquired thrombophilia patients, anti-*β*-2 glycoprotein antibodies were positive as biomarker in majority of acquired type of thrombophilia. Other markers useful are anticardiolipin antibodies and deficient LAC.

Key words: Hypercoagulability, Inherited thrombophilia, Pregnancy, Thrombin assay, Thrombophilia

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INTRODUCTION

The term thrombophilia was first introduced by Egerberg *et al*., in 1965, when he reported a Norwegian family who had a remarkable tendency to venous thrombosis because of a deficiency in the natural anticoagulant antithrombin. At present, this term is generally used to describe a laboratory

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abnormality (most often in the coagulation system) that increases the tendency to venous thromboembolism. Thrombophilic abnormalities can be acquired or inherited. Theoretically, the concept is that patients who have thrombophilia have an intrinsic prothrombotic state that in itself is insufficient to cause thrombosis, but may lead to an event when superimposed on (clinical) risk factors, including increasing age.[1,2] There is a growing view that inherited and acquired thrombophilia may predispose to adverse pregnancy outcome (APO). As already known for the acquired antiphospholipid antibody syndrome, most inherited thrombophilic disorders are also associated with pregnancy-related disorders such as (recurrent) fetal loss, stillbirth, intrauterine growth restriction, preeclampsia, and the hemolysis-elevated liver enzyme-low platelets syndrome of pregnancy.[3,4] Inherited thrombophilia types include antithrombin III deficiency, protein C deficiency, protein S deficiency, factor V Leiden (FVL) mutation, prothrombin 20210A mutation, and hyperhomocysteinemia. Antithrombin III, protein C, and protein S function as physiological inhibitors of coagulation cascade and are, therefore, referred to as natural anticoagulants. Deficiencies resulting in one of these proteins lead to an imbalance in basal coagulation activity toward a prothrombotic state. This has been confirmed in studies showing increased markers of thrombin generation in patients with one of this deficiencies.[5,6] More recently, deficiency of protein Z has also been linked to pregnancy complications including preterm delivery. The FVL mutation is the most common inherited thrombophilic defect and is found in approximately 20% of patients who have venous thromboembolism (VTE) and in 5% of Caucasian population. It is a point mutation gene coding for clotting factor V (G1691 A), causing replacement of arginine by glutamine in the cleavage site for activated protein C (APC, Q), thereby making activated factor V more resistant to inactivation by this physiologic anticoagulant (APC resistance).[7,8] The frequency of the FVL mutation varies among different ethnic groups. The mutation is present in 5.2% of Caucasians, 1.2% of African Americans,[9] and 5–9% of Europeans, while it is rare in Asian and African populations.[9,10] The FVL mutation is primarily inherited in an autosomal dominant fashion[8,10] Heterozygosity for the FVL mutation is present in 20–40% of non-pregnant patients with thromboembolic disease, while homozygosity, the rarer condition, is associated with a significantly higher (100-fold) risk of thromboembolism.[10] The prothrombin 20210A mutation is a point mutation that leads to a normal protein, but higher average levels of inactive factor II (prothrombin) compared with wild-type genotype, which is the presumed mechanism of the prothrombotic phenotype.[9] The most common acquired thrombophilia is due to antiphospholipid antibodies, which include lupus anticoagulant (LAC) and anticardiolipin antibodies and anti-B-2glycoprotein 1 antibodies.[8] Thrombophilias derived from a combination of hereditary and acquired components, such as the VIII C factor, hyperhomocysteinemia, and acquired APC resistance, are identified. In addition, pregnancy itself leads to a thrombophilic state as a result of hemostatic and fibrinolytic changes.[7] During pregnancy, procoagulant factors (such as VIII, XII, VII, and V) and the von Willebrand factor and fibrinogen are increased, protein S and the APC are reduced, and fibrinolytic activity is diminished.[9] All of these modifications, together with an enlarged plasmatic volume, prepare the mother to face the hemostatic state during delivery. Recently, anti-I3-2glycoprotein 1 antibody is also considered as the marker for acquired thrombophilia.[5] Although the relation of acquired thrombophilia to APO is well established, controversy still exists for inherited thrombophilia. Studies published in literature have shown varying results possibly because of ethnic differences.[11] Deficiency of antithrombin was the first recognized inherited thrombophilia. Antithrombin deficiency is inherited in an autosomal dominant pattern, and the prevalence of the heterozygous state in the general population is estimated at 1:2000–1:5000.[11,12] The prevalence of antithrombin deficiency in unselected patients with a history of VTE is approximately 1%[13,14] and increasing up to 4.9% in recurrent VTE or VIE in individuals <45 years of age.[15] The gene for antithrombin is at lq 23–25 and a database of mutations associated with antithrombin deficiency has been compiled and recently updated.[16] Protein C deficiency was the second inherited thrombophilia described. The prevalence of heterozygous protein C deficiency in the general population ranges from 0.15% to 0.8%.[17,18] Protein S serves as a cofactor for protein C, thereby acting as a natural inhibitor of the coagulation cascade. Protein S circulates in plasma in two forms - about 40% in the free active form and 60% bound to C4b-binding protein, a regulator of the complement system. Levels of C4b-binding protein are increased in pregnancy, with the combined oral contraceptive pill and in inflammation. An increase in the level of C4b-binding protein leads to a reduction in the level of free active protein S, possibly contributing to a thrombophilic state.[19] In 1996, Poort *et al*.[20] first described a mutation in the 3'-untranslated region of the prothrombin gene that was more common in individuals with venous thrombosis. The mutation is a single base-pair substitution of guanine to adenine at position 20210 (G20210A)[21] and is associated with increased levels of prothrombin. The prothrombin G20210A mutation is increased 2—5-fold in individuals with VTE (5.0–6.2%) compared with controls (1.2–2.6%).[22,23] This study was carried out with an aim to study the prevalence of acquired and inherited thrombophilia in women with APO.

Aims and Objectives

This study aims to study the prevalence of acquired and inherited thrombophilia in women with APO and their

role on pregnancy complications. The objective was also to study the type of thrombophilia associated with each APO.

Type of Study

This is a prospective, cross-sectional, observational study.

Period of Study

The study duration was from July 2006 to June 2008.

Institute of Study

This study was conducted in the Department of Obstetrics and Gynaecology, AIIMS, New Delhi.

MATERIALS AND METHODS

A total of 69 patients with a history of APO in previous pregnancies were included in this study to understand the prevalence of hemophilia in them. Ethical committee clearance was obtained before the commencement of the study. An ethical committee approved pro forma and consent forms were used while conducting the study.

Inclusion Criteria

Patients with a history of APO in previous pregnancies such as:

1. Severe preeclampsia <36 weeks
   a. Blood pressure more than 160/110
   b. Proteinuria >5 g/day
   c. Hemolysis
   d. Elevated liver enzymes
   e. Platelets <1 lakhs/mm^3
   f. Eclampsia.
2. Placental abruption.
3. Delivery of small for gestational age baby.
4. Unexplained intrauterine deaths.
5. Recurrent abortions (>3) were included in the study.

Exclusion Criteria

Patients with chronic hypertension, diabetes mellitus, cardiovascular disease, renal disease, multiple pregnancies, maternal drug or alcohol abuse, intrauterine infections, suspected chromosomal abnormalities, congenital malformations detected by ultrasound, and on anticoagulation therapy were excluded from the study.

Timing of Study

Patients with a history of APO fitting inclusion criteria were screened for thrombophilia in the preconception period or >6 weeks postpartum.

Method of Study

Detailed obstetric history was taken. Routine investigations such as hemogram and liver and renal function tests were done. Some special investigations such as TSH, glucose tolerance tests with 75 g glucose, hysteroscopy, parental blood karyotyping, and TORCH screen if indicated were done to exclude other causes of APO. After proper counseling and informed consent, 20 mL of blood was drawn from the patient and sent to the department of hematology, to investigate for thrombophilia in the same hospital. Blood sample mixed with 3.2% trisodium citrate (1:9 ratios). Sample centrifuged at 3500–4000 rpm and stored at −70°C. This sample was used for various thrombophilic studies.

Test for Anticardiolipin Antibodies

Anticardiolipin antibodies were measured by ELISA method (AIDA cardiolipin - GM Germany). Both IgG and IgM types of ACL were assayed.

Principle of the Test

Diluted serum samples are incubated with cardiolipin immobilized on microliter wells. After washing away unbound serum components, rabbit antihuman IgG or IgM conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3, 3', 5, 5'-tetramethylbenzidine and enzyme substrate is added to trace specific antibody binding. Addition of stop solution terminates the reaction and provides the appropriate pH for color development. The optical densities of the standards, positive control, and samples are measured using a microplate reader at 450 nm. Optical density is directly proportional to antibody activity in the sample. For the assessment of patient's sera diagnosis, the following ranges are recommended [Table 1].

LAC Test

This was performed using Kaolin clotting time (Sigma Diagnostics, USA).

Principle

LAC act against the prothrombinase, thereby prolonging all phospholipids dependent tests such as APTT, PT, and RVVT. When the APTT is modified by omitting platelet substituting reagent, it becomes particularly sensitive to the LAC. If the test is performed on a range of mixtures of normal and patient's plasma, different patterns of response are obtained, indicating the presence of LAC, deficiency of one or more of the coagulation factors, or the lupus cofactor effect.

Table 1: The reference range for quantitative results of ACL

Range	Cardiolipin IgG (GPLU/mL)	Cardiolipin IgM (MPLU/mL)
Normal	<11	<10
Positive	>11	>10
Moderate	20–40	20–40
High	>40	>40

Requirements

1. Kaolin (10 mg/mL in Tris buffer) pH 7.4 2.
2. Normal platelet poor plasma.
3. Patient's plasma.
4. Calcium chloride (0.025 moL/L).

Procedure

1. The following dilutions were made [Table 2].
    1. Take 0.2 mL of above dilution +0.1 mL Kaolin (10 mg/mL).
    2. Incubate for 2 min with constant shaking.
    3. Add 0.2 mL of calcium chloride and observe for clot formation.
    4. Note down the clotting. Repeat the test with all other four dilutions.

Normal values: The reference range is taken between 60 and 120 s. Interpretation: If the kaolin clotting time of PP is >120 s, then the following calculations are performed: Reference% =(NP+PP)-(NP)/(PP) ×100. Normal sample shows a reference range <15%. Anything above 15% is abnormal. Proglobal C assay: It is a clotting-based technique (Dade Behring, Germany). It is a screening test for thrombophilia. For this assay, two test tubes marked blank (B) and test (T), respectively. 100 μL of citrated plasma is taken each tube. 100 μL of buffer is added to blank tube and 100 μL of Proglobal C activator is added to test tube. Then, 100 μL of APTT reagent added to both tubes and incubated for 3–4 min. 100 μL of calcium chloride added to both tubes. Then, clotting time assessed in both tubes. Then, ratio (T/B) assessed. Ratio multiplied with coefficient factor which is specific for the sample kit. Normal value is >0.8. Low Proglobal C <0.8 warrants further tests for protein C, protein S, and APCR. Protein C assay: It is a sandwich ELISA. "READS protein C antigen 96-microwell Test Kit" manufactured by Corgenix, Inc., USA was used. A capture antibody specific for human protein C was coated to 96-microwell polystyrene plates. Diluted plasma was incubated in the wells allowing any available protein C to bind to the antihuman protein C antibody on the microwell surface. The plates were washed to remove unbound proteins and other plasma molecules. Bound protein C was quantified using horseradish peroxidase (HRP) conjugated antihuman protein C detection antibody. Following incubation, unbound conjugate was removed by washing. A chromogenic substrate of tetramethylbenzidine (TMB) hydrogen peroxide is added to develop a colored reaction. The intensity of the color is measured in optical density (O.D) units with spectrophotometer at 450 nm. Protein C antigen relative percent concentrations in PP are determined against curve prepared from the reference plasma provided with the kit. Reagents: Each READS protein C antigen 96-microwell test kit contains the following reagents.

1. 12 × 8 antihuman protein C antibody-coated microwells.
2. 60 mL sample diluents' (blue-green) solution contains sodium azide.
3. 3 vials ×0.5 mL lyophilized reference plasma, with assay sheet.
4. 12 mL antihuman protein C HRP conjugate (blue solution).
5. 13 mL substrate (TMB and H_2O_2) 15 mL stopping solution (0.36 N sulfuric acid).
6. 30 mL wash concentrate (×33 phosphate-buffered saline with 0.01% Tween 20).

Procedure

1. Reference plasma is prepared by adding 0.5 mL reagent grade water. Gently mix and allows standing for 10 min.
2. Predilute all plasmas (1:2 dilutions in sample diluent) as follows: Reference plasma: Add 100 μL reference plasma to 100 μL sample diluent.
3. Control and patient samples: Add 20 μL plasma to 20 μL sample diluents and mix well. These predilutions are utilized in preparing the working solutions in steps 4 and 5.
4. Using the 1:2 reference plasma dilutions from step 3, prepare six working reference dilutions.
5. Prepare working dilutions of control and patient samples by adding 20 uL of prediluted plasma (1:2 dilutions from step 3) to 500 pL sample diluent.
6. Mix thoroughly and add 10 μL of the working solutions (reference plasmas, controls, and patient samples) to the appropriate microwells.
7. Add 100 μL of sample diluent to the reagent blank well to leave the water blank well empty.
8. Incubate 40 min at room temperature. Then, invert the microwells and dump the sample fluid.
9. Wash 4 times with working solution.
10. Add 100 μL conjugate to each well.
11. Incubate for 10 min at room temperature. Then, invert the microwells and dump the conjugate solution.
12. Wash 4 times with wash solution.
13. Add 10 μL substrate to each well (except for the water blank well) and incubate for 10 min at room temperature. Blue color will develop in samples in wells with positive samples.

Table 2: The dilutions made

Normal platelet poor plasma (NP)	Normal platelet poor plasma+patient plasma (NP+PP)	PP
0.2 mL	0.1 mL+0.1 mL	0.2 mL

PP: Patient plasma

14. Add 100 μL stopping solution to each well except for the water blank well to stop the enzyme reaction. Blue substrates will turn yellow and colorless substrate will remain colorless. Do not add stopping solution to the water blank well. Read the O.D at each well at 450 nm against 650 nm reference filter.

RESULTS

1. Calculate the mean O.D. for the duplicates of the reference plasma dilutions, controls selected for use, and patient samples.
2. Plot the mean O.D. obtained for each dilution of the reference plasma (x-axis), against the corresponding value of the reference level (y-axis). A log-log or point-to-point graph is recommended, although a semi-log may also be used.
3. Using the mean O.D., determine the control and patient relative value from the graph, or alternatively use linear regression to calculate from the reference curve.
4. To calculate protein C antigen level in percentage of normal, multiply the control and patient relative values obtained from the reference curve by the assigned value for the REIDS reference plasma (see vial label).

For example:

- Patient relative value (from the reference curve): 40
- Reference plasma assigned value (from vial label): 105% of normal
- Actual patient protein C antigen value (as percentage of normal): 40 × 1.05 = −42%
- Normal range of protein C is 72–160%.

Protein S Assay

It is also a sandwich ELISA kit manufactured by the same company. Procedure and principle the same as previous test except for the antihuman protein S HRP conjugate. Normal level of protein S is 50–130%. APC resistance: It is a clot-based technique; kit is manufactured by "Stago Diagnostics, France." Reagent includes factor V deficient plasma, venom as the cofactor, and calcium chloride (0.025M). Procedure: Patients plasma is diluted with buffer in 1:10 ratio. Control was also prepared. 100 μL each of patient's plasma, factor V deficient plasma, and venom was mixed with each other and incubated for 4 min. Then, additional 100 μL of calcium chloride was kept in fully automated coagulometer and the clotting time noted. Normal value: For APCR, it was >120 s. If low, APCR was observed, then FVL mutation study was done. Antithrombin III assay: It is a chromogenic method. Kit is manufactured by Stago Diagnostics, France. Patient's plasma is diluted with 1:10 ratio with buffer. Reagents included substrate and thrombin. Procedure: Patients plasma, substrate, and thrombin 100 ptL each added and mixed and incubated for 3–4 min. O.D. measured at 450 nm taken and graph plotted. Normal value was taken as 70–130%. Anti-β-2 glycoprotein antibodies: It was measured by ELISA method. The kit was manufactured by AIDA, Germany. Normal range: 5–15 IU/mL. Homocysteine: ELISA method. Normal range: It was 5–15 IU/mL. Prothrombin gene mutation: Prothrombin gene mutation was not prevalent in Indian population as evidenced by the previous reports. Hence, it was not done in our laboratory. FVL mutation: Whole blood collected in EDTA vial and genomic DNA was extracted from whole blood as per standard protocols. The polymerase chain reaction (PCR) using known primers was used to amplify exon 10 of the factor V gene which contains the G→A mutation at nucleotide position (1691). Following amplification, a 200 aliquot of the product was digested overnight with 5 IU of the enzyme Mn11 (New England Biolabs, Hitchin, UK) at 37°C. Samples of the digested and undigested PCR product were separated electrophoretically in a 3% agarose gel and the bands visualized using ethidium bromide. The undigested PCR product measures 223 base pairs (bp) in size. Following cleavage with *Mnl1*, a normal allele produces bands of 37, 82, and 104 bp. A mutant allele produces bands of 82 and 141 by due to loss of one *Mnl1* cleavage site. Controls on each gel included a known heterozygote, a normal control known not to possess the FVL mutation and a water blank containing no input DNA.

Statistical Analysis

The prevalence of thrombophilia in each APO was calculated. Demographic data and clinical data are presented as patient group means with SE. SPSS software version 11.0 was used for analysis.

OBSERVATIONS AND RESULTS

A total of 69 patients with a history of APO were screened for inherited and acquired thrombophilia. The patients were enrolled over a period of 2 years from July 2006 to June 2008 from the OPD at the Department of Obstetrics and Gynaecology, AIIMS, New Delhi. Recurrent abortion in 32 (46.37%) patients was the most common APO in women screened for thrombophilia in the study. Other AOPs were unexplained IUD in 17 (24.63%), IUGR in 11 (15.94%), and severe preeclampsia in 9 (13.04%) [Table 3 and Figure 1].

Age Distribution

The mean age of the patients in this study was 27.34 ± 3.28 with a range of 20–36 years. The mean age of patients with recurrent abortion was 27.38 ± 2.25 with a range of 22–32 years while that for the patients with intrauterine deaths it was 27.70 ± 1.99 with a range of 24–31 years, with intrauterine growth restriction it was 27.81 ± 2.92 with a range of 24–34 years and preeclampsia it was 28.66 ± 3.35 with a range of 25–36 years [Table 4].

Distribution of Gravidity

The mean gravidity of the patients in the present study was 3.7 ± 1.25. The mean gestation of the patients with recurrent abortion was 3.81 ± 0.86, while that for patients with IUD was 3.41 ± 1.12, with IUGR it was 4 ± 0.89 and preeclampsia it was 4.33 ± 0.87 [Table 5].

The thrombophilic tests were positive in 33/69 (47.82%) patients in the study, and among them, there were 8/69 (10.14%) patients with inherited thrombophilia in the present study. Protein C deficiency was noted in 4 (5.79%) patients, hyperhomocysteinemia in 2 (2.89%), antithrombin III deficiency in 1 (1.44%), and FVL mutation was noted in 1 (1.44%) patient each [Table 6].

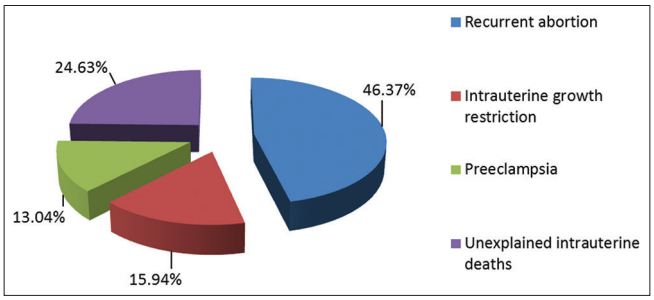

Figure 1: The incidence of adverse pregnancy outcome in the study (*n* = 69)

Prevalence of Acquired Thrombophilia

25/69 (36.23%) patients tested positive for acquired thrombophilia. 25 patients tested positive for anti-β-2 glycoprotein antibodies, nine patients for ACL, and two for LAC [Table 7].

The prevalence of thrombophilia was observed in patients with in recurrent abortion and found that 18/32 (56.25%) tested positive for thrombophilia. 16 of 32 (50%) patients with recurrent abortions were detected to have acquired thrombophilia. 2/32 (6.25%) patients were observed to have inherited thrombophilia. Anti-β-2 glycoprotein antibody was positive in 10/32 (31.25%) patients. ACL IgG was positive in 3/32 and ACL IgM was positive in 3/32 of the patients (6/32–18.75%) [Table 8].

Table 3: The incidence of APO conditions in the study (*n*-69)

Type of APO	Number of patients 69 (%)
Recurrent abortion	32 (46.37)
Unexplained intrauterine deaths	17 (24.63)
IUGR	11 (15.94)
Preeclampsia	9 (13.04)

IUGR: Intrauterine growth retardation, APO: Adverse pregnancy outcome

Table 4: The age distribution of patients with different APO

APO	Age mean±SD (range in years)
All patients	27.34±3.28 (20–36)
Recurrent abortion	27.38±2.25 (22–32)
Unexplained intrauterine deaths	27.70±1.99 (24–31)
Intrauterine growth restriction	27.81±2.92 (24–34)
Preeclampsia	28.66±3.35 (25–36)

APO: Adverse pregnancy outcome

Table 5: The distribution of gravidity in the study group

APO	Mean gravidity±SD (range)
All patients	3.7±1.25 (1–8)
Recurrent abortion	3.81±0.86 (3–6)
Unexplained intrauterine deaths	3.41±1.12 (1–5)
Intrauterine growth restriction	4±0.89 (3–6)
Preeclampsia	4.33±0.87 (3–6)

SD: Standard deviation

Table 6: The prevalence of inherited thrombophilia in the study group

Type of thrombophilia	Number of patients *n*=08/69 (%)
Protein C deficiency	4 (5.79)
Protein S deficiency	1 (1.44)
Antithrombin III deficiency	1 (1.44)
FVL mutation	1 (1.44)
Hyperhomocysteinemia	2 (2.89)
Total	8 (10.14)

FVL: Factor V Leiden

Table 7: The prevalence of acquired thrombophilia in the study group (*n*=69)

Laboratory test	Number of patients - 25
LAC	2
ACL IGG	6
ACL IGM	3
Anti-β-2 glycoprotein antibody	25
Total positive tests	36

LAC: Lupus anticoagulant

Table 8: The prevalence of thrombophilia among the patients with recurrent abortion (*n*=32)

Laboratory test	Number of patients (*n*=32) (%)
Protein C deficiency	0
Protein S deficiency	0
Antithrombin III deficiency	1 (3.12)
FVL mutation	0
Hyperhomocysteinemia	1 (3.12)
LAC	0
ACL IgG	3 (9.37)
ACL IgM	3 (9.37)
Anti-β-2 glycoprotein antibody	10 (31.25)
Inherited thrombophilia	2
Acquired thrombophilia	16
Total	18/32 (56.25)

LAC: Lupus anticoagulant

Prevalence of Thrombophilia in Unexplained IUD

There were 17/69 (24.63%) patients with preterm in intrauterine deaths. Among them, 2/17 (11.76%) were found to have inherited thrombophilia. Protein C deficiency was observed in 1/17 (5.88%) patients. 1 patient (5.88%) had FVL mutation. Acquired thrombophilia was seen in 10/17 (58.82%) patients. 7/17 patients (41.17%) had anti-β-2 glycoprotein antibody positive result [Table 9].

Prevalence of Thrombophilias in IUGR

In the present study, 11/69 (15.94%) patients had intrauterine growth restriction (birth weight below the 10th percentile). Seven of 11 patients screened positive for thrombophilia (63.63%). Inherited thrombophilia was present in 2/11 patients (18.18%). Protein C deficiency and hyperhomocysteinemia were present in 1 patient (9.09%) each with inherited thrombophilia in this group. Five of 11 patients (45.45%) screened positive for acquired thrombophilia. Anti-β-2 glycoprotein was present in 4/11 patients (36.36%) and ACL IgG was observed positive in 1/11 (9.09%) of the patients [Table 10].

Prevalence of Thrombophilia in Severe Preeclampsia

In this study, nine patients with severe preeclampsia were included in the study. 7/9 patients screened positive for thrombophilia (77.77%). Inherited thrombophilia was present in 2 (18.18%) of 11 patients and both showed positive for Protein C. One patient each was positive for protein S deficiency and 4 (44.44%) for anti-β-2 glycoprotein antibody test showing acquired thrombophilia in 5/9 patients (55.55%) [Table 11].

Table 9: The prevalence of thrombophilia in unexplained intrauterine deaths (*n*=17)

Laboratory test	Number of patients (*n*=17) (%)
Protein C deficiency	1 (5.88)
Protein S deficiency	0
Antithrombin III deficiency	0
FVL mutation	1 (5.88)
Hyperhomocysteinemia	0
LAC	1 (5.88)
ACL IgG	1 (5.88)
ACL IgM	0
Anti-β-2 glycoprotein antibody	7 (41.17)
Acquired thrombophilia	10 (58.82)
Inherited thrombophilia	2 (11.76)
Total	12 (70.58)

LAC: Lupus anticoagulant

Table 10: The prevalence of thrombophilia in intrauterine growth retardation group (*n*=11)

Laboratory test	Number of patients (%) (*n*=11)
Protein C deficiency	1 (18.18)
Protein S deficiency	0
Antithrombin III deficiency	0
FVL mutation	0
Hyperhomocysteinemia	1 (9.09)
LAC	0
ACL IgG	1 (9.09)
ACL IgM	0
Anti-β-2 glycoprotein antibody	4 (36.36)
Acquired thrombophilia	5 (45.45)
Inherited thrombophilia	2 (18.18)
Total	10 (90.90)

LAC: Lupus anticoagulant

Table 11: Prevalence of thrombophilia in severe preeclampsia patients (*n*=9)

Type of thrombophilia	Number of patients (*n*=9) (%)
Protein C deficiency	2 (18.18)
Protein S deficiency	1
Antithrombin III deficiency	0
FVL mutation	0
Hyperhomocysteinemia	0
LAC	0
ACL IgG	1 (11.11)
ACL IgM	0
Anti-β-2 glycoprotein antibody	4 (44.44)
Acquired thrombophilia	5 (55.55)
Inherited thrombophilia	2 (18.18)
Total	7 (77.77)

LAC: Lupus anticoagulant

DISCUSSION

There are various causes for APO. Patients with a history of APO such as recurrent miscarriages, unexplained intrauterine deaths, severe preeclampsia, and intrauterine growth restriction need to be evaluated in the preconception period or 6-week postpartum. The common causes for these pregnancy complications such as thyroid disorder, diabetes mellitus, hypertension, and chronic systemic illnesses should be ruled out with proper history, physical examination, and relevant laboratory investigations. Special investigations such as parental karyotyping and diagnostic hysteroscopy are needed in recurrent spontaneous abortions. In the absence of these common causes of pregnancy complications, the patients have to be investigated for thrombophilia. Definite relationship of acquired thrombophilia with different APO has been reported in previous studies. Studies are available evaluating inherited thrombophilia and pregnancy complications but with conflicting reports. This could be due to the difference in the prevalence of inherited thrombophilia in ethnic groups or difference in the methodology of the studies reported. This study was an observational study in which 69 patients with APO were included from the OPD of Obstetrics and Gynecology Department of AIIMS and investigated for acquired inherited thrombophilia. The prevalence of thrombophilias in APO: In the present study, 33/69 women with thrombophilia happening in APOs were observed (47.82%). In a comparative study shown in Table 12, the incidence was ranging from 32% to 66%.

The number of patients screened in the present study is comparable to Kupferminc *et al.*[24] and Ariel *et al.*[25] studies.

Prevalence of Inherited Thrombophilias in Recurrent Abortion

2/32 (6.25%) of the patients with recurrent abortion had inherited thrombophilia. Acquired thrombophilia was present in 16/32 (50%) of patients with recurrent abortion. Among these, majority (10/32 [31.25%]) were positive for anti-ß-2 glycoprotein antibodies. 6/32 patients were positive for anticardiolipin antibodies (18.75%); 3/32 (9.37%) patients had each positive for IgG and IgM anticardiolipin antibodies. None had LAC positive. A comparative study [Table 13] was made and found that the incidence of inherited thrombophilia in women with recurrent abortion in the present study was low compared to the other studies. This may be due to the low prevalence of thrombophilic mutations in Indian population or could be because of small sample size. The prevalence was comparable to Rai *et al's.* study, but he only screened for FVL mutation.

The prevalence of acquired thrombophilia in women with recurrent abortion in the present study was high (50%) comparable to some large studies by Kumar *et al.*,[35] Velayuthaprabhu *et al.*,[36] etc. Anti-β-2 glycoprotein antibodies 10/32 (31.25%) were the most commonly detected in our study which is comparable to that in Kumar *et al's.*[35] studies? There were some studies screened for more classes of antibodies against antiphospholipid which were not included in this study such as Yamada *et al.*[37] and Velayuthaprabhu *et al.*[36] [Table 14].

Prevalence of Thrombophilias in Unexplained IUD

Of 17 patients with intrauterine deaths screened for thrombophilia, 2/17 (11.76%) patients had inherited thrombophilia. Protein C deficiency was present in 1/17 (5.88%) patient and one patient had FVL mutation (5.88%). Antithrombin III deficiencies, hyperhomocysteinemia, and protein C deficiency were not detected in this group. Acquired thrombophilia was present in 10/17 (58.82%) of patients. Most prevalent positive test for thrombophilia was anti-β-2 glycoprotein antibodies in 7/17 (41.17%). 1 patient (5.88%) had ACL IgG positive. The prevalence of inherited thrombophilia in this study was lower, whereas it was higher in Kupferminc *et al.*[24] and Alfirevic *et al.*[27] studies. Similarly, the incidences of

Table 12: A comparative study of incidence of thrombophilia in APO

Study	Total number of patients screened	Prevalence of thrombophilia (%)
Kupferminc *et al.*[24]	110	65
Ariel *et al.*[25]	40	42
Sarig *et al.*[26]	145	66
Alfirevic *et al.*[27]	102	53
Ogunyemi *et al.*[28]	75	32
Zahed *et al.*[29]	91	55
Hvas *et al.*[30]	414	42
Present study (2008)	69	47.82

APO: Adverse pregnancy outcome

Table 13: Comparative study of prevalence of inherited thrombophilia in recurrent abortion patients

Study	Number of patients screened	Prevalence of inherited thrombophilia (%)
Foka *et al.*[31]	80	36
Rai *et al.*[32]	1111	8
Couto *et al.*[33]	88	76
Xu *et al.*[34]	112	38.4
Present study (2008)	32	6.25

Table 14: The prevalence of acquired thrombophilia in recurrent abortion

Study	Number of patients	Prevalence of acquired thrombophilias (%)	LAC N/N (%)	ACL N/N (%)	Anti-ß-2 glycoprotein antibodies (%)
Parazzini *et al.*[38]	220	15	16/220 (7)	19/99 (19)	-
Kumar *et al.*[35]	107	46%	11/107 (10.28)		33/82 (40.24)
Yamada *et al.*[37]	114	26.3*	2/114 (1.8)	9.7	5.3
Patarassi *et al.*[39]	64	48.4	-	31/64 (48.4)	-
Velayuthaprabhu *et al.*[36]	155	51.6**	-	62/155 (40)	-
Couto *et al.*[33]	88	13.6	1/88 (1.1)	11/88 (12.5)	-
Present study	32	31.25	0	6/32 (18.75)	10/32 (31.25)

*Included antiphosphatidyl ethanolamine antibody accounting 20%. **Included antiphosphatidyl serine antibody accounting 19%

acquired thrombophilia were lower in this study and higher in all other studies [Table 15].

Prevalence of Thrombophilias in IUGR

Of 11 patients with IUGR, two patients had inherited thrombophilia (18.18%). Inherited thrombophilia was proved by protein C deficiency in 1 patient (9.09%) and FVL mutation 1 patient (9.09%). Antithrombin III deficiencies, hyperhomocysteinemia, and protein S deficiency were not detected in this group. 4 patients (36.36%) showed anti-β-2 glycoprotein antibodies. One patient had anticardiolipin IgG antibodies (9.09%). Anticardiolipin IgM antibodies and LAC were not detected in patients with intrauterine growth restriction. The prevalence of inherited thrombophilia in IUGR in our study is comparable to the three previous studies[24,27,40] may be due to the similar and small sample sizes. The prevalence differs from Van Pampus MG *et al.*'s[43] studies because he included only FVL mutation. High prevalence of acquired thrombophilia in the present study compared to the previous studies may be due to the high prevalence of anti-β-2 glycoprotein antibodies in our study population, which marker was not screened in these previous studies [Table 16].

Prevalence of Thrombophilias in Severe Preeclampsia

Nine patients with severe preeclampsia were screened for thrombophilia. Seven patients screened positive for thrombophilias (77.77%). Inherited thrombophilia was present in 2 of 9 patients (22.2%). Both were positive for protein C deficiency. Acquired thrombophilia was present in 5 (55.5%) patients. Four patients had anti-β-2 glycoprotein antibody accounting (44.44%) [Table 17]. Regarding inherited thrombophilias, the prevalence in the present study was comparable to the study of Van Pampus *et al.*,[43] but the present study differs from having high prevalence of acquired thrombophilia. It may be due to the difference in the sample size. Dekker *et al's.*[44] and Kupferminc *et al.*[24] studies differ from the present study

Table 15: The comparative study of prevalence of thrombophilia in women with IUD

Study	Total number of patients with IUD	Prevalence of inherited thrombophilia (%)	Prevalence of acquired thrombophilia (%)
De Vries *et al.*[40]	18	22.2	22.2
Gris *et al.*[41]	232	81	14.22
Kupferminc *et al.*[24]	12	50	-
Ariel *et al.*[25]	40	42.5	0
Alfirevic *et al.*[27]	18	55.5	5.5
Present study (2008)	17	11.76	58.82

Table 16: Studies comparing prevalence of thrombophilia in IUGR (*n*=11)

Study	Total number of patients with IUGR	Prevalence of inherited thrombophilia (%)	Prevalence of acquired thrombophilia (%)
Kupferminc *et al.*[24]	44	50	4.5
De Vries *et al.*[40]	13	46.1	7.6
Alfirevic *et al.*[27]	25	40	24
Verspyck *et al.*[42]	203	8	-
Present study (2008)	11	18.18	45.45

IUGR: Intrauterine growth retardation

Table 17: The comparison between studies with data of prevalence of thrombophilia in patients with severe preeclampsia

Study	Number of patients with severe preeclampsia	Prevalence of inherited thrombophilia (%)	Prevalence of acquired thrombophilia (%)
Dekker *et al.*[44]	101	60	28.4
Kraus *et al.*[45]	21	41	-
Mello *et al.*[46]	46	32.6	3
Kupferminc *et al.*[24]	97	60	2
Lin *et al.*[47]	50	22	-
Horstkamp *et al.*[48]	70	7.1	-
Degroot *et al.*[49]	163	12.9	-
Ozcan *et al.*[50]	44	42.3	-
Van Pampus *et al.*[43]	345	17.7	20.9
Ganzevoort *et al.*[51]	206	26	10
Mello *et al.*[46]	406	37.9	12.8
Present study (200)	9	22.2	55.5

in having high prevalence of inherited thrombophilias and comparatively low prevalence of acquired thrombophilias. It may be due to the highly prevalent thrombophilic mutations in those ethnic groups.

The present study has limitations. The major limitation is being the small sample size. The present study was done in a tertiary hospital so some degree of referral and selection bias cannot be excluded in the study. The study design is observational study.

CONCLUSIONS

Thrombophilia is common among patients with APO. Inherited thrombophilia is less common than the acquired ones. Biomarkers such as protein C deficiency, hyperhomocysteinemia, antithrombin III deficiency, and FVL mutation help to diagnose inherited type and treat these patients early. Among the acquired thrombophilia patients, anti-β-2 glycoprotein antibodies were positive as biomarker in majority of acquired type of thrombophilia. Other markers useful are anticardiolipin antibodies and LAC test.

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