

ORIGINAL ARTICLE

Association of thrombophilia and polycystic ovarian syndrome in women with history of recurrent pregnancy loss

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Purpose: To evaluate the prevalence of thrombophilic disorders in polycystic ovarian syndrome (PCOS) women with history of recurrent pregnancy loss (RPL). **Materials and methods:** This study was carried out in 184 women with history of RPL, of which 92 of them were diagnosed with PCOS and 92 patients were without known PCOS. The prevalence of thrombophilic disorders was compared between the two mentioned groups. **Results:** According to the findings, 70.7% of PCOS women with history of RPL had thrombophilic disorders. The prevalence of protein C deficiency was significantly higher in PCOS group compared to the non-PCOS group (21.7% vs. 10.9%, $p = 0.04$). There was a trend toward higher prevalence of protein S deficiency in PCOS group compared to the control group, but the difference did not reach statistical significance (23.9% vs. 13%, $p = 0.05$). The prevalence of other thrombophilic disorders such as antithrombin III deficiency, homocysteine elevation, antiphospholipid antibody and Factor V Leiden was comparable between groups. **Conclusion:** The prevalence of thrombophilic disorders was more common in PCOS women than the normal group. The protein C deficiency is associated with PCOS in women with history of RPL. There was a trend toward higher prevalence of protein S deficiency in PCOS women, which needs further study.

Keywords: Polycystic ovarian syndrome, recurrent pregnancy loss, thrombophilia, thrombophilic factor

Introduction

Polycystic ovarian syndrome (PCOS) is the most common heterogeneous endocrine disorder [1] which affects about 5%–10% of reproductive-aged women [2,3]. This syndrome is considered as the most common cause of anovulatory infertility and hirsutism [1] and characterized by oligomenorrhea, amenorrhea, anovulation, abnormal gonadotropins, androgen excess, polycystic ovarian morphology, type 2 diabetes mellitus, obesity, acne, skin hyperpigmentation and hyperinsulinemia [4–7].

PCOS is also associated with pregnancy complications such as recurrent miscarriage. The prevalence of PCOS in women with history of recurrent pregnancy loss (RPL) has been reported in a wide range of 10%–82% in different studies [8–10]. But the possible mechanisms by which PCOS could cause recurrent miscarriage remain controversial.

Recent studies suggest an association between hereditary thrombophilia and pregnancy loss. Activated protein C (APC) resistance due to Factor V Leiden mutations is associated with sporadic and RPL [11,12]. Furthermore, *MTHFR* C677T homozygosity, protein S, protein C and antithrombin III (ATIII) deficiencies and antiphospholipid syndrome could be responsible for most of the RPL [13,14]. Uteroplacental vascular insufficiency due to thrombotic effects of thrombophilia predispose pregnant women to pregnancy failure and fetal loss [15].

On the other hand, several studies have been made to evaluate the association of thrombophilia, PCOS and RPL [12,16–20]. However, there are still no sufficient evidences whether thrombophilic disorders can be considered as a causal factor of recurrent miscarriage in PCOS women.

If women with PCOS are really predisposed for thrombophilic disorders, they will probably have poor prognosis in pregnancy and treatment strategies should be considered before any decision for pregnancy. In this study, we aimed to evaluate the prevalence of thrombophilic disorders in PCOS women with history of RPL.

Methods

This case control study was carried out in 184 women aged 20–40 years during 2009–2010 in recurrent miscarriage clinic of Roointan-Arash Hospital, Tehran, Iran. The study population was women with history of RPL who were referred for diagnosis and treatment. Ninety-two patients were diagnosed with PCOS and 92 were without known PCOS.

This study was approved by Tehran University of Medical Science's Ethics Committee and informed consent was obtained from all subjects. Primary evaluation was performed through physical examination, menstrual and obstetrical history, medical and surgical history, family history and measurement of blood pressure and body mass index.

The inclusion criteria included women with history of RPL who were free of other etiologies for RPL such as uterine structural abnormalities (assessed by sonogram, hysterosalpingogram, hysteroscopy or laparoscopy), myoma, Asherman's syndrome and cervical incompetence. Any women with inflammatory, hematological and thromboembolic diseases, hypothyroidism, malignancy, Cushing's syndrome and liver diseases were excluded from the study. Current pregnancy, delivery or miscarriage occurring

within the preceding 3 months, recent surgery, recent sex steroid therapy and recent use of anticoagulation drugs (warfarin, heparin and aspirin) and diabetic drugs (metformin) were other exclusion criteria.

Diagnosis of PCOS was established based on Rotterdam criteria [21] and presence of at least two criteria defined as PCOS. RPL was defined by three or more consecutive pregnancy losses below 20 weeks of gestation.

Laboratory assays

Venipuncture was performed on all fasting women on day 3 of menstrual cycle in order to assess thrombophilic factors. Venous blood was collected on both 3.2% trisodium citrate anticoagulant and clot activator by means of Aysel vacuum blood collection tubes (Aysel Plastik Tekstil ve Elektronik, Adana, Turkey). The tubes were immediately centrifuged at 3500 rpm for 15 min at room temperature to obtain relatively platelet-free plasma/serum. Plasma/serum was then frozen and stored in small aliquots at -70°C until assayed.

Protein S, protein C and Factor V Leiden were assessed using the clotting assay kits (HEMOCLOT; HYPHEN Biomed, Neuville-Sur-Oise, France). AT activity was measured using a chromogenic assay (BIOPHEN AT 2.5). Homocysteine and antiphospholipid antibodies were measured by means of the enzyme immunoassay kits (Axis Homocysteine EIA; Axis-Shield Diagnostics Ltd., Dundee, UK, and AESKULISA Phospholipid-Screen-GM; AESKU.Diagnostics, Wendelsheim, Germany).

Statistical analysis

Statistical analysis was performed by SPSS software version 13 (SPSS Inc., Chicago, IL, USA). In order to compare the mean age and mean body mass index of the two groups, an independent T analysis was used. A chi square test was used to compare hormonal abnormalities and abnormal glucose tolerance test (GTT) between groups. We also used a chi square test or Fisher's exact test to compare all thrombophilic disorders between two groups. A p value <0.05 was considered statistically significant.

Results

In this case control study, the prevalence of thrombophilic disorders in 92 PCOS and 92 non-PCOS women with history of RPL was compared. According to the findings, there were no significant differences in the mean age ($p=0.92$) and body mass index ($p=0.78$) of women between groups (Table I). Among women with PCOS, 49 patients (53.3%) were found to have testosterone levels higher than normal range versus 22 patients (23.9%) in non-PCOS group ($p < 0.001$), 38 patients (41.3%) had luteinizing hormone/follicle-stimulating hormone ratio > 2 which was significantly higher than 24 women (26.1%) in control group ($p = 0.029$) and 20 women (21.7%) in each group had abnormal levels of prolactin ($p = 1.00$). Forty patients (43.5%) in the PCOS group and 10 (10.9%) in the control group had abnormal GTT 1 h with 75 g glucose ($p < 0.001$). Overt diabetes of 4.3% was observed in PCOS women, which was significantly higher than the control group ($p = 0.043$).

Nearly 70.7% of PCOS women with history of RPL had thrombophilic disorders, which were significantly different with 47.8% in non-PCOS women group ($p = 0.002$). About 21.7% of women with PCOS had protein C deficiency versus 10.9% in the control group ($p = 0.04$) (Table II).

Table I. Characteristics of women in two studied groups.

	PCOS ($n = 92$)	Non-PCOS ($n = 92$)	p value
Age (years)	29.16 ± 2.32	29.13 ± 2.33	NS
Body mass index (kg/m^2)	23.87 ± 4.44	24.06 ± 4.5	NS

Data are expressed as mean \pm SD.

PCOS, polycystic ovarian syndrome; NS, not statistically significant.

Table II. Abnormal thrombophilic factors in two studied groups.

	PCOS ($n = 92$)	Non-PCOS ($n = 92$)	p value
Protein S deficiency (%)	22 (23.9)	12 (13)	NS (0.05)
Protein C deficiency (%)	20 (21.7)	10 (10.9)	0.04
Antithrombin III deficiency (%)	4 (4.3)	4 (4.3)	NS
Factor V Leiden (%)	3 (3.3)	3 (3.3)	NS
Homocysteine elevation (%)	8 (8.7)	7 (7.6)	NS
Antiphospholipid antibody (%)	8 (8.7)	8 (8.7)	NS

PCOS, polycystic ovarian syndrome; NS, not statistically significant.

Although protein S deficiency was not significantly different between groups, there was a trend toward higher prevalence of protein S deficiency in PCOS women compared to the control group (23.9% vs. 13%, $p = 0.05$) (Table II).

The prevalence of other thrombophilic disorders such as ATIII deficiency, Factor V Leiden, abnormal homocysteine and antiphospholipid antibody was comparable between groups ($p = 1.00, 1.00, 0.78$ and 1.00 , respectively) (Table II).

Discussion

Thrombophilias are a group of acquired or genetic blood coagulation disorders which predispose patients to vascular thrombosis and pregnancy complications, including early pregnancy loss [12,22,23].

Deficiencies of natural anticoagulants protein C, protein S and AT and also hyperhomocysteinemia, Factor V Leiden and APC resistance are thrombophilic defects that increase the risk of thrombosis [24].

According to the findings, 70.7% of PCOS women with history of RPL had thrombophilic disorders, which were significantly different with 47.8% in non-PCOS women group. The prevalence of protein C deficiency in the PCOS group was 21.7%, which was significantly different with 10.9% in the non-PCOS women group. There was a trend toward higher prevalence of protein S deficiency in the PCOS group compared to the control group, even though it didn't achieve statistical significance. Therefore, it seems that in further study with greater population, significant difference of protein S might be observed between groups.

AT is the most important inhibitor of blood coagulation [25]. Inherited ATIII deficiency is associated with a long-term complication of venous thromboembolism [25]. High incidence of poor pregnancy outcome among AT-deficient women was also reported [26]. The prevalence of inherited AT deficiencies in the general population is very uncommon (between 1 in 600) [27]. In this study, ATIII deficiency was found in four women (4.3%) of each group, which was more common compared to the general population.

In contrast to our findings, in the study by Tsanadis et al. [16], all patients in PCOS and non-PCOS groups showed normal median proportions of APC, protein S and ATIII. They reported

that recurrent fetal loss in PCOS women might be due to other mechanisms rather than thrombophilic disorders. Tsanadis et al. conducted a preliminary study with 30 PCOS women and 45 non-PCOS group from Greece, whereas the present study was based on a greater sample of 184 women (92 PCOS and 92 non-PCOS) who were all Iranian population with history of recurrent miscarriage.

Factor V Leiden is a genetic state that results in APC resistance and is considered as an important risk factor for systemic venous thrombosis. In a previous study, women with PCOS had the same prevalence of APC resistance compared to the general population [19]. Similarly, the low insignificant prevalence of Factor V Leiden in this study (3.3% in each group) demonstrated that this factor probably does not play any role in thrombotic disorders and fetal loss in PCOS patients.

It is now widely accepted that hyperhomocysteinemia could be a thrombotic risk factor in PCOS women. In a study by Cerqueira et al. [28], higher serum level of homocysteine was reported in PCOS patients compared to the control group. In the current study, the prevalence of abnormal level of homocysteine in both groups was within the normal range expected for general population and no significant difference was found.

However with respect to the current study, serum homocysteine level would not be a valuable marker for prediction of pregnancy thrombotic events due to PCOS such as fetal loss. On the other hand, in most cases, blood homocysteine concentration is probably affected by several factors including vitamin status, diet, age and genotype of the patients [29].

We recently evaluated thrombophilic factors such as protein S, protein C, ATIII, APC resistance and homocysteine levels in PCOS and non-PCOS patients and no association between thrombophilia and PCOS in infertile women was observed [17].

Antiphospholipid antibody syndrome is regarded as the most frequently acquired risk factor for thrombophilia which is responsible for many pregnancy losses [30]. Most of the cases in the current study had normal levels of this factor. These results indicate that antiphospholipid antibody does not play a role in PCOS and RPL. However, no previous study regarding antiphospholipid antibody as a thrombophilic factor in PCOS women is available in the literature and further research of this kind would be required.

Ultimately, the prevalence of thrombophilic disorders was more common in PCOS women than the normal group. The protein C deficiency is associated with PCOS in women with history of RPL. There was a trend toward higher prevalence of protein S deficiency in PCOS women but the difference did not reach statistical significance. Thus, the statistically significant difference might be achieved with further study with a greater population.

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References

- Dunaif A, Chang RJ, Franks S, Legro RS. Polycystic ovary syndrome. Totowa, NJ, USA: Humana Press; 2008.
- Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab* 2004;89:2745–2749.
- Tehrani FR, Simbar M, Tohidi M, Hosseiniapanah F, Azizi F. The prevalence of polycystic ovary syndrome in a community sample of Iranian population: Iranian PCOS prevalence study. *Reprod Biol Endocrinol* 2011;9:39.
- Kelekci KH, Kelekci S, Incki K, Ozdemir O, Yilmaz B. Ovarian morphology and prevalence of polycystic ovary syndrome in reproductive aged women with or without mild acne. *Int J Dermatol* 2010;49:775–779.
- Hickey M, Doherty DA, Atkinson H, Sloboda DM, Franks S, Norman RJ, Hart R. Clinical, ultrasound and biochemical features of polycystic ovary syndrome in adolescents: implications for diagnosis. *Hum Reprod* 2011;26:1469–1477.
- McGowan MP. Polycystic ovary syndrome: a common endocrine disorder and risk factor for vascular disease. *Curr Treat Options Cardiovasc Med* 2011;13:289–301.
- Li L, Yang D, Chen X, Chen Y, Feng S, Wang L. Clinical and metabolic features of polycystic ovary syndrome. *Int J Gynaecol Obstet* 2007;97:129–134.
- Rai R, Backos M, Rushworth F, Regan L. Polycystic ovaries and recurrent miscarriage—a reappraisal. *Hum Reprod* 2000;15:612–615.
- Cocksedge KA, Saravelos SH, Metwally M, Li TC. How common is polycystic ovary syndrome in recurrent miscarriage? *Reprod Biomed Online* 2009;19:572–576.
- Sagle M, Bishop K, Ridley N, Alexander FM, Michel M, Bonney RC, Beard RW, Franks S. Recurrent early miscarriage and polycystic ovaries. *BMJ* 1988;297:1027–1028.
- Rai R, Shlebak A, Cohen H, Backos M, Holmes Z, Marriott K, Regan L. Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage. *Hum Reprod* 2001;16:961–965.
- Glueck CJ, Gogenini S, Munjal J, Tracy T, Prankoff J, Wang P. Factor V Leiden mutation: a treatable etiology for sporadic and recurrent pregnancy loss. *Fertil Steril* 2008;89:410–416.
- Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet* 2003;361:901–908.
- Kupfermanc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, Fait G, Lessing JB. Increased frequency of genetic thrombophilia in women with complications of pregnancy. *N Engl J Med* 1999;340:9–13.
- Glueck CJ, Awadalla SG, Phillips H, Cameron D, Wang P, Fontaine RN. Polycystic ovary syndrome, infertility, familial thrombophilia, familial hypofibrinolysis, recurrent loss of *in vitro* fertilized embryos, and miscarriage. *Fertil Steril* 2000;74:394–397.
- Tsanadis G, Vartholomatos G, Korkontzelos I, Avgoustatos F, Kakosimos G, Sotiriadis A, Tatsioni A, et al. Polycystic ovarian syndrome and thrombophilia. *Hum Reprod* 2002;17:314–319.
- Moini A, Sarrafion F, Ziaei S, Faghizadeh S, Arabipour A. A possible role for familial thrombophilia in women with polycystic ovarian syndrome. *Gynecol Endocrinol* 2011;27:80–85.
- Glueck CJ, Wang P, Bornovali S, Goldenberg N, Sieve L. Polycystic ovary syndrome, the G1691A factor V Leiden mutation, and plasminogen activator inhibitor activity: associations with recurrent pregnancy loss. *Metab Clin Exp* 2003;52:1627–1632.
- Atiomo WU, Condon J, Adekanmi O, Friend J, Wilkin TJ, Prentice AG. Are women with polycystic ovary syndrome resistant to activated protein C? *Fertil Steril* 2000;74:1229–1232.
- Sucak A, Acar K, Celen S, Danisman N, Sucak GT. Outcome of pregnancies in women with thrombophilic disorders. *J Obstet Gynaecol* 2010;30:847–851.
- Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–25.
- Ivanov PD, Komsa-Penkova RS, Konova EI, Kovacheva KS, Simeonova MN, Popov JD. Association of inherited thrombophilia with embryonic and postembryonic recurrent pregnancy loss. *Blood Coagul Fibrinolysis* 2009;20:134–140.
- Kutteh WH, Triplett DA. Thrombophilias and recurrent pregnancy loss. *Semin Reprod Med* 2006;24:54–66.
- Makris M. Thrombophilia: grading the risk. *Blood* 2009;113:5038–5039.
- Patnaik MM, Moll S. Inherited antithrombin deficiency: a review. *Haemophilia* 2008;14:1229–1239.
- Sabadell J, Casellas M, Alijotas-Reig J, Arellano-Rodrigo E, Cabero L. Inherited antithrombin deficiency and pregnancy: maternal and fetal outcomes. *Eur J Obstet Gynecol Reprod Biol* 2010;149:47–51.
- Tait RC, Walker ID, Perry DJ, Islam SI, Daly ME, McCall F, Conkie JA, Carrell RW. Prevalence of antithrombin deficiency in the healthy population. *Br J Haematol* 1994;87:106–112.

28. Cerqueira JM, Costa LO, Nogueira Ade A, Silva DC, Torres Dde O, Santos AC. [Homocysteinemia in polycystic ovary syndrome women]. *Rev Bras Ginecol Obstet* 2010;32:126–132.
29. Saw SM, Yuan JM, Ong CN, Arakawa K, Lee HP, Coetzee GA, Yu MC. Genetic, dietary, and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. *Am J Clin Nutr* 2001;73:232–239.
30. Pierangeli SS, Leader B, Barilaro G, Willis R, Branch DW. Acquired and inherited thrombophilia disorders in pregnancy. *Obstet Gynecol Clin North Am* 2011;38:271–95, x.