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ORIGINAL ARTICLE

Study of Thrombophilia in Recurrent Pregnancy Loss

Hansda Jayasree · Roychowdhury Joydeb

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Abstract

Objectives Recently, it has been found that women who have thrombophilia have increased risk of fetal loss. This study was designed to corroborate the association of elevated factor VIII level, protein C and protein S deficiencies, and the presence of LAC in women with recurrent pregnancy loss.

Materials and Methods 53 patients with history of two or more pregnancy losses and 47 healthy age-matched subjects with no history of pregnancy loss and who have delivered at least one term infant without any complication were enrolled into the study.

Results Thrombophilic defect was present in 64.15 % of patients of study group. Protein S deficiency (50.94 %) was the most common thrombophilic defect observed. Spontaneous abortion (SA), preterm birth (PTB), and intrauterine growth retardation (IUGR) were the most important pregnancy complications observed. The strongest associations of pregnancy complications were observed with protein S deficiency (87.5 %) and with elevated factor VIII (66.66 %) level.

Conclusion This study observed strong association of thrombophilia with unexplained recurrent pregnancy loss.

Hansda J., Senior Resident
NRS Medical College, Kolkata, India

Hansda J. (✉), Senior Resident
C/O-Sri R C Hansda, 2/2 Krittibas Road (South), B-Zone, Durgapur, Burdwan, 713205, WB, Kolkata, India
e-mail: hansdajayasree@yahoo.in

Roychowdhury J., Professor & Head
Department of OBGY, ESI-Postgraduate Institute and Medical Research, Joka, Kolkata, India

Introduction

Recurrent spontaneous miscarriage affects 1–3 % of women of reproductive age [1]. At least, one-third recurrent miscarriage is unexplained, and the rest have a persistent underlying cause for their pregnancy losses. Identifiable causes can be found in only about 30–50 % of these women [2]. RPL (recurrent pregnancy loss) could also include pregnancy losses up to gestational week 28. The most common causes of recurrent miscarriage are uterine anomalies, endocrine disorders, parental chromosomal abnormalities, and immunological factors, including those associated with the APS (antiphospholipid antibody syndrome) and infections. Even after a thorough evaluation, however, the potential cause remains unexplained in about one third of cases [3, 4].

A number of studies have reported an increased risk of RPL in women with inherited thrombophilia [5–7]. In the European prospective cohort study on thrombophilia (EPCOT), a significant association between thrombophilia and miscarriage was reported [8]. The term thrombophilia is generally used to describe a laboratory abnormality (most often in the coagulation system) that increases the tendency

to venous thromboembolism (VTE) in any site or pulmonary embolism.

Thrombophilic abnormalities can be acquired or hereditary. Hereditary thrombophilia comprise a number of conditions, such as antithrombin (AT) III deficiency, protein S (PS) and protein C (PC) deficiencies, factor V Leiden, prothrombin 20210A mutation, elevated factor VIII level, and mutation of gene encoding the enzyme methylenetetrahydrofolate reductase (MTHFR).

The evidence for pregnancy loss having a thrombophilic mechanism rests on three pillars: increased prevalence of thrombophilia in RPL, a higher incidence of pregnancy loss in the presence of thrombophilia and the demonstration of thrombosis in decidual vessels. However, it is still uncertain if heritable thrombophilia causes recurrent miscarriage, and routine testing in women with recurrent miscarriage is preferable but not currently advocated [9]. The principal acquired thrombophilic states include APS and hyperhomocysteinemia [10]. Thrombophilia has been suggested to be a cause for microembolism in the placenta resulting in abortion or adverse outcome of pregnancy.

The purpose of the study was to evaluate the association between the common markers of thrombophilia and recurrent pregnancy loss.

Materials and Methods

During the period from May 1, 2008 to July 31, 2009, a prospective observational case–control study was carried out in the Department of Gynaecology and Obstetrics in collaboration with the Department of Haematology of Nilratan Sircar Medical College and Hospital, Kolkata. Two groups of women were enrolled: 53 patients with history of two or more pregnancy losses as cases—the pregnancy loss may be in the 1st, 2nd or 3rd trimester—and 47 healthy, age-matched subjects, with at least one successful pregnancy and without pregnancy complications (intrauterine growth restriction, stillbirth, and abruptio placentae), or any abortion—were enrolled as control. 37 patients of study group and 30 control subjects were pregnant during the study period. Age >35 years; women with anatomical abnormality of the genital tract; pregnancy loss caused by maternal infection; medical disorders like hypertension; endocrinal disorders like diabetes, thyroid disorder; personal and family history of chromosomal abnormality; personal and family history of venous thromboembolism; and single pregnancy loss were excluded from the study.

Data were collected at entry to the study, by personal interview. In order to evaluate the causes of RPL, search was made for genital tract abnormalities, chromosomal abnormalities, medical disorders, endocrinal disorders, infections and venous thromboembolism. Any family history of chromosomal abnormality was enquired. The uterine cavity was evaluated anatomically by transvaginal ultrasound scan and hysterosalpingography (HSG) to detect mullerian malformations or the presence of fibroids or polyps. Endocrinological assessment included screening for diabetes, hypothyroidism. Thyroid-stimulating hormone (TSH) and fasting glucose were evaluated in all patients. Endocervical swabs were taken to detect chlamydia, mycoplasma, and bacterial vaginosis. Careful maternal history regarding age, parity, number of pregnancy loss, number of living issue, last menstrual period, socioeconomic status, education level, and drug history were recorded thoroughly. Thorough physical examinations like height, weight, BP, pulse, edema, etc. were done. Blood tests for protein C, protein S, and factor VIII level and lupus anticoagulant (LAC) were done in both groups. Pregnancy outcome in the study group was also observed.

Whole blood samples were collected from all the selected subjects in the study to screen for thrombophilia. Blood sample was collected in 3.2 % citrate (anticoagulant) in a ratio of 9:1 from the patients and controls for protein C, protein S, factor VIII level estimation, and LAC detection. Protein C and Protein S levels were estimated by means of Berichrome PC and PS kits in Sysmex CA-550 Analyser. Protein C level was considered normal between 78 and 118 %, and protein S level was considered normal between 64 and 96 %.

The one-stage assay for factor VIII was employed using CA-550 Analyser. Factor VIII level was considered normal between 62 and 94%.

Test for LAC was done by kaolin cephaline clotting time (KCCT). KCCT was done on patient's plasma (a), control's plasma (c), and 1:1 mixture of patient's and control's plasma (b).

If $(c - a)/b \times 100 \geq 1.5$, then it indicates positive test for LAC.

Data collected in this study were analyzed in Microsoft Excel and Epi-inao (version 3.5.4). When p value <0.05, the result is considered statistically significant.

Results

A majority of the patients of this study belong to 20–29 years of age (79.25 % in cases and 74.47 % in controls). In the study group, 37 patients out of 53 (69.81 %) were pregnant during the study period, and the rest of 16 (30.19 %) subjects were non-pregnant. In control group, 30 patients out of 47 (63.83 %) were pregnant.

In the study population, the maximum number of patients 32/53 (60.38 %), had h/o 1st trimester abortion (Table 1).

A total of 34/53 (64.15 %) patients with RPL had thrombophilic defect, whereas these defects were present in only five (10.64 %) control subjects, which is statistically significant ($p = 0.000$) (Table 2). Protein S deficiency was detected in 27/53 (50.94 %) patients in study group versus 1/47 (2.12 %) subjects in control, which is statistically significant ($p = 0.000$). Statistically significant elevated factor VIII level was also documented in study group.

Analysis of thrombophilic defect in pregnant women in this study showed a total of 26/37 (70.27 %) and 3/30 (10 %) pregnant women had thrombophilic defect in the case and control groups, respectively, which was statistically significant ($p = 0.000$). Statistically significant Protein S deficiency and elevated factor VIII level were detected in the pregnant patients in the study group (Table 3).

During the study period, 25 babies were born among the study group. 12/25 (48 %) babies were born with low birth weight (Table 4).

During the study period, outcome of 30 pregnancies were observed as seven mothers were yet to be delivered of baby. Among those 30 cases, 10 pregnancies were uneventful, and other 20/30 (66.67 %) patients developed pregnancy complications. The most common complication was IUGR, which affected 8/30 (26.67 %) patients. Other common pregnancy complications observed among the study population were abortion and preterm birth (Table 5). Table 6 shows out of eight pregnant patients with Protein S deficiency, 7 (87.5 %) had varied pregnancy complications like IUGR, SA, or PTB. 66.66 % patients with elevated FVIII developed preterm birth and IUGR.

Similar complications were also observed amongst pregnant mothers with combined defects like protein C and protein S deficiencies in 50 % of cases, and 60 % of cases were observed with protein S and Factor VIII defect.

Discussion

This is a prospective observational case–control study. During this study, prevalence of pregnancy losses was analyzed among the study group. It was observed that in the study population, 60.38 % patients had h/o 1st trimester pregnancy loss, followed by 26.41 % patients who had pregnancy loss in all the three trimesters. The similar observation was also found in a study by Maria et al. [11], who evaluated the prevalence of thrombophilia in women with spontaneous pregnancy loss. In their study population, 60 % patients had two or more first trimester pregnancy loss, and 19.2 % patients had pregnancy loss in all the three trimesters. Alteration of hemostasis with a trend toward thrombophilia has been frequently associated to RPL. Several published studies seem to differ not only on the frequency of specific alteration but also on the inclusion and exclusion criteria of the enrolled patients. For this reason, we may observe also great differences about the involvement of thrombophilia during the evaluation of patients with RPL [12]. However, the incidence of thrombophilia seems to increase if any causes of miscarriage, such as endocrine alterations and uterine malformations, are also excluded from the study population [13].

Table 1 Distribution of pregnancy loss in study group

	<12 weeks	12–28 weeks	>28 weeks	Combined
Case ($n = 53$)	32/53 (60.38 %)	1/53 (1.89 %)	6/53 (11.32 %)	14/53 (26.41 %)

The data of this study confirmed this trend and showed a strong association of thrombophilia with RPL, and 64.15 % women with RPL had shown one or more thrombophilic defects if other common causes of miscarriage are excluded, which is statistically significant. Before evaluating thrombophilia in selected patients of this study, we excluded common conditions associated with miscarriage. 35.85 % patients were found to have isolated thrombophilic defect in the study group compared with 10.64 % of control group. Combined thrombophilic defects were present in 28.30 % in the study group. High prevalence of thrombophilia was also detected in patients with RPL in several other studies [14–16]. A study by Sarig et al. [13] showed isolated thrombophilic defect in 66 % of study group compared with 28 % in controls, and combined thrombophilic defects were documented in 21 % women who experienced pregnancy loss, compared with 5.5 % of control subjects. Study by Maria et al. [11] reported that 56 % of patients had thrombophilic defects in their series of patients with RPL.

The evaluation of the analyzed thrombophilic conditions in our study revealed that protein C deficiency was present in 15.09 % of patients with RPL. Protein S deficiency was present in 50.94 % of patients with RPL ($p = 0.000$). Similar statistically significant defect was observed in case of elevated factor VIII level ($p = 0.02$). The strongest association of thrombophilia with RPL was observed with protein S deficiency. These data seem to be in agreement with the results of several studies [7, 14, 17, 18]. Marietta et al. [18], in their study, evaluated statistically significant association ($p = 0.0002$) between elevated factor VIII level and early pregnancy failures. Karimi et al. [17], in their study, also observed 5 and 11.25 % patients with recurrent abortion had protein C and protein S deficiency, respectively. Nazli H et al. [19] observed that 45 % of patients were found deficient for protein C and S in their

Table 2 Frequency of thrombophillic alterations in the study and control group

	Study group ($n = 53$)	Control group ($n = 47$)	p Value ($p < 0.05 = S$)	Odds ratio	Relative risk
P C deficiency	8/53 (15.09 %)	0/47 (0 %)	–	–	–
P S deficiency	27/53 (50.94 %)	1/47 (2.12 %)	0.000	47.77	23.94
Elevated factor VIII level	14/53 (26.41 %)	4/47 (8.51 %)	0.02	3.86	3.10
LAC	2/53 (3.77 %)	0/47 (0 %)	–	–	–
Isolated defect	19/53 (35.85 %)	5/47 (10.64 %)	0.003	4.69	3.37
Combined defect[a]	15/53 (28.30 %)	0/47 (0 %)	–	–	–
Total defect	34/53 (64.15 %)	5/47 (10.64 %)	0.000	15.03	6.03
No defect	19/53 (35.85 %)	42/47 (89.36 %)	0.000	0.07	0.40

[a] Combined defect reflects presence of more than one thrombophilic defect

Table 3 Frequency of thrombophilic alterations in pregnant women with RPL and in pregnant control

	Case ($n = 37$)	Control ($n = 30$)	p Value ($p < 0.05 = S$)	Odds ratio	Relative risk
P C deficiency	7/37 (18.92 %)	0/30 (0 %)	–	–	–
P S deficiency	21/37 (56.76 %)	1/30 (3.33 %)	0.000	38.06	17.03
Elevated FVIII level	11/37 (29.73 %)	2/30 (6.67 %)	0.02	5.92	4.46
LAC	2/37 (5.41 %)	0	–	–	–
Combined defect	13/37 (35.14 %)	0	–	–	–
Total defect	26/37 (70.27 %)	3/30 (10 %)	0.000	21.27	7.03

Table 4 Baby outcome and birth weight

Birth weight	Case ($n = 25$)	Control ($n = 30$)	p Value ($p < 0.05 = S$)	Odds ratio	RR
<2 kg	3/25 (12 %)	0	–	–	–
2–2.5 kg	9/25 (36 %)	5/30 (16.67 %)	0.10	2.81	2.16
>2.5–3 kg	10/25 (40 %)	17/30 (56.67 %)	0.22	0.51	0.71
>3 kg	3/25 (12 %)	8/30 (26.66 %)	0.18	0.38	0.45

Table 5 Pregnancy outcome in study group

	Uncomplicated	Complicated 20 (66.67 %)					
		PIH[a]	PIH + PTB	IUGR + PTB	SA	PTB	IUGR
Case ($n = 30$)	10 (33.33 %)	1 (3.33 %)	1 (3.33 %)	1 (3.33 %)	5 (16.67 %)	4 (13.33 %)	8 (26.67 %)

[a] Pregnancy-induced hypertension

Seven mothers yet to be delivered at end of study

case series. Analysis of thrombophilic defect in pregnant women in this study showed that, in total, 70.27 and 10 % pregnant women had thrombophilic defects in case and control groups, respectively, which was statistically significant ($p < 0.001$). Among the babies born during the study period, 48 % were born with low birth weight.

In our study, out of 30 patients, 20 (66.67 %) developed pregnancy complications. The incidence of pregnancy complications like spontaneous abortion, preterm birth, and intrauterine growth retardation were found in 16.67, 13.33, and 26.67 % of pregnancies, respectively. Similar observation was found in the series published by Tulppala et al. [20] who conducted a prospective study of 32 deliveries in 63 women with RPL and found that pregnancy complications like preterm delivery and IUGR were 9.7 and 20 %, respectively, in these women.

Analysis of the correlation of individual thrombophilic defects with poor pregnancy outcome is listed in (Table 6). 87.5 % patients with Protein S deficiency had IUGR/spontaneous abortion/preterm birth, and 66.66 % pregnant patients with elevated FVIII level had pregnancy complications. Sanson et al. [5] in their series found 22.3 % of the

Table 6 Correlation of individual thrombophilic defects with poor pregnancy events

Case ($n = 30$)	Isolated PC↓ (a) 0	Isolated PS↓ (b) 08[a]	Isolated F VIII↑ (c) 03	Isolated LAC (d) 0	Combined (a&b) 2	Combined (b&c) 5	Combined (b&d) 1	Combined (c&d) 1	Combined (a&b&c) 2[b]	Normal test 8
IUGR (9)	–	5	1	–	–	–	–	1	–	2
SA (5)	–	1	–	–	1	–	1	–	–	2
PIH (2)	–	–	–	–	–	1	–	–	1	–
PTB (6)	–	2	1	–	–	2	–	–	1	–
Uncomplicated (10)	–	1	1	–	1	2	–	–	1	4

[a] One patient with protein S deficiency had pregnancy complications both IUGR and preterm birth

[b] Out of two patient with protein C, protein S and Factor VIII defect one developed PTB and PIH

pregnancies in thrombophilic women resulted in miscarriage or stillbirth as compared with 11.4 % of non-deficient subjects.

Conclusion

In substantial number of cases where the underlying cause remains unexplained, the congenital and acquired thrombophilic defects now largely can explain many of those pregnancy losses due to underlying placental vasculopathy. This small prospective observational study also observed strong association of thrombophilic defects in many patients with unexplained recurrent pregnancy loss. However, because of lack of required genetic laboratory facilities, other congenital thrombophilic defects could not be analyzed. Since this study was carried out over a very short period of time with a small group of patient population, it demands a larger and long-term comprehensive prospective case–control study to prove a definite stronger association of congenital and acquired thrombophilic defects with unexplained recurrent pregnancy loss. This will help in subsequent management of these patients in pregnancy by adequate antithrombotic therapy which can be initiated from very early days of pregnancy to prevent placental vasculopathy and coagulation defects, and thereby improve maternal and perinatal outcomes of these pregnancies to a great extent.

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