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Protein S deficiency complicated pregnancy in women with recurrent pregnancy loss

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Abstract

This prospective study aimed to evaluate pregnancy outcome and complications in women with recurrent pregnancy loss (RPL) and protein S (PS) deficiency, who received low dose aspirin (LDA) or LDA plus heparin (LDA/H) therapies. Clinical characteristics, pregnancy outcome, and complications of 38 women with two or more RPL and <60% of plasma free PS antigen were compared among three groups: antiphospholipid antibody (aPL)-negative women who received LDA (group A), aPL-negative women who received LDA/H (group B), and aPL-positive women who received LDA/H (group C). Gestational weeks (GW) at delivery in group C (median 32GW) were earlier than 40GW in group A and 38.5GW in group B ($p<0.05$). The birth weight in group C (median 1794g) was less than 2855g in group B ($p<0.05$). The incidences of fetal growth restriction (37.5%), pregnancy-induced hypertension (37.5%), and preterm delivery (62.5%) in group C were higher than those (4.5%, 0%, and 4.5%, respectively) in group B ($p<0.05$). Women with RPL, PS deficiency, and positive aPL had high risks for adverse pregnancy outcome and complications, even when they received LDA/H therapy. Among women with RPL, PS, and negative aPL, there was no difference in these risks between LDA alone and LDA/H therapies.

Introduction

Protein S (PS) is a vitamin K-dependent glycoprotein that is present in platelets and is synthesized within the liver and endothelial cells. PS is part of the natural anticoagulant system by acting as a cofactor to activated Protein C (PC) in the proteolytic inactivation of procoagulant factors Va and VIIIa. In addition, PS has direct activated PC-independent anticoagulant activity by inhibiting formation of prothrombin and proteinase complexes. PS forms a complex with the compliment regulatory protein, C4b-binding protein. Approximately 60% of the total plasma PS circulates bound to C4b-binding protein, while the remaining 40% circulates as free PS. Only free PS has anticoagulant function.

PS deficiency causes failure of the coagulation and hemostasis system, and is associated with venous thromboembolism and prenatal complications of pregnancy involving miscarriage, fetal death, fetal growth restriction FGR, abruptio placenta, and pregnancy-induced hypertension [1-5]. PS deficiency is classified into two types, which include primary (inherited) PS deficiency and secondary PS deficiency in which plasma PS levels physiologically decrease because of hormonal factors, such as estrogen [6].

Low-dose aspirin (LDA) alone or LDA plus heparin (LDA/H) can be used for the treatment of PS deficiency-complicated pregnancies [7]. However, whether LDA or LDA/H therapy is effective for pregnancies in women with a history of recurrent pregnancy loss (RPL) and PS deficiency is still unclear [7, 8]. This cohort study aimed to evaluate the

outcome and complications of pregnancy in women with RPL and PS deficiency under LDA or LDA/H therapy in relation to the presence or absence of antiphospholipid antibody (aPL).

Material and methods

This prospective cohort study was approved by the institutional ethical boards of Kobe University Hospital. Informed consent was obtained from all of the patients. Between June 2009 and March 2014, women who had a history of two or more RPLs and visited the infertility clinic of the university hospital were enrolled. All RPL women underwent measurement of chromosomal karyotypes. We also measured thyroid, liver, and kidney function, as well as hemostatic coagulation factors, including d-dimer, factor XII, protein C, protein S, lupus anticoagulant (LA), anticardiolipin antibody (aCL), and β2-glycoprotein I-dependent anticardiolipin antibody (aβ2GPI) prior to pregnancy and/or early in pregnancy. Women who carried a chromosomal abnormality of the couple were excluded from the study.

Plasma levels of free PS antigen were measured by latex agglutination tests using the LIA test free PS II (reference range, 71–113%; Roche Diagnostics Japan, Tokyo). PS deficiency was defined as <60% of plasma free PS antigen early in pregnancy. RPL women with PS deficiency received either LDA (aspirin 81 mg/day until 27 gestational weeks [GW]) alone or LDA/H (unfractionated heparin 5000–10,000 units/day until 36 GW) therapy. They were informed about adverse effects of unfractionated heparin therapy including the bleeding

and osteoporosis, and also informed about scanty evidence of the efficacy for repeated early pregnancy losses. Then, they selected to receive LDA or LDA/H therapy with informed consent. If RPL women with PS deficiency had a positive test for aPL, they were recommended to receive LDA/H (aspirin 81 mg/day until 27 GW; heparin 10,000–15,000 units/day until delivery) therapy.

According to the criteria of antiphospholipid syndrome [9], positive tests for aCL, aβ2GP1, and LA were defined as follows: an IgG and/or IgM isotype of aCL is present in serum or plasma with a medium to high titer (>40 index or > the 99th percentile), as measured by a standardized ELISA; IgG of aβ2GP1 is present in serum or plasma with a titer > the 99th percentile, as measured by a standardized ELISA; and LA is present in plasma, as detected according to the guidelines of the International Society on Thrombosis and Hemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies).

The patients were divided into three groups as follows: aPL-negative women who received LDA alone (group A), aPL-negative women who received LDA/H (group B), and aPL-positive women who received LDA/H therapy (group C). Clinical characteristics, and outcome and complications of pregnancy were compared among these three groups.

Statistical analysis was performed by JMP 11(JMP, Tokyko, Japan). Differences between groups were analyzed using the Student's t test or Welch's test for independent samples where variables had a normal distribution, or the Wilcoxon criterion if distribution of

data was not normal. A p value < 0.05 was considered statistically significant.

Results

During the study period, 259 consecutive women with RPL were enrolled. Forty-five (17.4%) women had PS deficiency early in pregnancy. Forty of the 45 women completed their pregnancies, in which two pregnancies of aPL-negative women with LDA/H therapy ended in miscarriages with abnormal chromosomal karyotypes of fetuses. These two pregnancies were excluded from the study analyses. The other 38 singleton pregnancies, including eight pregnancies in group A, 22 in group B, and eight in group C, resulted in live births beyond 22 GW and were analyzed in this study. One pregnancy in group B and four in group C were complicated by systemic lupus erythematosus. In group B, 15 women received 10,000 units/day of unfractionated heparin and 7 received 5000 units/day depending on their pregnant histories. In group C, 3 women received 15,000 units/day of unfractionated heparin and 5 received 10,000 units/day depending on positive aPL tests or pregnant histories including stillbirth.

The clinical characteristics of the 38 women with RPL and PS deficiency are shown in Table I. The body weight at delivery or number of previous live birth in group C was significantly higher than that in group B ($p<0.05$). The frequency of primary RPL in group B (90.9%) was significantly higher than that in group A (62.5%, $p<0.05$) and in group C (50%,

p<0.01). There were no significant differences in maternal age at pregnancy, body mass index, numbers of previous gestation and previous pregnancy loss, GW at blood sampling, or plasma levels of free PS antigen among the three groups.

The outcome and complications of pregnancy are shown in Table II. GW at delivery in group C (median, 32; range, 26–40 GW) was significantly earlier than that in group A (40; 34–41 GW, p<0.05) and in group B (38.5; 34–41 GW, p<0.05). Birth weight in group C (1794; 750–3384 g) was significantly less than that in group B (2855; 1716–3890 g, p<0.05). The incidence of complications of pregnancy, including fetal growth restriction (37.5%), pregnancy-induced hypertension (37.5%), and preterm delivery (62.5%) in group C was higher than that in group B (4.5%, 0%, and 4.5%, respectively, p<0.05). There was no significant difference in GW at delivery, birth weight, or the incidence of complications of pregnancy between groups A and B. No women experienced thromboembolism during their pregnancies.

Discussion

In the Japanese population, thrombophilia, such as gene mutations of factor V Leiden and prothrombin G20210A, is almost absent [10-12]. However, the incidence of PS deficiency in Japan is approximately 2.0% [11, 13], which is higher than that in the European population (0.03–0.13%) [14]. The point mutation of PS Lys155Glu, which is named PS

Tokushima, is found at a high frequency of 1 in 55 Japanese people [13]. A recent multicenter study of the Ministry of Health, Labour and Welfare of Japan (H20-Kodomo-Ippan-002) demonstrated that PS deficiency is a risk factor of RPL in Japanese women, accounting for 7.8% of etiologies. In the present study, we found a relatively high frequency of PS deficiency (17.4%) in RPL women. This is because patients who have a history of autoimmune disease, thromboembolism, and severe complications of pregnancy are often referred to university hospitals.

In Europe and the United States, PS deficiency is a risk factor of RPL, especially for late pregnancy loss [15, 16]. PS deficiency may induce thrombosis in decidual vessels and impair placentation through hypercoagulability and inflammation, causing an adverse outcome and complications in pregnancy [1]. Recent studies have reported the efficacy of anticoagulant therapy during pregnancy in RPL women with PS deficiency [8, 17, 18]. The authors from these studies recommended the use of heparin therapy rather than no therapy or LDA alone. However, the number of subjects in these studies was relatively small. Therefore, standard medication during pregnancy in RPL women with PS deficiency has still not been established.

In the present study, among aPL-negative women, there was no significant difference in the outcome or complications of pregnancy between women with LDA alone (group A) and women with LDA/H (group B). Heparin therapy might not be necessary for

pregnancies of RPL women with PS deficiency who have a negative test for aPL or no history of thromboembolism. However, aPL-positive women with LDA/H (group C) had earlier GW at delivery and a smaller birth weight than aPL-negative women with LDA/H (group B). The incidence of fetal growth restriction, pregnancy-induced hypertension, and preterm delivery in group C was higher than that in group B. Women with RPL, PS deficiency, and a positive test for aPL had a high risk of complications in pregnancy, even when they received LDA/H therapy. Careful managements are necessary during pregnancies of these women.

However, the present study enrolled a relatively small number of subjects and included several limitations. Genetic analyses were not performed in all of the women with PS deficiency; and PS deficiency was diagnosed from plasma levels early in pregnancy. The selection of RPL women receiving LDA or LDA/H therapy was not randomized. Instead of low-molecular-weight heparins, unfractionated heparin was administered to patients because only the latter is covered by Japanese health insurance. Half of the women in group C had systemic lupus erythematosus of which disease activity might affect the outcome of pregnancy. Further studies are necessary to confirm the conclusions of the present study.

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Declaration of Interest Statement

The authors declare no conflicts of interest.

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Table I. Clinical characteristics of 38 women with recurrent pregnancy loss and protein S deficiency

	Group A n=8	Group B n=22	Group C n=8	*P* value
Age (years old)	37.5 (29-40)	36 (26-42)	32 (29-40)	N.S.
Body weight at delivery (kg)	57 (48-84)	58 (51-68)	63 (57-70)	<0.05 [b,c]
Body mass index at delivery (kg/m^2)	22.9 (19.6-35.0)	22.9 (19.7-26.1)	23.1(21.7-27.0)	N.S.
Number of previous gestation	3 (2-4)	3 (2-12)	3 (2-5)	N.S.
Number of previous live-birth	0 (0-1)	0 (0-1) [b]	0.5 (0-2) [c]	<0.05 [b,c]
Number of previous pregnancy loss before 22 weeks	2 (0-3)	2 (2-12)	2 (2-5)	N.S.
Number of previous pregnancy loss at or beyond 22 weeks	0 (0-2)	0 (0-1)	0 (0-1)	N.S.
Primary recurrent pregnancy loss (%)	62.5 [a]	90.9 [b]	50 [c]	<0.05 [a,b] <0.01 [b,c]
Gestational weeks at blood sampling	7 (5-11)	10 (5-12)	8 (4-14)	N.S.
Plasma level of free protein S antigen (%)	41.5 (35-57)	47 (27-59)	51.5 (37-59)	N.S.

Data are expressed as the median (range) or percentage. N.S., not significant.

Table II. The pregnancy outcome and complications

	Group A n=8	Group B n=22	Group C n=8	*P* value	
Gestational week at delivery	40 (34-41) [a]	38.5 (34-41) [b]	32 (26-40) [c]	<0.05 [b,c]	<0.05 [a,c]
Birth weight (g)	2,916 (2,250-3,314) [a]	2,855 (1,716-3,890) [b]	1,794 (750-3,384) [c]	<0.05 [b,c]	0.061 [a,c]
Pregnancy complications (%)					
Fetal growth restriction	0	4.5 [b]	37.5 [c]	<0.05 [b,c]	
Pregnancy induced hypertension	0	0 [b]	37.5 [c]	<0.01 [b,c]	
Preterm delivery	25	4.5 [b]	62.5 [c]	<0.01 [b,c]	

Data are expressed as the median (range) or percentage.