Decreased maternal protein S activity is associated with fetal growth restriction

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Abstract

Introduction: Protein S (PS) activity has been shown to decrease during normal pregnancy. The aim of this study was to determine any correlation between decreased maternal PS activity and fetal growth restriction (FGR).

Methods: We carried out a retrospective study of maternal PS activity and complement 4b-binding protein (C4BP) concentration in 102 patients with FGR and 58 patients with fetuses that had normal growth. Among pregnancies affected by FGR, 14 diagnoses were made in the second trimester and 88 in the third trimester. Patients whose fetuses had normal growth were matched with FGR subjects for maternal age and gestational age at sampling (29 cases each in the second and third trimester).

Results: Mean PS activity of the control group in the third trimester was significantly lower than in the second trimester (56.5 ± 16.5% vs 35.8 ± 13.8%). PS activity in women with FGR was significantly decreased in both the second trimester (36.6 ± 13.2%) and third trimester (30.2 ± 12.2%) compared with control group levels. Plasma concentrations of C4BP for the control group were significantly higher in the third trimester than in the second trimester (90.5 ± 17.5% vs 81.1 ± 13.6%). However, in women with FGR, plasma C4BP concentrations in both the second trimester (84.0 ± 14.8%) and the third trimester (86.0 ± 17.7%) were comparable with concentrations of the control group.

Conclusions: Maternal PS activity decreased as normal pregnancies progressed but decreased over time in cases with FGR. Excessive decreases in PS activity during pregnancy could contribute to development of FGR.

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KEYWORDS
Fetal growth restriction; Pregnancy; Protein S
Introduction

Fetal growth restriction (FGR) is a common complication of pregnancy associated with a failure of normal placental invasion and development [1]. The consequences of this placental dysfunction carry a significant increased risk of fetal morbidity and mortality as well as possible later deficits in a child's neuropsychological development [2]. Recently, several studies have focused on a link between maternal inherited thrombophilia and FGR: Researchers found that the frequency of maternal inherited thrombophilia, which may be accompanied by deficiencies in factors such as antithrombin (AT), protein C (PC), or protein S (PS), was significantly increased in the FGR group of mothers compared with the control group [3–5]. In cases of maternal thrombophilia associated with FGR, maternal floor infarction of the placenta, which is characterized by deposition of fibrinoid material, could be found not only in the maternal surface but also in intervillous spaces of the placenta [6]. Thus, both maternal thrombophilia and infarction of intervillous spaces of the placenta could be causes of FGR.

On the other hand, it has been reported that PS activity showed a progressive decrease during pregnancy in women without inherited PS deficiency, and this condition is called "acquired PS deficiency" [7–9]. It has also been reported that the level of C4BP increases during pregnancy [10]; hence, an increased C4BP level may contribute to reduced PS activity during pregnancy. When protein S activity falls below 35%, clotting time is shortened, thus increasing the risk of developing venous thromboembolism [10]. However, it has not been revealed whether decreased PS activity during pregnancy could contribute to development of FGR. In the present study, we investigated maternal PS activity in patients who fetuses demonstrated FGR to determine if there was a correlation between FGR and decreased PS activity.

Materials and methods

Study population

We studied 102 subjects whose fetuses demonstrated FGR and 58 subjects whose fetuses had normal growth. All were followed at the Maternity and Perinatal Care Unit of Kyushu University Hospital from January 1997 to December 2004. The gestational ages were calculated from the date of the last menstrual period and confirmed sonographically between 9 and 11 weeks of gestation. The Institutional Ethics Committee approved the study, and all mothers gave informed consent prior to participation. Fetal growth was considered normal when the birth weight was appropriate for the gestational age (within the mean±10th percentile for the gestational age). FGR was defined as having both estimated fetal body weight and birth weight lower than the 10th percentile for gestational age according to Japanese standards [11,12]. None of the cases in our analysis involved maternal or fetal complications such as intrauterine fetal death, fetal genetic aberrations, eclampsia, pre eclampsia, abruptio placentae, gestational hypertension, preterm labor, preterm rupture of membranes, or other medical disorders. No mothers had a history of stroke or thromboembolic disease.

After verifying that fetuses were viable, we obtained blood samples from subjects with FGR at the time of diagnosis. Among FGR pregnancies, 14 were diagnosed in the second trimester (14–28 weeks), and 88 were diagnosed in the third trimester (≥28 weeks). We also obtained a total of 58 blood samples from subjects whose fetuses had normal growth, and these were matched for maternal age and gestational age at sampling (29 cases, second trimester; 29 cases, third trimester).

Measurement variables

PS activity was determined by the coagulometric method (Staclot Protein S kit, Diagnostica Stago, Asnieres, France). Plasma concentrations of complement 4b-binding protein (C4BP) were determined by latex agglutination (Liatest C4b-BP kit, Diagnostica Stago, Asnieres, France). Normal values of PS activity in non-pregnant women were set between 59% and 128% [13]. When

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<th>Table 1 Clinical profiles of control and FGR subgroups throughout pregnancy</th>
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<tr>
<td><strong>Second trimester</strong></td>
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<td>Maternal age (years)</td>
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<td>PT-INR (%)</td>
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<td>APTT (seconds)</td>
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FGR, fetal growth restriction; GA, gestational age; PT-INR, prothrombin time-international normalized ratio; APTT, activated partial thromboplastin time.

<sup>*</sup> In the second trimester, the mean gestational age at delivery and infant birth weight were significantly lower in FGR groups compared to the control groups (ANOVA, P<0.003).

<sup>**</sup> In the third trimester, the mean gestational age at delivery and infant birth weight were significantly lower in FGR groups compared to the control groups (ANOVA, P<0.001).
measurements of PS activity were below the lower limit of the normal range, we re-evaluated PS activity at 1 month postpartum, and inherited defects were considered when repeat tests showed values similar to the original measurements. In addition, subjects with a deficiency of AT or PC, or the presence of anticardiolipin antibodies and lupus anticoagulant, were excluded from the present study.

Statistical analysis

All data are presented as mean±SD. Two-way analysis of variance (ANOVA) was used to analyze for differences in PS activity and C4BP among subgroups. If there were statistically significant differences, the Bonferroni method was applied for multiple comparisons. A P value of less than 0.05 was considered to be statistically significant; note that P values in text and figures are for tests done without the Bonferroni correction. Statistical analyses were performed with a statistical software package (StatView Version 5.0; SAS Institute, Cary, N.C., USA).

Results

Table 1 shows the maternal age, gestational age at blood draw, and platelet count and coagulation data in each trimester for the 58 women of the control group and the 102 women whose pregnancies showed FGR. For each trimester, there were no statistically significant differences between these two groups except for gestational age at delivery and infant birth weight (Table 1).

In the normal control group, mean PS activity in the third trimester (37.5±11.5%) was significantly lower than levels measured in the second trimester (56.5±16.5%; ANOVA, P<0.0001, 0.1% significance with Bonferroni correction) (Fig. 1). In the FGR group, PS activity did not decrease significantly between the second and third trimesters (ANOVA, second trimester 36.6±13.2% vs. third trimester 30.2±12.2%; P=0.146). In the comparison of PS activity during each trimester between control and FGR groups, PS activity levels for the FGR group in both the second and third trimester were significantly decreased compared with levels in the control group (ANOVA, P<0.0001 and P=0.010, 0.1% and 5% significance with Bonferroni correction, respectively).

Plasma concentrations of C4BP for the control group in the third trimester were significantly higher than those measured in the second trimester (ANOVA, second trimester 75.5±9.6% vs. third trimester 93.4±14.0%; P=0.004, 5% significance with Bonferroni correction) (Fig. 2). In the FGR group, plasma C4BP concentrations did not increase significantly between the second and third trimesters (ANOVA, second trimester 84.0±14.8% vs. third trimester 86.0±17.7%; P=0.759). When plasma concentrations of C4BP during each trimester were compared between control and FGR groups, there were no significant differences in either the second or third trimester between groups (ANOVA, P=0.283 and P=0.092, respectively).

Two of 98 subjects (2.0%) with FGR had a confirmed PS deficiency after reevaluation of PS activity at 1 month postpartum, whereas none of the control group had a PS deficiency.

Discussion

PS is a vitamin K-dependent glycoprotein that is synthesized primarily in hepatocytes, endothelial cells and megakaryocytes [14]. About 40% of protein S is present as free PS, and it participates in both activated protein C (APC)-dependent and APC-independent mechanisms of anticoagulation, both of which result in down-regulation of thrombin and factor Xa generation. However, approximately 60% of PS forms a complex with C4BP, which has no anticoagulation function.

Inherited PS deficiency has been identified in 1–7.5% of patients with deep venous thromboembolism (DVT) and in 0.03–0.13% of the general
Caucasian population [15]. However, Japanese people have a higher prevalence of PS deficiency, both in DVT patients (12.7%) and in the general population (0.48–0.63%) [7]. In studies, the frequency of PS deficiency was significantly increased in FGR groups compared with control groups [16–18]. According to a review by Alfirievic et al. [16], women with FGR had associated PS deficiency more often than did controls, with a pooled odds ratio and 95% confidence interval (CI) of 10.2 and 1.1–91, respectively. In the present study, 2 of 98 subjects (2.0%) with FGR had a PS deficiency, whereas none of the control group members had a PS deficiency. These findings suggest that decreased maternal PS activity may lead to development of FGR.

Mean PS functional levels decline strikingly from the first to third trimester of pregnancy [19]. The present result, demonstrating that PS activity was significantly decreased from the second to third trimester in normal pregnancies, is consistent with previous reports [7–9]. On the other hand, it has been reported that the level of C4BP increases during pregnancy [10]. Our study showed that the C4BP level of subjects in the third trimester was significantly higher than that in the second trimester among women with normal pregnancies. This suggests that an increased C4BP level may reduce PS activity during pregnancy.

On the other hand, PS activity of the FGR group in this study was significantly lower than that of the control group in both the second and third trimesters. However, the plasma C4BP concentrations in each trimester did not differ between groups. This suggests that the decreased PS activity observed in women with FGR is not only associated with increased C4BP level but also with other factors. Patients with AT deficiency, PC deficiency, anticardiolipin antibodies, or lupus anticoagulant were excluded from this study. In addition, no included subject exhibited any thromboembolic symptoms, and family histories were examined carefully to exclude patients with thromboembolic tendencies. Our results indicate that FGR is associated with decreased maternal PS activity. Thus, decreased PS activity may contribute to the development of FGR. In this study, we could neither prove a causal relationship between decreased PS activity and development of FGR nor determine how the degree of decrease in PS activity during pregnancy may indicate a greater risk for FGR. Clarification of these matters requires further interventional study with larger groups; enrollment would have to precede development of FGR and also be based on the degree of decrease in PS activity.

The etiology and mechanisms underlying FGR are not clearly understood. It has been suggested that FGR is associated with abnormal placental vasculature and disturbances of hemostasis leading to inadequate maternal–fetal circulation [20,21]. Placental histological examination showed that patients with FGR had more lesions of uteroplacental insufficiency or chronic villitis than did placentas of patients with preterm infants that had grown appropriately [22]. Arias et al. found that thrombotic lesions were present in 7 of 13 placentas of patients who had an adverse pregnancy outcome together with evidence of a thrombophilic state in the mother [23]. Among subjects with low free protein S levels, three of eight placentas had placental infarction and three had intervillous thrombosis [24]. These findings suggest that a similar pathogenic mechanism might have caused FGR in our subjects with decreased PS activity. Whether abnormal clotting occurs in the fetal, maternal, or both placental circulations remains to be examined.

Because uteroplacental thrombosis is a feature of FGR in women with thrombophilia, prophylaxis with heparin has been offered as anticoagulant therapy in pregnancy to prevent adverse pregnancy outcomes including FGR in women with antiphospholipid syndrome or other thrombophilias [25]. If it is confirmed by further studies that vascular injury and thrombin generation due to decreased PS activity causes FGR in addition to inherited thrombophilia, such prophylactic antenatal anticoagulation for patients with decreased PS activity might be anticipated.

Finally, two notes are in order about our study design. We were limited to identifying appropriate matched pregnancies from the total number of normal pregnancies delivered at our institution during the study period. We were able to identify 29 second-trimester control cases compared with 14 FGR pregnancies, but only 29 third-trimester control cases compared with 88 third-trimester cases. A larger number of third-trimester control cases might well have strengthened our findings. The strength of our findings is also affected by our choice in statistical tests. We chose to do our primary analysis with two-way ANOVA, with adjustment during multiple comparisons with use of a Bonferroni correction. A peer suggested that this may have been too conservative and that use of either Neuman–Keuls or Scheffes testing would have been appropriate and have yielded stronger findings. We reviewed our decision-making and decided to remain with our chosen testing, but it is always worth noting that statements of statistical significant depend on the choices made in statistical testing.

In conclusion, FGR pregnancies that were diagnosed in both the second and third trimester had PS activity levels that were lower than those measured in normal pregnancies. This suggests that excessively
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References