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The Normal Anticoagulant System and Risk of Placental Abruptio: Protein C, Protein S and Resistance to Activated Protein C

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Abstract

Objective—To examine the association between maternal thrombophilia associated with anticoagulation (proteins C and S and activated protein C resistance ratio, APCR) and risk of placental abruptio.

Methods—Data were derived from a case-control study — The New Jersey-Placental Abruptio Study (2002–07). Maternal blood was collected from abruptio cases and controls and was assayed for the thrombophilias. Decreased protein C, S and APCR was defined as values <5% and <10% among controls.

Results—Of a total of 132 cases and 127 controls, 3 were heterozygous for the factor V Leiden mutation (1 case and 2 controls). Mean ($\pm$ standard deviation) protein C (114.2 ± 25.6 vs 121.4 ± 27.6 ; $P=0.009$), protein S (39.9 ± 18.4 vs 35.7 ± 15.2 ; $P=0.043$) and APCR (2.86 ± 0.29 vs 2.88 ± 0.27 ; $P=0.039$) were different between cases and controls. Abruptio cases were associated with an odds ratio of 3.2 (95% CI 1.2, 9.9) in relation to decreased protein C (<5th centile). Decreases in both protein S and activated protein C resistance ratio were not associated with abruptio.

Conclusions—A decrease in protein C was associated with an increased risk for abruptio, suggesting an important role for the physiologic anticoagulant system in the etiology of placental abruptio.

Keywords

Thrombophilia; placental abruptio; protein C; protein S; activated protein C resistance

Normal pregnancy is a prothrombotic state with a marked increase in procoagulant activity¹ characterized by an elevation of coagulation factors VII, VIII, X, fibrinogen and von Willebrand factor ² In parallel, there is a decrease in the physiologic anticoagulants, manifest by a significant decrease in protein S activity and an acquired resistance to activated protein C.^{3,4} The risk for thrombosis in women during pregnancy is substantially

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increased,⁵ and thrombosis represents the greatest cause of maternal morbidity and mortality during pregnancy, puerperium and the postpartum periods.^{6,7}

Inherited thrombophilias have been linked to an array of adverse pregnancy outcomes, including preeclampsia (especially the severe form), fetal growth restriction, stillbirth and placental abruption.^{8–12} It is believed that disruption of the maternal-fetal circulation as a result of thrombosis is an important risk factor for these untoward obstetrical complications.¹³ Abnormalities in the innate anticoagulant system associated with decreased protein S and protein C activities and activated protein C resistance predispose to thrombosis.^{1,3,4,7}

The purpose of our study was to examine if placental abruption was associated with an increased risk of an abnormality in the natural anticoagulant system. We compared the distributions of proteins C and S, and resistance to activated protein C ratio in a multicenter case-control study of placental abruption, and examined if these thrombophilias were associated with increased abruption risk.

Material and Methods

New Jersey—Placental Abruption Study

Data for this study were derived from a multicenter case-control study of placental abruption. Subjects were recruited from Robert Wood Johnson University Hospital (July 2003 through April 2007) and at Saint Peter's University Hospital (August 2002 through June 2004) in New Brunswick, NJ. Both hospitals are tertiary, level III referral centers and deliver approximately 8,000 deliveries annually. Further details of the study are described elsewhere.^{14–16} Ethics approval from the institutional review boards of both hospitals, as well as that of UMDNJ-Robert Wood Johnson Medical School, NJ, was received.

Cases eligible for inclusion were women with a clinical diagnosis of placental abruption (at ≥ 20 weeks) before or during delivery, by the attending obstetrician. The definition of placental abruption included painful vaginal bleeding or hemorrhage accompanied by documented fetal distress, uterine pain or tenderness, or uterine hypertonicity. In the absence of any of these clinical hallmarks for abruption, if the delivered placenta showed signs of retroplacental bleeding or retroplacental hematoma/clots on the placental surface or disc, then these patients were also eligible for inclusion as potential cases. In addition, if there was an antepartum sonographic diagnosis of placental abruption, then these cases were also considered.¹⁷ Placental abruption cases were identified by daily review of hospital delivery logs at both hospitals, and/or by referral by the physician, nurse, or obstetrics and gynecology residents.

Controls were identified from reviewing daily delivery logs in both hospitals and were matched to cases on parity (nulliparous, primiparous, or parity ≥ 2), and maternal race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, or other race/ethnicity). If more than one patient met the eligibility criteria for controls, we chose the first consenting patient with a date of delivery closest to the abruption case. Women with a diagnosis of placenta previa in the index pregnancy or with abruption in any of their previous pregnancies were excluded from the eligible pool of controls.

All cases and controls were recruited immediately following delivery. Upon obtaining consent, patients were approached during their postpartum period, and a structured in-person interview questionnaire was administered (lasting approximately 30 minutes) to collect details regarding maternal and paternal demographic, lifestyle, behavioral and general health conditions. Upon completing the interviews, a sample of maternal peripheral blood was

drawn. In addition, consent was also obtained to abstract the medical and prenatal care records from the index and all previous pregnancies and outcomes. A majority of patient contact and recruitment were completed within 24 hours, and all within 72 hours following delivery.

Hemostatic assays

Blood for hemostatic assays was collected in 3.2% sodium citrate in a 9 to 1 blood to anticoagulant ratio and processed within one hour of venipuncture. Protein C activity was determined using the chromogenic method (Diagnostics Stago, Parsippany, NJ). Protein S activity was measured using a clotting assay for the measurement of a functional protein S level (Diagnostics Stago, Parsippany, NJ). Activated protein C resistance was determined using an APTT-based activated protein C resistance assay using an excess of Factor V deficient plasma (Beckman Coulter, Fullerton, CA). Testing was performed on an ACL coagulation analyzer system (Instrumentation Laboratories, Lexington, MA). Normal ranges were calculated based on 2 standard deviation about the mean using plasma from 25 normal men and women. The calculated ranges for protein C activity was 62–154%, protein S activity 56–140%, and activated protein C resistance 0.75–1.89. Coefficients of variation were 21%, 21%, and 17%, respectively, for protein C, protein S and activated protein C resistance.

Factor V Leiden mutation (G1691A) was performed by invader DNA assay (Third Wave Technologies, Madison, WI)

Statistical analysis

We examined the distributions of maternal socio-demographic characteristics between abruption cases and controls. Analysis of proteins C and S, and activated protein C resistance ratio were first examined for normality. Since the normality assumption was met, the distributions were compared between abruption cases and controls. These assays were also analyzed as categorical factors based on quartiles, and as values $<10^{\text{th}}$ and $<5^{\text{th}}$ centiles. The cut-offs for these variables were derived from the control population. Finally, these data were also analyzed as <2 standard deviation units for protein C, protein S and activated protein C resistance ratio of the laboratory reference values.

To address bias due to confounding, we adjusted the associations between proteins C and S, and activated protein C resistance ratio and placental abruption for several potential confounders through a multivariable regression analysis. We fitted general linear models and logistic regression models, respectively, for analyzing proteins C and S, and activated protein C resistance ratio as continuous and categorical variables. In all analyses, we adjusted for the following confounders: maternal age (<19 , 19–24, 25–34, and ≥ 35 years), maternal education (less than high school or high school or more of completed schooling), marital status (single or married), lack of prenatal care, smoking before and during pregnancy (yes/no) and prepregnancy body-mass index. Body-mass index (BMI) was calculated as the ratio of weight (in kilograms) over squared-height (in meters). BMI was first tested for potential non-linear association with case-control status by including a quadratic term for BMI. Since the quadratic term was not significant, we included BMI as a continuous variable.

Results

Of a total recruitment of 217 placental abruption cases and 210 controls as of April 2007, protein C, protein S and activated protein C resistance ratio assays were completed in 132 abruption cases and 127 controls. The distribution of socio-demographic characteristics

between the full study and that restricted to this study were similar and comparable (not shown). Maternal socio-demographic characteristics in relation to the 132 cases and 127 controls are shown in Table 1.

Distributions of proteins C and S activities, and activated protein C resistance ratio between abruption cases and controls are shown in Figure 1. Protein C levels were, on average, -7.2% (95% confidence interval -13.7% , -4.3%) lower among abruption cases than controls, and after adjusting for potential confounders, the mean difference was even larger (-9.1% , 95% CI -13.9% , -4.3%). Contrarily, mean protein S activity levels were higher among abruption cases than controls (adjusted mean difference 4.6% , 95% confidence interval 0.1% , 9.2%). Although the distribution of activated protein C resistance ratio was similar between cases and controls, the difference was modest (0.06 , 95% CI 0.01 , 0.11) following adjustment for confounders.

We examined the association between extremes of proteins C and S activities, and activated protein C resistance ratio and abruption (Table 2). In comparison to controls, abruption cases were more likely to have protein C activity $<5^{\text{th}}$ centile (odds ratio 3.2 , 95% confidence interval 1.2 , 9.9), and values below 2 standard deviations of the laboratory's reference range (odds ratio 5.2 , 95% confidence interval 1.6 , 48.6), although the analysis was affected by imprecision based on the wide confidence intervals. Although protein S distributions were similar between cases and controls, abruption cases were more likely to have increased activated protein C resistance ratio above the 90^{th} centile (odds ratio 2.4 , 95% CI 1.1 , 5.4) than controls. Interestingly, of the 2 abruption cases and 2 controls who had an activated protein C resistance ratio below the laboratory's reference range, 3 subjects (1 case and 2 controls) were heterozygous for the factor V Leiden mutation.

Comment

Placental abruption has been associated with abnormal placental vasculature and disturbances of hemostasis. Several thrombophilic mutations have been associated with an increased risk of obstetrical complications that are related to inadequate maternal-fetal circulation. It is well known that the normal physiologic anticoagulant activities of protein C, protein S, and activated protein C resistance ratio plays an integral role in holding the prothrombotic state of pregnancy in check.

Our study demonstrated that protein C activity was, on average, lower in placental abruption cases than controls, and that decreased protein C activity was associated with over a 5-fold increased odds of abruption. In addition, we observed a trend toward an inverse relationship between activated protein C resistance ratio and abruption, with cases more likely to demonstrate decreased anticoagulant activity. Interestingly, protein S activity was similar between cases and controls. These general findings corroborate previous studies that have demonstrated an association of abruption with protein C deficiency.^{8,18–20} However, our findings are at variance with other studies^{8,19,20} that showed an association between abruption and protein S deficiency. The lack of an association between abruption and an acquired activated protein C resistance ratio may be attributable to the specificity for the Factor V Leiden mutation of the activated protein C resistance assay utilized in this study. In many of the earlier studies, the study groups included patients with one or more of several adverse pregnancy outcomes, and not specifically placental abruption. Presumably, the association between the effects of proteins C and S and abruption risk was attenuated due to combining different outcomes.

Redline²¹ proposed a plausible biologic mechanism by which heritable thrombophilias can potentially lead to placental abruption. He speculated a series of events beginning with

abnormal clotting in the placental circulation, leading to complete thrombotic occlusion of the spiral artery, villous infarction, and ultimately resulting in placental abruption. There is recent evidence to suggest the importance of protein C and activated protein C at the level of the uteroplacental interface in the maintenance of normal pregnancy through the thrombomodulin-protein C system.²²

Thrombomodulin inhibits the pro-coagulant activity of thrombin by forming a high-affinity thrombin-thrombomodulin complex and drives the formation of the natural anticoagulant activated protein C.²³ Furthermore, the endothelial protein C receptor enhances the formation of activated protein C through this thrombin-thrombomodulin complex when it binds protein C.²⁴ Women with late fetal loss are more likely to have mutations in the glycoprotein receptors thrombomodulin and endothelial protein C receptor.²⁵ These receptors are expressed mainly on the endothelial surface of blood vessels and also in the placental syncytiotrophoblast.²⁶ It has been proposed that mutations in these glycoprotein receptors play a key physiologic role in the protein C anticoagulant pathway by interfering with the formation of thrombin-thrombomodulin complexes.²⁵

Furthermore, Isermann et al.²² suggested that thrombomodulin-protein C system is essential in controlling growth and survival of trophoblast cells in the placenta. They argued that thrombomodulin functions as an inhibitor of coagulation and, in particular, fibrinolysis, limiting the production of fibrin degradation products, which can trigger trophoblast apoptosis and inhibit trophoblastic cell growth. The interaction between thrombomodulin and protein C is an intriguing mechanism leading to vascular thrombosis and/or uteroplacental ischemia and placental abruption.

Strengths and limitations of the study

Our study has several strengths. Placental abruption cases were carefully ascertained utilizing rigorous clinical diagnostic criteria, lab personnel carrying out the assays were blinded to case-control status, tight matching of controls to abruption cases (on parity and maternal race/ethnicity), adjustment for a variety of confounders, and sufficiently large study size. However, this study was limited by an inability to examine race-specific changes in proteins C and S activities and activated protein C resistance ratio, and the ethnic distribution of our population may limit generalizability.

Another important consideration in our study design is the time when blood samples were drawn for assays. We recruited abruption cases and controls following their delivery, and blood was drawn immediately following recruitment. Thus, the observed changes in proteins C and S may represent pregnancy associated changes (i.e., acquired) rather than baseline levels. The association of placental abruption with lower, but not necessarily truly deficient protein C levels, compared to controls suggests the possibility of acquired changes in protein C activity or the possibility of genetic determinants of variation in protein C levels. Polymorphisms in the protein C gene and other genetic determinants of variation in protein C level have been identified.^{27,28}

Conclusions

Our findings highlight a potential role for the protein C pathway in the pathophysiology of placental abruption and demonstrate that patients with abruption may be more likely to have a defect in these pathways. These findings underscore the need for further studies to elucidate the relative contributions of genetic determinants and changes in protein C and S activities in relation to placental abruption. Additional studies to evaluate pharmacologic interventions, such as heparin, in individuals identified with defects in the protein C pathway

may be useful to assess whether the risk of placental vascular thrombosis and subsequent placental abruption can be mitigated.

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Appendix

Investigators that participated in the New Jersey — Placental Abruption Study included Cande V. Ananth, PhD, MPH (Principal investigator), Darios Getahun, MD, MPH, Neela Srinivas, MD, MPH, Celeste DeMarco, RN, BSN, Denise Elsasser, MPH, Yu-Ling Lai, RN and Shelby Pitts, RN (Division of Epidemiology and Biostatistics); John C. Smulian, MD, MPH, Wendy L. Kinzler, MD, Morgan R. Peltier, PhD, and Marian Lake, RN, MPH (Division of Maternal-Fetal Medicine); Department of Obstetrics, Gynecology, and Reproductive Sciences; Claire Philipp, MD (Department of Medicine), all at UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; and George G. Rhoads, MD, MPH (Department of Epidemiology) and Dirk F. Moore, PhD (Department of Biostatistics), UMDNJ-School of Public Health, Piscataway, NJ.

Other investigators that were involved with the study included Jacques Genest, MD (McGill University, Montreal, Canada), Susan Shen-Schwarz, MD (Department of Pathology, Saint

Peter's University Hospital, New Brunswick, NJ), and Vinay Prasad, MD (Department of Pediatric Pathology, Nationwide Children's Hospital, Ohio State University, Columbus, OH

Fig. 1. Distributions (mean and standard deviation) of protein C activity (%), protein S activity (%), and activated protein C resistance ratio between placental abruption cases and controls. Unadjusted difference (95% confidence interval) between cases and controls for each anticoagulant assay are also shown

Table 1

Distribution of placental abruption cases and controls in relation to selected maternal socio-demographic characteristics

Maternal characteristics	Cases (n=132)		Controls (n=127)		P-value
	n	%	n	%	
Maternal age (years) [†]					0.323
<20	3	2.3	1	0.8	
20-24	23	17.4	20	15.8	
25-34	67	50.8	63	49.6	
≥35	39	29.6	43	33.9	
Parity					0.613
Nulliparous	49	37.1	47	37.1	
Primiparous	49	37.1	41	32.3	
Parity ≥2	34	25.8	39	30.6	
Maternal race/ethnicity					0.172
Caucasian	23	17.4	24	18.9	
African-American	44	33.3	55	43.3	
Hispanic	43	32.6	31	24.4	
Other race/ethnicity	22	16.7	17	13.4	
Education below high school	58	43.9	35	27.6	0.006
Single marital status	36	27.3	31	24.4	0.600
Smoking during pregnancy	19	14.4	4	3.2	0.002
Factor V Leiden mutation [‡]	1	0.8	2	1.6	0.540
Pre-pregnancy body-mass index [‡]	25.3 (6.1)		24.8 (5.5)		0.524

[†] Only 3 subjects (1 abruption case and 2 controls) in the study were heterozygous for the Factor V Leiden mutation

[‡] Data denoted as mean (standard deviation)

Table 2
Associations between deficiencies in protein C and S activities, activated protein C resistance and placental abruption

	Cases (n=132) (%)	Controls (n=127) (%)	Odds ratio (95% confidence interval)	
			Unadjusted	Adjusted [†]
Protein C activity (%)				
Quartile 4 (≥ 138)	20.5	25.2	1.0 (Reference)	1.0 (Reference)
Quartile 3 (120–137)	20.5	23.6	1.1 (0.5, 2.2)	1.2 (0.5, 2.8)
Quartile 2 (104–119)	25.0	26.0	1.2 (0.6, 2.4)	1.6 (0.7, 3.6)
Quartile 1 (<104)	34.1	25.0	1.7 (0.8, 3.3)	2.4 (1.0, 5.7)
Bottom 10% (<84) vs ≥10%	11.4	8.7	1.4 (0.6, 3.1)	1.6 (0.6, 4.0)
Bottom 5% (<82) vs ≥5%	10.6	3.9	2.9 (1.0, 8.3)	3.2 (1.2, 9.9)
Below lab reference range	3.8	0.8	5.0 (0.6, 43.1)	5.2 (1.6, 48.6)
Protein S activity (%)				
Quartile 4 (≥45)	37.1	27.6	1.7 (0.9, 3.2)	1.7 (0.8, 3.8)
Quartile 3 (36–44)	17.4	21.3	1.0 (0.5, 2.1)	1.2 (0.5, 2.7)
Quartile 2 (25–35)	21.2	25.2	1.2 (0.6, 2.4)	1.0 (0.4, 2.3)
Quartile 1 (<25)	21.2	26.0	1.0 (Reference)	1.0 (Reference)
Bottom 10% (<15) vs ≥10%	6.8	8.7	0.8 (0.3, 1.9)	0.83 (0.3, 2.5)
Bottom 5% (<12) vs ≥5%	0	2.4	—	—
Below lab reference range	62.9	72.4	0.6 (0.4, 1.1)	0.6 (0.3, 1.1)
Activated protein C resistance ratio				
Quartile 4 (≥3.1)	34.9	28.4	1.0 (Reference)	1.0 (Reference)
Quartile 3 (2.9–3.1)	26.5	36.2	0.6 (0.3, 1.1)	0.4 (0.2, 0.9)
Quartile 2 (2.8–2.9)	12.9	11.8	0.9 (0.4, 2.0)	0.5 (0.2, 1.3)
Quartile 1 (<2.8)	25.8	23.6	0.9 (0.5, 1.7)	0.3 (0.1, 0.7)
Upper 90% (>3.2) vs ≤90%	18.2	15.8	1.2 (0.6, 2.3)	2.4 (1.1, 5.4)
Upper 95% (>3.4) vs ≤95%	2.3	5.5	0.4 (0.1, 1.6)	0.7 (0.2, 3.6)
Below lab reference range	1.5	1.5	1.0 (0.1, 6.9)	— [‡]

[†] Odds ratios were adjusted for maternal age, education, marital status, smoking during pregnancy, and prepregnancy body-mass index

[‡] Number of cases and controls too few for meaningful analysis