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Elevated first trimester soluble fibrin polymer is associated with adverse pregnancy outcome in thrombophilic patients

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Inherited and acquired thrombophilic conditions are associated with maternal thromboembolic events and a variety of adverse perinatal outcomes, including, preeclampsia, intrauterine growth restriction (<10th percentile), second and third trimester fetal loss, and abruptio placentae [1,2]. Currently, there is a paucity of functional or global tests that will provide insight as to the prediction of adverse pregnancy outcome [3,4]. Activation markers are of limited predictive value for adverse outcome [5]. Bombeli et al. [6] found that thrombin–antithrombin complexes (TAT) or D-dimers did not correlate with risk stratification in pregnant women based upon personal or family history of thrombosis and the presence of a thrombophilic condition. However, these authors did find that women with ongoing thrombosis during pregnancy had significantly elevated TAT and D-dimers with or without anticoagulant therapy. Coagulation activation markers such as prothrombin fragment 1.2 and TAT are increased with the progression of pregnancy to levels as seen in those patients with active thrombosis [4]. Bremme et al. [7] found that normal pregnancy (n=26 women) was associated with both increased thrombin activity, increased soluble fibrin levels (9.2–13.4 nmol/l), as well as fibrinolysis, as evidenced by increased levels of fibrin D-dimer (91–198 µg/l). Soluble fibrin polymer (SFP) has been shown to be a specific and sensitive marker of active clotting and thrombin generation in a group of nonpregnant individuals at high risk for thromboembolic event [8]. The purpose of this study was to determine if elevated SFP is an additional risk for adverse pregnancy outcome in thrombophilic patients.

We conducted a retrospective cohort study among 39 patients with inherited and acquired thrombophilia (41 pregnancies) and 50 uncomplicated gestations. Thrombophilic women previously underwent a uniform evaluation for inherited and acquired thrombophilic conditions. A reference laboratory was used to evaluate the relevant thrombophilic conditions. Factor V Leiden, methylenetetrahydrofolate reductase gene mutation, and prothrombin gene mutation 20210 assay were performed by using multiplex allele-specific primers' PCR amplification. We defined protein S as deficient if protein levels were below 40%, as determined by functional assay. Protein C and antithrombin were measured by chromogenic and antigenic assays, and respective levels below the laboratory's normal

reference range were considered abnormal. Homocysteine testing was evaluated by high performance liquid chromatography (HPLC), and values above the laboratory's normal reference range were considered abnormal. Antiphospholipid antibody syndrome was diagnosed by established criteria [9]. Anticardiolipin and anti-beta 2 glycoprotein I antibodies were performed by enzyme-linked immunosorbent assay (ELISA), and values above the laboratory's reference range were considered abnormal. The presence of the lupus anticoagulant was detected by screening with a sensitive activated partial thromboplastin time (APTT) and confirmed by the dilute Russel viper venom time with correction by adding the high concentration of phospholipids. This is felt to be a reliable confirmatory test for the lupus inhibitor. PAI-1 antigen and activity were measured by ELISA and chromogenic assay respectively, and levels three-fold above the upper limit of the reference range were considered abnormal.

Thrombocytosis, as part of the myeloproliferative syndrome, was determined by the presence of an elevated platelet count, associated with abnormal platelet function studies, platelet flow cytometry, and compatible bone marrow findings, clinical presentation, such as splenomegaly, and other white cell or red cell abnormalities.

Following informed consent and institutional review board approval, pregnant patients enrolled in the maternal fetal medicine faculty practice of New York University School of Medicine who underwent phlebotomy for other routine laboratory tests as part of their obstetrical care were asked to donate an additional aliquot of blood for SFP evaluation. Specimens were collected at routine blood draw and the plasma samples were stored at -80°C until analysis. Maternal plasma SFP concentrations were measured with a commercial ELISA [thrombus precursor protein (TpP); American Biogenetic Sciences, Inc., Copiague, New York, USA], as previously described [8]. The standard curve consistently had a R value of more than 0.98, the interassay and intraassay errors were less than 7%.

Using a computerized obstetrical database, all thrombophilic patients, with an available blood sample obtained during their first prenatal visit and who met the criteria for thrombophilia, were identified and recruited. All charts were reviewed in detail to confirm the accuracy of the diagnosis. To establish a reference range for SFP in pregnancy, blood specimens from the first, second, and third trimesters were obtained from a group of healthy pregnant women with normal pregnancy outcome.

Patients with thrombophilia were identified as having the following conditions: protein S deficiency (N = 8), factor V Leiden mutation R506Q (N=12), prothrombin gene mutation 20210A (N=6), antiphospholipid antibody syndrome (N=17), hyperhomocysteinemia (N=1), and thrombocythemia and myeloproliferative disorder with predominant manifestation of thrombocytosis (N=3). Five patients had two defects.

Preeclampsia was defined by American College of Obstetricians and Gynecologist's criteria [10]. Severe preeclampsia was defined when the following were present: systolic blood pressure (SBP) of at least 160mmHg or diastolic blood pressure (DBP) of at least 110mmHg and plus two or more protein on urinary dipstick. Severe preeclampsia was considered present if one of the blood pressure or proteinuria requirements was met along with either thrombocytopenia (platelet count<100 000/ml) or elevated liver enzymes (serum glutamic oxalo-acetic transaminase or serum glutamic pyruvic transaminase). The data were analyzed by Wilcoxon rank sum, t-test (DF, 1), and χ^2 (DF, 1) where appropriate. Level of significance was set at a P value less than 0.05. Receiver operator characteristic (ROC) curve analysis was performed to determine the optimum cutoff for SFP, to predict adverse pregnancy outcome.

Thrombophilic patients had significantly lower birth weights and delivered earlier than controls, respectively: 2943 ± 829 g vs. 3498 ± 527 g and 37.0 ± 4.5 weeks vs. 39.4 ± 1.5 weeks ($P < 0.01$). First trimester mean SFP maternal levels (mg/ml) were significantly higher among thrombophilic patients compared with controls (21.5 ± 3.7 vs. 3.7 ± 0.8 mg/ml; $P < 0.02$). The ROC curve of SFP to predict adverse pregnancy outcomes by using 3 mg/ml in the first trimester as cutoff ($P = 0.03$) produced a sensitivity of 83%, specificity 64%, positive predictive value of 57%, and negative predictive value of 97% for adverse pregnancy outcome. Five thrombophilic patients had at least two thrombophilic conditions, and their SFP values were higher than cases with one (26.4 ± 16.0 ; $P < 0.01$). Even with heparin therapy, cases also had higher SFP values than controls in the second trimester (31.7 ± 8 vs. 10.9 ± 2 ; $P < 0.01$) and third trimester (35.1 ± 16.1 vs. 12.9 ± 3 ; $P < 0.01$). Table 1 shows the distribution of the adverse pregnancy outcomes in the thrombophilia group.

There were no significant differences between the thrombophilic patients ($n=39$) and controls ($n=50$) regarding mean maternal age, years_{SD} (32.6 ± 5.8 vs. 33.1 ± 5.6), median gravidity and range [3 (1–11) vs. 2 (0–9)], median parity and range [1 (0–4) vs. 0 (0–5)], nulliparity (17 vs. 16%), multiparity (83 vs. 84%), race expressed as percent Caucasian (80 vs. 83%), respectively. There was no significant difference in gestational age at blood draw [median (range) between the thrombophilic patients ($n=41, 40, 39$) and controls ($n=50, 50, 50$)] in the first trimester [10.2 (4.0–14.0) vs. 9.0 (4.6–13.0)], second trimester [21.3 (15.0–27.4) vs. 17.6 (15.3–26.9)], or the third trimester [33.5 (27.3–40.4) vs. 29.7 (27.7–40.0)], respectively.

Our study confirms the progressive increase of thrombin generation as pregnancy proceeds with a markedly accelerated thrombin generation in thrombophilic patients. Higher first-trimester SFP increases the risk for adverse pregnancy outcomes. Even some normal pregnant patients with uneventful pregnancies have elevated SFP values. The significance of this is not known at this time and may be reflective of normal biologic variability or indicative of indolent processes. SFP levels are inversely related to gestational age at delivery and birth weight confirming our previous data regarding multiple gestations [11]. Testing for SFP may be useful in predicting patients at risk for thrombophilia-induced poor pregnancy outcome and monitoring anticoagulation efficacy. After the completion of this study, the ELISA test for SFP became unavailable commercially. It employs a very specific antibody to SFP. Perhaps, other tests may be developed that will exhibit similar properties. Our data indicate that reintroduction of SFP measurement may have clinical utility, particularly if confirmed in a larger prospective study.

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Table 1

Distribution of adverse pregnancy outcome

APO	Protein S	FVL	PGM	APS	HCT
	8	6	12	7	1
Preeclampsia	2	1	1	1	2
IUGR		1		1	2
Stillbirth				1	
SAB					1
pPROM			1		
Mat TEP					1