Fibrinogen Proteolysis and Platelet α-Granule Release in Preeclampsia/Eclampsia

By Z. Borok, J. Weitz, J. Owen, M. Auerbach, and H. L. Nossel

Serial measurements of the plasma concentration of fibrinopeptide A, thrombin-increasable fibrinopeptide B (reflecting Bβ 1-42), desarginyl fibrinopeptide B, beta thromboglobulin, and platelet factor 4 were made before, during, and after delivery in patients with preeclampsia/eclampsia. The data were correlated with routine coagulation studies, hematologic and renal status, as well as with the clinical manifestations. In 11 patients with mild preeclampsia, there were small increases in the fibrinopeptides at the time of delivery, but no other hematologic changes. In 5 patients with severe preeclampsia/eclampsia, there were marked increases in plasma levels of fibrinopeptides and platelet alpha granule proteins, which correlated in time with the clinical manifestations. When the changes in these patients were compared with those occurring in patients undergoing intraamniotic hypertonic saline infu-

Although the etiology of preeclampsia/eclampsia is unclear, there is much evidence for intravascular coagulation in the disease. Widespread fibrin deposition has been a prominent histologic finding in fatal cases of eclampsia.1 Electron microscopy of renal biopsy material from patients with preeclampsia has shown fibrinoid material in the glomeruli, and this material stained with fluorescent-labeled antiserum to fibrinogen.2 Increased fibrinolytic activity and levels of fibrinogen degradation products (FDP), changes in the factor VIII level, thrombocytopenia, decreased platelet survival, and increased β-thromboglobulin (BTG) levels have been reported.3-8 Abnormal perfusion lung scans, thought to reflect pulmonary emboli, were observed in 10/19 patients with preeclampsia and 6/8 patients with eclampsia.9

Because of these findings, we undertook serial studies in a group of patients with preeclampsia/eclampsia, focusing on fibrinopeptide and platelet alpha granule protein levels as indices of fibrinogen proteolysis and platelet release. Fibrinopeptide A (FPA) reflects fibrin I formation by thrombin proteolysis of fibrinogen; Bβ 1-42, measured as thrombin-increasable fibrinopeptide B (TIFPB), reflects plasmin degradation of fibrin I to form fragment X; and desarginyl FPB (B1-13) reflects fibrin II formation by thrombin proteolysis of fibrin I. Beta thromboglobulin (BTG) and platelet factor 4 (PF4) reflect alpha granule release from platelets.

MATERIALS AND METHODS

Patients

The patients studied were admitted to hospital with a diagnosis of preeclampsia or eclampsia. The criterion for entry to the study was a blood pressure of at least 140/90 mm Hg in patients who had been normotensive in the first half of pregnancy, or an increase in systolic pressure of 30 mm Hg or diastolic of 15 mm Hg over baseline values. Informed consent was obtained from all patients.

Study Protocol

Blood samples for measurement of fibrinopeptides and platelet proteins were collected daily from the time of admission to the study through delivery and until discharge from hospital. Samples for blood counts, coagulation tests, and FDP levels were collected 3 times a week. Daily urine samples were tested for protein, and creatinine and protein were determined twice weekly on 24-hr urine collections. Records were kept of blood pressure, and the presence of edema, hyperreflexia, or changes in mentation were noted.

Blood Collection and Processing

Samples were collected from an antecubital vein using a 21-gauge butterfly needle. Four and one-half milliliters blood for TIFPB and B1-13 measurement was collected via a syringe into a plastic tube containing 0.5 ml anticoagulant solution, consisting of Trasylol 1,000 U/ml, heparin 1,400 U/ml, adenosine 10 mM, and theophylline 20 mM in HEPES-buffered saline, pH 7.4. For FPA, PF4 and BTG measurement, 9 ml was then collected via the same needle into a siliconized vacutainer tube containing 1/10 volume 3.8% trisodium citrate for coagulation and FDP determination, and a tube of clotted blood was collected for thrombocytopenia and increased platelet protein levels were seen in these patients and were disproportionate to the degree of increase in the fibrinopeptide A level, suggesting that a mechanism other than thrombin must have contributed to the platelet changes; and (4) in two patients with severe preeclampsia/eclampsia, high desarginyl fibrinopeptide B levels preceded renal insufficiency, possibly reflecting fibrin II formation in renal vessels.
was separated by centrifugation for 15 min at 1,700 g. Plasma for assay of FPA, PF4, and BTG was centrifuged at 50,000 g for 10 min, and 0.5 ml of platelet-poor plasma was removed and stored at –80°C, for assay of the platelet proteins. The remaining plasma was stored at –80°C, and fibrinogen was adsorbed with bentonite prior to FPA assay. Plasma for assay of TIFPB and B1-13 was precipitated with ethanol as previously described. Samples for TIFPB assay were treated with thrombin after neutralizing heparin, as previously described. Samples for B1-13 assay were adsorbed with bentonite and treated with carboxypeptidase B, as previously described.

Radioimmunoassays
FPA, B1-13, TIFPB, BTG, and PF4 were assayed as previously described. The normal range for BTG in our laboratory is between 6 and 48 ng/ml, with a median value of 18 ng/ml, and for PF4 is between 1.7 and 21 ng/ml, with a median value of 6.0 ng/ml. In our laboratory, the level in normals for FPA is <1.5 pmole/ml, for TIFPB is <4 pmole/ml, and for B1-13 is <0.6 pmole/ml.

Other Tests
Hemoglobin, leukocyte, and platelet counts were measured with a Model S plus Coulter counter. Fibrinogen levels were measured as described by Ellis and Stransky. Serum FDP was measured by the tanned red cell hemagglutination inhibition method, as described by Merskey et al. A computerized sequential multiple analyzer (Technicon, Tarrytown, NY) was used for the blood chemistry determinations.

Statistics
The distribution of values of fibrinopeptides and platelet proteins was skewed, but was adequately described by logarithmic gaussian distributions. Analyses were carried out on logarithmically transformed data, with significance of differences determined by analysis of variance performed according to Sokal and Rohlf.

RESULTS
Sixteen patients were studied (median age 28 yr), 11 of whom had mild preeclampsia and 5 severe disease. In the group with mild disease, 7 were delivered vaginally and 4 by caesarian section.

Mild Disease
The data for the 11 patients with mild preeclampsia are shown in Figure 1. The data for the 7 days before and for the 7 days after delivery were compared by analysis of variance, and there were no statistically significant differences in fibrinogen concentrations, platelet counts, or platelet protein levels. The FPA levels increased from a mean of 1.4 pmole/ml before delivery to a mean of 2.3 pmole/ml after delivery (p = 0.006). The mean value for TIFPB increased from 4.6 pmole/ml to 8.0 pmole/ml (p < 0.001), and the mean value for B1-13 increased from 0.5 pmole/ml to 0.8 pmole/ml after delivery (p < 0.001). The FDP level increased from a mean of 2 μg/ml to 4 μg/ml (p = 0.001), and the hemoglobin concentration decreased from 13 g/dl to 12 g/dl after delivery (p < 0.001).

Severe Disease
Five patients had severe preeclampsia/eclampsia, as defined by severe hypertension, cerebral or visual disturbances, thrombocytopenia, or impaired hepatic function. Two patients were admitted with severe preeclampsia, two with eclampsia, and one developed severe preeclampsia in the postpartum period. All five patients had thrombocytopenia and increased FDP levels. The levels of fibrinopeptides and platelet proteins were higher in these patients than in those with mild disease. The coagulation abnormalities coincided in time with the clinical manifestations of the disease, and as the symptoms cleared, the coagulation changes also resolved.

Case 1 (E. N.—Fig. 2)
This 29-yr-old gravida 3 para 2 (G3P2) was admitted at 32 wk gestation with a blood pressure of 180/120 mm Hg, temporary blindness due to retinal edema, peripheral and facial edema, hyperreflexia with clonus, and 3+ proteinuria. The serum creatinine was 0.9 mg/dl (normal, 0.6–1.5 mg/dl), the uric acid was 7.4 mg/dl (normal, 2.5–7.0 mg/dl), and serum transaminases and alkaline phosphatase were increased. The initial coagulation studies were normal except for FDP of 3 μg/ml and an increased BTG level. The platelet count decreased from 108,000/μl on admission to 47,000/μl over the course of a few hours, prompting caesarian section. For the next week, the patient had severe thrombocytopenia and hemolysis with fragmented red cells. Fibrinogen concentrations were over 400 mg/dl, although the FDP level reached 81 μg/ml. On day 8, while receiving plasma infusions, prednisone (100 mg/day), aspirin (1,200 mg/day), and dipyridamole (400 mg/day), the platelet count abruptly increased from 24,000 to 254,000/μl without associated changes in the PF4 or BTG levels. The FPA, B1-13, and TIFPB levels all began to decrease on day 8 and were normal by day 21. The hypertension, edema, and proteinuria gradually resolved, and 6 mo later, there was no evidence of disease.

Case 2 (E. J.—Fig. 3)
This 17-yr-old G2P0 was admitted at 36 wk gestation after a generalized seizure, with a blood pressure of 150/110 mm Hg, gross hematuria, oozing from venipuncture sites, generalized edema, and 1+ proteinuria. The platelet count was 40,000/μl, the fibrinogen concentration 180 mg/dl, and the FDP level 165 μg/ml. The serum creatinine was 1.2 mg/dl and the urine output was 30 ml/hr. The serum uric acid was 11.3 mg/dl and serum transaminases and alkaline phosphatase were increased. PF4 level was 400 ng/ml,
the BTG level 390 ng/ml, and both FPA and TIFPB levels were increased. A second sample collected within 1 hr showed almost identical values. Caesarian section was performed, and the platelet count rose to 90,000/μl. The BTG level remained increased at 24 hr postpartum, whereas the PF4 level decreased rapidly and was normal at 24 hr. Renal function progressively deteriorated and hemodialysis was instituted, but the patient died on the fourth postpartum day following cardiac arrest. An autopsy was not performed.

Case 3 (M. B.—Fig. 4)

This 27-yr-old G2P0 was admitted at 34 wk gestation after a generalized seizure, with a blood pressure of 150/110 mm Hg, bleeding gums, peripheral and facial edema, hyperreflexia, and 3+ proteinuria. The platelet count was 74,000/μl, the FDP level was 10 μg/ml, and the serum creatinine was 1.6 mg/dl. Caesarian section was performed, and the following day, the platelet count was 10,000/μl and the hemoglobin was 5.9 g/dl without red cell fragmentation. FPA,
TIFPB, and platelet protein levels were increased initially and increased further as the platelet count fell following delivery. The B1-13 levels paralleled the FPA level, except for a peak of 27 pmole/ml on day 2. At 8 wk postpartum, physical examination and laboratory studies were normal.

Case 4 (M. D.)

This 18-yr-old G2P0 was admitted at 36 wk gestation in labor and had a normal vaginal delivery. Postpartum, her blood pressure increased to 170/110 mm Hg and hyperreflexia and oozing from the gums were present. The FDP level was 82 µg/ml and the platelet count decreased to 59,000/µl. The fibrinopeptide levels were increased postpartum and the platelet protein levels were normal. By 6 days postpartum, physical examination and laboratory studies were normal.

Case 5 (I. E.)

This 21-yr-old G2P0 was admitted at 33 wk gestation with a blood pressure of 160/110 mm Hg, peripheral edema, hyperreflexia, and altered mentation. Cesarian section was performed 2 days after admission. On the second postpartum day, the platelet count fell to 103,000/µl and the FDP level increased to 21 µg/ml. Fibrinopeptide levels were increased from the first to the seventh postpartum day, whereas the platelet protein levels were normal throughout. By the fifth postpartum day, the platelet count and FDP level were normal, and 6 wk later, the blood pressure was normal.

DISCUSSION

In the patients with mild preeclampsia, the mean fibrinopeptide and platelet protein levels prior to
Fig. 3. Clinical and laboratory data in case 2. (A) Platelet counts, fibrinopeptide and platelet protein levels. Platelet (Pits) and FFP infusions are shown by the arrows. (B) Platelet counts, fibrinogen, FDP, and hemoglobin concentrations. Transfusions with packed red cells (RBC) and whole blood (blood) are shown by the arrows.

Fig. 4. Clinical and laboratory data in case 3. (A) Platelet counts, fibrinopeptide and platelet protein levels. Platelet and FFP infusions are shown by the arrows. (B) Platelet counts, fibrinogen, FDP, and hemoglobin concentrations. Transfusions with packed red cells (RBC) are shown by the arrows.
delivery were within our normal range. Douglas et al.\textsuperscript{18} reported increased levels of fibrinopeptide A (9 pmole/ml) and BTG (50 ng/ml) in patients with preeclampsia as compared to mean FPA levels of 1 pmole/ml and BTG levels of 30 ng/ml in the third trimester of normal pregnancy. The differences between the present findings and those of others\textsuperscript{18,19} is thought to reflect differences in diagnostic criteria. More severe disease is associated with renal insufficiency, which could be responsible for the higher BTG levels.\textsuperscript{20-22}

The five patients with severe disease had marked coagulation changes. In general, the clinical and hematologic disturbances correlated in time and in degree. In comparing these changes with those occurring after intraamniotic hypertonic saline infusion, both similarities and differences are evident. The changes occurring after intraamniotic hypertonic saline infusion were interpreted as indicating that intravascular coagulation occurs in stages—an initial stage in which thrombin action predominates, a later stage in which plasmin action predominates, and a still later stage in which FDP levels are increased and both the fibrinogen levels and the platelet counts are decreased, but new proteolysis is not occurring.\textsuperscript{11} In the patients with severe preeclampsia/eclampsia, the FPA level exceeded the TIFPB level initially in case 1, but thereafter, in that case and in the other four cases, the TIFPB level initially was higher, which is consistent with the idea that most cases of intravascular coagulation manifest at a stage when thrombin and plasmin action on fibrinogen are balanced or the latter is predominant. The high TIFPB levels were paralleled by increased FDP levels. The degree to which the fibrinopeptide and FDP levels were increased was similar to that occurring after infusion of hypertonic saline, but the abnormalities were present for up to 7 days, rather than for several hours, indicating that the stimulus to intravascular coagulation is more persistent in preeclampsia/eclampsia. Plasma B1-13 levels are thought to reflect in vivo formation of fibrin II, which may form more persistent thrombi than fibrin I.\textsuperscript{23} As the three cases with increased levels of B1-13 subsequently developed increased serum creatinine levels, with case 2 manifesting renal failure and the highest B1-13 levels, one may speculate that fibrin II formed in the renal blood vessels. This speculation should be tested by analyzing the fibrin in these vessels. In interpreting increased fibrinopeptide and platelet protein levels as indicative of fibrinogen proteolysis and platelet release, the possible effect of renal failure on clearance rates must be considered. BTG\textsuperscript{29-32} and Bβ 1-42 (D. Lane, personal communication) levels are reported to be increased in renal failure. However, the small increase in serum creatinine in our patients is an inadequate explanation for the very high levels observed. FPA\textsuperscript{24} and PF4\textsuperscript{21,22} levels are often normal in severe renal failure, whereas B1-13 levels have not been measured. Clearance rates in normals and in patients with renal failure are necessary to definitively establish that the increased levels reflect increased production.

The PF4 and BTG levels in patients with mild preeclampsia/eclampsia were within the normal range, and their relative concentrations, with the BTG about 4 times the PF4 level, are typical of our experience with well collected blood samples. In contrast, cases 1 and 2, the two patients with the most severe disease, had markedly different results. Faulty venipuncture cannot be absolutely excluded; however, all venipunctures were thought to be clean and blood flow was good. Cases 1 and 2 represent the only patients in whom we have found comparable elevation of PF4 and BTG levels in the absence of heparin therapy or faulty venipuncture. These changes cannot be explained solely on the basis of thrombin-induced platelet release, as the increase in platelet protein levels was disproportionate to the degree of increase in FPA levels. Renal insufficiency does not account for the increased PF4 levels. The platelet transfusion given in case 2 immediately prior to delivery does not explain the increased platelet protein levels on the two blood samples taken before the transfusion. Although renal insufficiency and thrombin-mediated platelet release may have been contributing factors, other mechanisms are required to explain the increase in platelet protein levels in these two patients. In case 1, the abrupt increase in the platelet count without changes in the platelet protein or fibrinopeptide levels may have been due to markedly increased platelet production after a period of suppression or to release of sequestered platelets. In case 2, increased platelet protein release coupled with decreased PF4 clearance is necessary to explain the findings. The increased platelet release could be due to thrombin stimulation of platelets; however, the lack of correlation between the FPA and the platelet protein levels suggests that another mechanism for platelet release is involved. The clearance of PF4 is normally very rapid and is thought to be mediated by binding to vascular endothelium.\textsuperscript{25,26} One possible mechanism for decreasing the clearance of PF4 could be an abnormality of PF4 binding to endothelial cells. An effect of a noxious agent on the endothelial cells lining the vascular tree, particularly the small vessels, could be responsible for the hematologic changes and organ dysfunction occurring in the disease. Thus far, however, there is no direct evidence for endothelial cell injury nor is there evidence that fibrin formation and platelet activation directly contribute to the organ dysfunction.
COAGULATION CHANGES IN PREECLAMPSIA

ACKNOWLEDGMENT

The helpful criticism of Dr. Karen Kaplan and the expert technical assistance of Betty Grossman, Thomas Lim, and Carol Wilkins are gratefully acknowledged. Furthermore, we are grateful for the invaluable help given by the nurses and house officers in the Sloane Hospital for Women. We thank Amy Winter for typing this manuscript.

REFERENCES

Fibrinogen proteolysis and platelet alpha-granule release in preeclampsia/eclampsia

Z Borok, J Weitz, J Owen, M Auerbach and HL Nossel