Serial measurements of circulating tissue plasminogen activator and fibrin(ogen) degradation products predict outcome in gestational proteinuric hypertension

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Abstract **Gestational proteinuric hypertension (GPH), a major cause of maternal death, may be characterised by hypertension and proteinuria alone or may progress to disturbed coagulation and multiorgan failure. Since the condition can only be reversed by termination of pregnancy, there is a need for reliable indicators of severity. We found circulating levels of tissue plasminogen activator (tPA) (27,98 ± 2,12 v. 7,17 ± 0,81 ng/ml, mean ± SEM), fibrin(ogen) degradation products (FDP) (7,55 ± 1,99 v. 1,92 ± 0,47 µg/ml) and fibronectin (221 ± 15,2 v. 120 ± 15,2 µg/ml) to be significantly increased in 21 patients with severe GPH when compared with 21 normotensive, age- and gestational age-matched pregnant controls. More importantly, patients who developed severe GPH showed a progressive increase in tPA and FDP levels with time. This was in contrast to patients who had hypertension and proteinuria alone, in whom tPA and FDP concentrations did not increase. Parallel measurements did not reveal a fall in platelet count or an increase in urinary protein excretion in patients who subsequently progressed to severe disease. Our findings may be of assistance to clinicians faced with the need to prolong pregnancy in patients with GPH in order to ensure fetal viability.**

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Gestational proteinuric hypertension (GPH) is a major cause of maternal death in the Western world.[1] Patients may present with hypertension and proteinuria alone or may develop fulminant disease with disturbed coagulation, vascular injury and multisystem failure.[2,3] The reason for this progression is unclear and there are currently no early clinical or laboratory predictors of severity that are generally acceptable.

We evaluated plasma and serum concentrations of tissue plasminogen activator (tPA) and fibrin(ogen) degradation products (FDP) prospectively in patients with GPH and present data suggesting that the rate of increase of tPA and FDP in the circulation may assist in predicting the outcome in such patients.

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Patients and methods

The study was conducted in two phases. In the first phase, circulating tPA, FDP and fibronectin (Fn) concentrations were measured in 21 consecutive patients with severe progressive (fulminant) GPH and 21 healthy, normotensive pregnant women matched for age, race, gestational age and parity. All control patients remained normotensive throughout pregnancy and the puerperium. In the second phase 22 consecutive patients with GPH admitted to the Groote Schuur Hospital region underwent twice weekly estimations of full blood count, platelet count, 24-hour urinary protein excretion, circulating tPA, FDP and Fn concentrations from the time of presentation to the time of delivery. Eleven of these patients progressed to severe maternal disease, while the remainder were delivered for fetal indications. All patients and control subjects were examined and followed up by one physician (J.A.) who was responsible for defining and recording the outcome without knowledge of the trial data. GPH was considered to be present in any patient with a blood pressure of 140/90 mmHg or more who had a 24-hour urinary protein excretion in excess of 0,3 g. Any patient with a sustained blood pressure greater than or equal to 160/110 mmHg or a platelet count of less than 150×10^9/l or a count that fell by more than 50×10^9/l over a period of 3 days or with organ dysfunction ascribed to GPH was considered to have severe or progressive disease.[4]

The protocol was approved by the Ethics and Research Committee of the University of Cape Town. All patients were fully informed and agreed to have part of routinely collected blood used for this study.

Blood collection and preparation of serum

To limit *in vitro* fibrinolysis, blood samples were collected in cold polystyrene tubes containing 3,8% sodium citrate and 0,025M aminocaproic acid. Plasma was separated within 4 hours of collection, and stored at –70°C until analysis. Serum was prepared by clotting the plasma in a mixture containing thrombin and soybean trypsin inhibitor for 4 hours at 0°C, as recommended by Merskey *et al.*[5]

Laboratory investigations

Fibrin(ogen) degradation products were determined in serum by an enzyme-linked immunoassay (ELISA) developed in our laboratory.[6] Tissue plasminogen activator was measured in plasma using the Imubind-5-tPA ELISA kit (American Diagnostica Inc., Greenwich, Conn., USA).[7] Fn concentrations in serum were measured by means of ELISA with polyclonal rabbit anti-human Fn IgG as capturing antibody, mouse anti-human Fn monoclonal antibody, CJ2, raised in our laboratory, and goat antimouse immunoglobulin conjugated to peroxidase.

Statistical methods

In phase 1 of the study mean values for each group were

compared by means of Student's *t*-test. Non-parametric distribution-free tests were used to confirm the existence of differences between groups. In phase 2, multiple observations allowed parallel linear regressions (different intercepts but common slope and common variance) to detect linear changes in some responses with regard to increasing gestational age.

Results

The concentrations of FDP (mean ± SEM) (7,55 ± 1,99 v. 1,92 ± 0,47 µg/ml; $P = 0{,}008$), tPA (27,98 ± 2,12 v. 7,17 ± 0,81 ng/ml; $P < 0{,}0001$) and Fn (221 ± 15,2 v. 120 ± 15,2 µg/ml; $P < 0{,}0001$) were significantly higher in patients with gestational proteinuric hypertension than in the normotensive control subjects (Table I).

TABLE I.
Concentrations of FDP, tPA and Fn (mean ± SE) in patients with severe GPH and age- and gestational age-matched normotensive pregnant control subjects

	FDP (µg/ml)	tPA (ng/ml)	Fn (µg/ml)
Normotensive control pregnant subjects (21)	1,92 ± 0,47	7,17 ± 0,81	120 ± 15,2
Severe GPH (21)	7,55 ± 1,99*	27,98 ± 2,12*	221 ± 15,2*

* Significantly different from controls; $P < 0{,}005$.

In the sequential study, data were analysed according to gestational age, from admission through to delivery. Control data gave no evidence of any linear regression of serum FDP, Fn or tPA levels with gestational age. A common slope and variance model was fitted within each of the two GPH groups. This fitting revealed a marked relationship between serum FDP concentration and gestational age in patients with severe, progressive GPH. This relationship amounted to a change of 12,38 ± 4,40 µg FDP/ml serum/wk ($P = 0{,}007$). In contrast, there was no relationship between FDP concentration and gestational age in patients who had hypertension and proteinuria alone (0,09 ± 1,07 µg FDP/ml serum/wk; $P = 0{,}935$).

Analysis of the tPA-gestational age relationship revealed similar changes — 6,82 ± 2,17 ng tPA/ml plasma/wk; ($P = 0{,}003$) in severe disease and 1,02 ± 0,53 ng tPA/ml plasma/wk ($P = 0{,}059$) in the patients with hypertension and proteinuria alone.

There was no relationship between serum Fn concentration and gestational age in either group of patient data. Parallel measurements did not reveal a fall in platelet counts or an increase in urinary protein excretion in those patients who subsequently progressed to severe disease.

Discussion

Fulminant GPH is a progressive disorder reversed only by termination of pregnancy. The high maternal morbidity and occasional mortality associated with severe forms of this condition are an absolute indication for immediate delivery. Prolongation of the pregnancy beyond 28 - 32 weeks' gestational age may benefit the fetus and is the goal of management in milder cases where the condition is characterised by hypertension and proteinuria alone. Unfortunately, the course of the disease is often unpredictable and physicians are forced to rely on the degree or rate of increase of hypertension, the amount of proteinuria, the platelet count and tests of renal and hepatic function, all of which are relatively late signs of progression.

Since endothelial damage is thought to occur at an early stage,[8] we have examined markers of such injury in the hope that these may prove to be useful indicators of severity.

The results of phase 1 of this study confirm previous work suggesting that Fn concentrations are significantly higher in pregnancies complicated by GPH.[9] The finding that serum FDP and plasma tPA concentrations are increased lends additional support to the contention that endothelial damage, disordered coagulation and fibrinolysis play a central role in the pathogenesis of this condition.

Of importance is our interpretation of the estimated rates of change of serum FDP and plasma tPA. When FDP rates of change greater than 2,50 µg/ml/wk are sustained in the same individual after several observations, there is an increased probability of progression to severe disease. This increased probability also holds for sustained tPA rates of change of 2,20 ng/ml or more per week. However, these increased probabilities are not so marked as to be in any sense clinically definitive. Similarly, sustained rates of change below the stated FDP and tPA rates will be associated with decreased probability of progression and, in the absence of other indications, suggest that pregnancy may not need to be terminated. Our finding that neither a fall in platelet count nor an increase in degree of proteinuria reliably predict progression to severe disease further emphasises the possible importance of the above conclusions. The observation that both FDP and tPA rates of change are of the requisite order in the same patient is reassuring. Since these criteria represent different components of the same process, the use of both criteria only marginally increases the predictive power of one criterion alone. While the rate of increase of FDP and tPA should clearly not be considered in isolation, we believe that these measurements will provide a valuable additional prognostic index. Our data indicate that GPH is unlikely to progress in patients with static levels of FDP and tPA and suggest that pregnancy be maintained in these patients when fetal viability is at stake.

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