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NEPHROLOGY FORUM

The kidney in preeclampsia

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CASE PRESENTATION

A 27-year-old Asian woman was admitted to the hospital for swelling of her legs over the previous 2 to 3 days. A primigravida, she was 30 weeks pregnant. She had been well and had no prior history of hypertension, diabetes, or renal disease. She saw her physician every month and had had no previous swelling. Her prior blood pressure measurements and urinalyses had been normal. The initial serum creatinine when she was 6 weeks pregnant was 0.6 mg/dL. When she saw her obstetrician the morning of admission, she felt otherwise well. The examination was unremarkable except for a blood pressure of 160/100 mm Hg, which was unchanged 30 minutes later, and significant pretibial edema. Her reflexes were normal. Urinalysis demonstrated 3+ protein without blood, sugar, leukocytes, or nitrate. Laboratory tests revealed a serum creatinine of 0.9 mg/dL, uric acid of 5.0 mg/dL, and normal coagulation studies and liver enzymes. A spot urine protein-to-creatinine ratio was 1.8.

The patient was admitted to the hospital for bed rest. Fetal ultrasound showed the fetus to be without defect; the estimated gestational age was 29 weeks. Serial blood pressure measurements demonstrated a range of 150 to 180/95 to 105 mm Hg. The patient was given labetalol, 100 mg twice daily. Serial non-stress testing showed that the fetus continued to do well, but the patient's blood pressure became difficult to control over the following week. Her blood pressure climbed to 160/100 mm Hg despite maximal doses of labetalol and alpha-methyldopa. Her 24-hour urine protein content had increased to approximately 3 g, and the serum creatinine had risen to 1.1 mg/dL, with a creatinine clearance of 85 mL/min. During her second week in the hospital, the patient was treated with corticosteroids to accelerate fetal lung maturity. She continued to be mildly hypertensive with moderate edema and normoreflexia. A cesarean section was undertaken at the end of the second week, when laboratory studies demonstrated a mild increase in aspartate aminotransferase (AST) and amino alanine transferase (ALT) and her serum creatinine increased to 1.2 mg/dL.

A baby boy weighing 3 lbs, 2 oz was delivered and did reasonably well. He required supplemental oxygenation and was cared for in the neonatal intensive care unit. He was discharged 4 weeks later without significant complication. The patient's swelling abated quickly after delivery. Her blood pressure was normal. Within one week, the serum creatinine was 0.8 mg/dL, and the urine protein-to-creatinine ratio was 0.15. Six months later, she was seen by her primary medical doctor. Her examination was normal: the blood pressure was 115/75 mm Hg, her serum creatinine was 0.8 mg/dL, creatinine clearance was 104 mL/min, and urinary protein excretion was 38 mg/day.

DISCUSSION

DR. RICHARD LAFAYETTE (*Associate Professor of Medicine, Stanford University Medical Center, Clinical Chief, Division of Nephrology, Stanford, California*): Preeclampsia remains a major cause of maternal and fetal morbidity and mortality in the United States and the world. Approximately 1 in 20 pregnancies is complicated by this disorder. Although there are many systemic manifestations of preeclampsia, its clinical hallmarks of hypertension, edema, and proteinuria reflect significant kidney dysfunction. This review aims to detail the

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pathophysiologic changes of the kidney that distinguish preeclampsia from normal pregnancy.

Definition

Preeclampsia has been defined as the onset of hypertension (blood pressure ≥140/90 mm Hg) with proteinuria (urine dipstick ≥2+ or urine protein >300 mg/day) and edema. This clinical triad, occurring in the second half of pregnancy, fulfills the criteria of the American Congress of Obstetrics [1, 2]. It must be distinguished from worsening renal disease during pregnancy (usually by history) and from pregnancy-induced hypertension without proteinuria, another common entity. In women with underlying hypertension, preeclampsia generally is identified by elevation of prevailing blood pressure levels and the development of proteinuria. Women are at increased risk when a first-degree female blood relative has had preeclampsia; if they have high blood pressure, diabetes, kidney disease, or migraine; they are over 35 years old; or they are multigravid [3, 4]. The major clinical risk is progression to eclampsia, an acute seizure disorder, but the disease also can lead to multiorgan failure with catastrophic consequences. Within the kidney, the predominant change occurs in glomerular endothelial cells. There may also be hemodynamic changes. To best understand the latter, let's review the normal renal physiology in pregnancy.

The kidney in normal pregnancy

Pregnancy produces marked physiologic changes throughout the body. Systemic hemodynamics are altered, with dramatic increases in total circulating blood volume, increased cardiac output, and reduced systemic resistance. The net result is a high-output state with a mildly reduced blood pressure [5].

Studies of human pregnancy that have utilized inulin, the most reliable filtration marker, have demonstrated that glomerular filtration rate (GFR) increases progressively during the first half of pregnancy [6–10]. It then reaches a peak level that exceeds nongravid GFR by 40% to 60%, after which it remains constant until the end of pregnancy [9–11]. This level of hyperfiltration extends into the first postpartum day, and hyperfiltration of lesser magnitude (± 20%) persists into the second postpartum week [11, 12].

The GFR can be equated with the product of K_f and the net pressure for ultrafiltration

$$GFR = (\Delta P - \Pi_{GC}) \times K_f$$

where ΔP is the transcapillary hydraulic pressure difference, Π_{GC} is the mean glomerular intracapillary oncotic pressure, and K_f is the glomerular ultrafiltration coefficient, the product of hydraulic permeability (k) and filtration surface area (S) [13]. Determinations of renal plasma flow (RPF), filtration fraction, and arterial oncotic pressure (πA) during peak gestational hyperfiltration in late pregnancy and on postpartum day 1 reveal that Π_{GC} is depressed by between 3.3 and 6.7 mm Hg [10]. In the event that neither ΔP nor K_f is altered by gestation, an equivalent increase in the net filtration pressure for ultrafiltration could account exclusively for the increased level of glomerular filtration. However, other workers have interpreted an alteration in the transglomerular sieving of macromolecules as indicating that, in addition to depression of πGC, an approximate 15% increase in K_f is required to account for the 38% increase in GFR observed by them in late pregnancy [6]. That this might be so is consistent with micropuncture determinations of K_f in the healthy pregnant rat [14]. Because the rats in this study were at filtration pressure equilibrium, unique values of K_f could not be determined, and conclusions regarding K_f could not be drawn. Evidence that K_f elevation might be implicated is provided from a separate, more recent study in which filtration dynamics were compared in gravid versus nongravid rats with a remnant kidney [15]. Because this experimental model is associated with filtration disequilibrium, unique values for K_f could be calculated. The authors reported a greater than 2-fold increase in this measure, which accounted for the hyperfiltration in the pregnant animals, and they suggested that K_f elevation could well be implicated in gestational hyperfiltration.

One mechanism by which K_f might be increased in pregnancy is through glomerular hypertrophy. Glomerular volume has not been determined during normal pregnancy. However, given the demonstration of increased kidney size and volume during pregnancy [16, 17], it is possible that glomeruli hypertrophy and that this change is accompanied by an increase in filtration surface area and hence, K_f. Our own study supports this finding: we noted a glomerular volume of 5.23 ± 2.16 μm/μm^3 $\times 10^6$ in women with preeclampsia, a value 2.3-fold larger than the corresponding value in healthy, nongravid female controls [18]. Our preeclamptic patients also exhibited considerable endocapillary cell proliferation and hyperplasia, more markedly in glomerular endothelial than in mesangial cells. We surmise that the expansion of the endocapillary cell compartment contributed significantly to the glomerular enlargement. Any glomerular hypertrophy attributable to pregnancy alone is thus likely to be of substantially smaller magnitude, such as less than by a factor of 2.0.

That K_f enhancement by a factor of ± 50% could be associated with pregnancy is also suggested by our recent study of healthy mothers on postpartum day 10 [11]. We found that glomerular capillary oncotic pressure had been restored to normal at this time, consequent upon reversal of the pregnancy-mediated hemodilution. Using a mathematical model of glomerular filtration, we computed that an increase of K_f by 50% could account

exclusively for the maintenance of the increased GFR by 20% in postpartum week 2 [11].

It seems reasonable to conclude that elevation of ultrafiltration pressure due to a low glomerular capillary oncotic pressure is the predominant cause of gestational hyperfiltration. An additional contribution by K_f owing to gestational-induced hypertrophy of glomeruli seems likely, but this remains to be proven. At present, there is no way to estimate ΔP in the human kidney. If anything, however, one would speculate that relative hypotension during pregnancy could be transmitted into the glomerular capillary, thereby lowering ΔP, and that this change would lower, not elevate, the GFR.

Preeclampsia

Preeclampsia is characterized by gestational hypertension, a phenomenon that has been attributed to an excess of vasoconstrictor over vasodilator influences in the systemic circulation [19–21]. A parallel imbalance in the renal circulation would be expected to lower the glomerular perfusion rate. Another characteristic of preeclampsia is abnormal glomerular morphology [22, 23], which could lower the intrinsic ultrafiltration capacity of glomerular capillaries. Limited glomerular ultrafiltration capacity on its own, or more particularly, in combination with glomerular underperfusion, results in depression of the GFR. Similar to the observations of others during late pregnancy, the GFR in our gravid control subjects was elevated above normal non-gravid levels by approximately 50%, averaging 149 ± 34 mL/min/1.73m^2 [8, 12, 18]. When compared to this high value, the corresponding GFR in patients with preeclampsia was significantly depressed, averaging only 91 ± 25 mL/min/1.73m^2. Of note, the corresponding levels of serum creatinine in the control and preeclampsia groups were 0.60 ± 0.10 and 0.83 ± 0.22 mg/dL, respectively. Because of the hyperbolic relationship between this quantity and GFR, the highest serum creatinine level in the preeclampsia group was only 1.2 mg/dL, despite simultaneous depression of corresponding GFR to 44 mL/min/1.73m^2 [18].

Unlike the GFR, renal plasma flow in gravid controls was not elevated above normal non-gravid levels in our study, averaging 624 ± 108 mL/min/1.73m^2 [18]. The corresponding renal plasma flow in the preeclampsia group was similar, averaging 648 ± 257 mL/min/1.73m^2 (P = NS). The selective decline of GFR resulted in marked depression of the filtration fraction. This latter finding indicates that determinants of GFR other than renal plasma flow must have been altered to explain the observed level of hypofiltration.

A consistently reported finding of hypoalbuminemia in pregnancy has been attributed to a combination of hypervolemia (hemodilution) and altered protein metabolism [24–26]. We found serum albumin concentration to be markedly depressed in women with normal pregnancy and with preeclampsia, slightly more so in preeclampsia, 1.87 ± 0.20 versus 1.95 ± 0.22 g/dL, respectively [18]. In fact, measured oncotic pressure is low in preeclampsia, resulting in a depression in πGC. This phenomenon is likely related to the significant proteinuria that defines the disease. However, as πGC is the force opposing the formation of filtrate, the trend toward depression of this quantity should have increased ultrafiltration pressure and hence, increased GFR [13]. It follows that depression of either the glomerular transcapillary hydraulic pressure difference and/or the glomerular ultrafiltration coefficient (Kf) must be invoked to explain the hypofiltration observed in preeclampsia.

It seems doubtful that ΔP depression generally could make a major contribution to the observed hypofiltration in preeclampsia, however. One reason for doubt is that mean arterial pressure in preeclampisa is usually quite elevated. Despite the mild trend to higher renovascular resistance in preeclampsia, it is difficult to imagine a selective increase in preglomerular segmental resistance of sufficient magnitude to prevent some fraction of the excess in arterial pressure from being transmitted into glomerular capillaries.

Our own morphometric analysis of glomeruli suggests that an important and perhaps predominant reason for the hypofiltration in preeclampsia is impaired hydraulic permeability of glomerular capillary walls, owing mainly to reductions in endothelial cell fenestral density and size, and to the accumulation beneath the endothelial monolayer of fibrinoid deposits.

Examination of the preeclamptic kidney by light microscopy reveals the typical prominence of endocapillary (mesangial and endothelial) cells. This pattern has been well described by others for more than 80 years but had not previously been directly linked to renal dysfunction [22, 27]. Our study [18] disclosed conspicuous endothelial cellularity in most cases, evidenced by up to 3 endothelial cell nuclei in some capillary lumens. Definite mesangial hypercellularity was difficult to discern. We also found a prominent infiltration of macrophages and a lesser number of lymphocytes and polymorphonuclear leukocytes within capillary lumens in 4 of the 11 patients. The hypertrophy of endothelial cells alone, or in combination with leukocyte infiltrates, resulted in obvious loss of capillary patency in all cases. Foamy macrophages were found in 2 of the patients. We also noted variable thickening of glomerular capillary walls, which was related to mesangial interposition in all cases, and prominent subendothelial hyaline deposits in one. An additional finding was the presence of early focal segmental glomerular sclerosis in 4 of 7 of the women, affecting 19 ± 11% of glomeruli, on average. Transmission electron microscopy confirmed the finding of endothelial cell hyperplasia, and revealed exudation of foamy macrophages, lymphocytes,

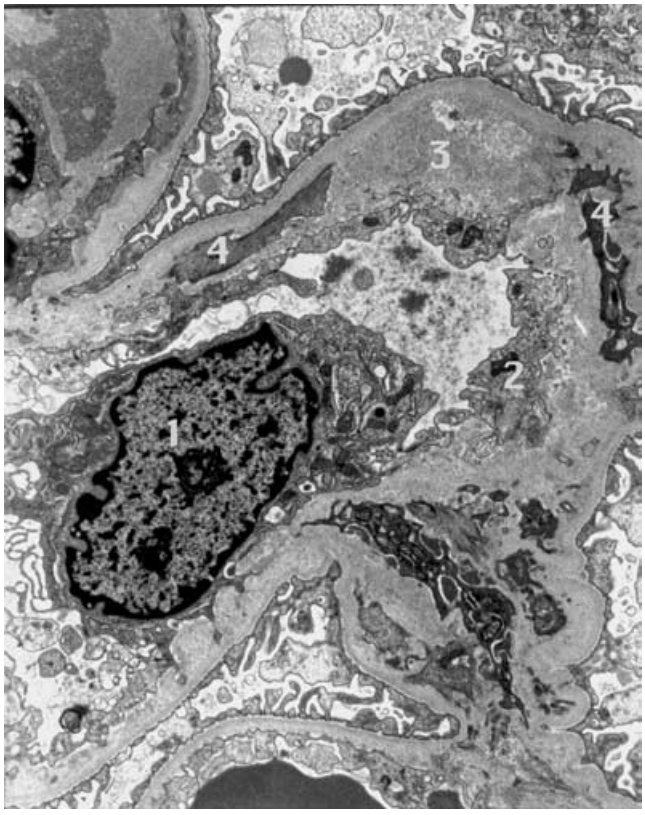

Fig. 1. Transmission electron microscopy of a representative glomerular capillary enumerating pathologic changes associated with preeclampsia.

and polymorphonuclear leukocytes within the capillary lumina and mesangium. In addition, capillary wall abnormalities were apparent (Figs. 1 and 2). These included hypertrophied endothelial cells, swollen segments of endothelial cytoplasmic rim in which fenestrae were not discernible, subendothelial fibrinoid and granular deposits, and interposition of mesangial cells.

Glomerular volume in preeclampsia was significantly larger than in controls, 5.23 ± 2.16 versus 3.29 ± 1.19 $\mu m/\mu m^3 \times 10^6$, respectively ($P < 0.05$) [18]. We defined "effective" filtration surface as that part of the peripheral GBM that was apposed to the fenestrated endothelial cytoplasmic rim. The remaining part of the peripheral GBM that was apposed to endothelial cell bodies or interposed mesangial cells (numbers 1 and 4, respectively, of Fig. 2) was excluded on the grounds that transcellular filtration is negligible. This latter component, which comprises "ineffective" filtration surface density, was increased by a factor of 3 in preeclampsia versus controls (0.024 ± 0.001 versus 0.008 ± 0.001, $\mu m^2/\mu m^3$, $P = 0.001$) mostly as a consequence of interposed mesangial cells. As a result, effective filtration surface density (Sv) was reduced in preeclampsia versus controls, averaging 0.055 ± 0.002 versus 0.100 ± 0.013, $\mu m^2/\mu m^3$ ($P < 0.001$). However, a trend to larger glomerular volume in preeclampsia offset the reduction in Sv. As a result, actual filtration surface area in preeclampsia was reduced below control by only 10%.

We did find, however, extensive dense deposits of fibrinoid material in a subendothelial location [18]. These deposits produced a proportionately greater thickening of the entire filtration pathway, from fenestral interface to slit diaphragm in preeclampsia. Scanning and transmission electroscopy demonstrated that fenestral density was lower in women with preeclampsia than in controls. A smaller area-to-perimeter ratio also attested to a reduction in endothelial fenestral size in preeclampsia. Together, reduced fenestral density and size in preeclampsia resulted in a smaller fraction of GBM occupied by fenestrae than in controls. This phenomenon is clearly visible by inspection of the digitized tracings that are taken from a control (Fig. 3, left) and a representative subject with preeclampsia (Fig. 3, right).

We calculated that overall k in preeclampsia was reduced below control by 30%. In contrast, effective filtration surface area (S) was only slightly reduced. From the product of S and k, we compute that SNK_f in controls averaged 6.78 nL/(min×mm Hg).The corresponding value in preeclampsia was substantially lower, 4.26 to 4.67 nL/(min×mm Hg). We found a 37% reduction in estimated SNKf; this change is remarkably similar to the corresponding reduction of 39% in observed GFR in this disorder. The above rationale suggests that K_f depression is indeed the predominant cause of hypofiltration in preeclampsia, and that it is unnecessary to invoke a substantial role for simultaneous depression of ΔP or RPF in these patients.

Pathogenesis of the renal injury

Unraveling the complex injury of preeclampsia has been an elusive task [28]. The disorder appears to have immunologic, genetic, and biochemical components that have made the identification of a common, mediating pathogenetic factor difficult. To date, no single gene mutation has been strongly associated with the disorder despite a fairly strong familial tendency [29]. Because of the prominent findings of endothelial injury with vasoconstriction and clotting activation, a number of hormonal, neurogenic, and other circulating factors have been implicated in this process (Table 1).

Unfortunately, no specific, successful therapy has been discovered for preeclampsia. To the contrary, allegedly promising therapies, such as aspirin and supplemental calcium, have proved of little or no value [30, 31].

Earlier research focused on prostaglandins, and researchers sought an imbalance leading to a deficiency of vasodilatory, antithrombotic prostanoids relative to vasoconstrictor and prothrombotic prostanoids [32]. While some data favor a possible imbalance in prostanoid tone, treatment with aspirin has proved of questionable value,

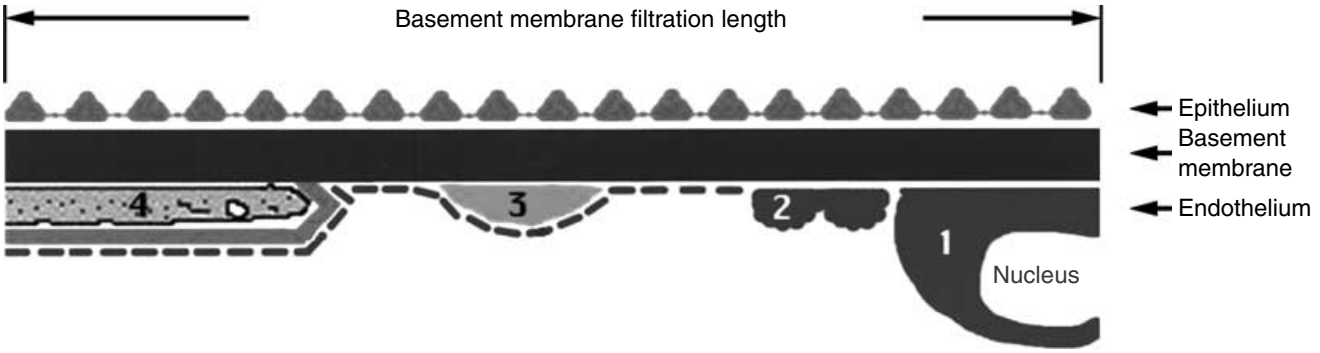

Fig. 2. Ultrastructural changes in preeclampsia seen in Figure 1. 1, endothelial cell body; 2, swollen, non-fenestrated endothelium; 3, subendothelial fibrinoid deposition; 4, mesangial cell interposition.

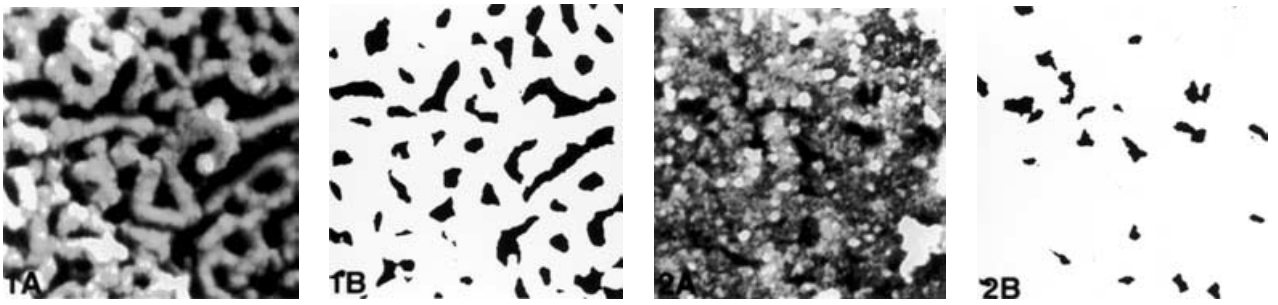

Fig. 3. Scanning electron microscopy and digitized tracings of fenestrae in control (1A and 1B) and in subject with preeclampsia (2A and 2B).

and other potentially causative factors were subsequently evaluated [32]. Recently, a major effort was made to link disorders in the nitric oxide (NO) system with the clinical features of preeclampsia [33]. Indeed, up-regulation of the NO system has been reported in normal pregnancy and is believed to contribute to the hyperperfusion and hyperfiltration, as well as to the systemic hemodynamic changes of normal pregnancy [33]. Preliminary evidence suggested down-regulation of nitric oxide synthase in the placenta and in blood and decreased activity of its second messenger, cyclic GMP [34]. The findings are conflicting, however, and failure of L-arginine to up-regulate the NO system or influence glomerular injury in a recent trial suggests that the NO system might not have a major role (personal data, presented at the 36th annual meeting of the American Society of Nephrology, 2005).

Efforts at understanding preeclampsia are underpinned by the knowledge that abnormalities in the placenta are universal in preeclampsia. Placental insufficiency, defined by inadequate development of uterine spiral arteries into high-capacity vessels to feed the placenta, is present in patients with preeclampsia [35]. Delivery of the fetus rapidly resolves the clinical syndrome. Thus efforts to understand placental insufficiency might unravel the pathophysiology of preeclampsia. Notable are studies by Fisher et al demonstrating basic defects in trophoblast function [36, 37]. The normal process of placental development requires invasion of trophoblasts into the uterine vasculature, with loss of differentiation from an epithelial to an endothelial phenotype. This differentiation, which appears to be significantly defective in women with preeclampsia, can lead to inadequate oxygen delivery to the placenta and the release of mediators of endothelial injury. In turn, the hypoxic, injured placenta may cause many downstream events, such as the release of inflammatory cytokines or the development of angiotensin II receptor autoantibodies, which can propagate endothelial injury [38, 39]. This sequence of events also might be

Table 1. Pathogenetic processes implicated in preeclampsia

Increased activity of the sympathetic nervous system
Increased responsiveness to angiotensin II
Hypocalciuria
Defects in atrial natriuretic peptide (ANP)
Increased angiotensin II agonistic autoantibodies
Failure to increase placental-derived vascular growth factors (PlGF) due to soluble inhibitors
Increased endothelin
Decreased activity of the nitric oxide system
Platelet activation through changes in clotting factors and platelet-related molecules, such as antithrombin III, vWF, platelet-activating factor
Increased ratio of thromboxane-to-prostacyclin
Defects in adrenocorticoid regulation
Increases in inflammatory cytokines
Placental vascular insufficiency
Insufficient differentiation of trophoblasts to endothelial cells
Other circulating toxins, including uric acid, parathyroid hormone, and "trophotoxins"
Reduced levels of relaxin

associated with the recent finding that normal pregnancy is associated with high levels of a placental-derived vascular growth factor (PlGF), similar to vascular endothelial growth factor, VEGF [40]. It appears that many women with preeclampsia, although not all, have high levels of a circulating inhibitor of this hormone (a soluble receptor, sFlt) [41]. The resultant deficiency of vascular growth factor activity might contribute to the hypertension, proteinuria, and renal injury that defines preeclampsia.

Still, great uncertainty remains. What is the primary change in preeclampsia? Which of the described abnormalities are downstream changes caused by the initial injury? Ongoing and future trials might better determine the pathogenesis of this disorder and lead to specific therapy. Currently, supportive care with control of blood pressure and prophylaxis against seizures is the only therapy shy of delivery of the child, as in this case.

QUESTIONS AND ANSWERS

DR. JOHN T. HARRINGTON (*Dean Emeritus, Tufts-New England Medical Center, Boston, Massachusetts*): Are the lesions elsewhere in the body in preeclampsia comparable to those in the kidney?

DR. LAFAYETTE: Data are limited, but there are some necropsy data from patients who have died from preeclampsia and its complications [42]. We originally hypothesized that the predominant pathology in preeclampsia was thrombotic microangiopathy. It appears that diffuse endothelial dysfunction causes platelet aggregation that leads to ischemic and necrotic changes. Broad evidence for this in these autopsy series demonstrates vascular injury in multiple organs, including the brain, liver, and lungs. Clearly, it is not solely the kidney that suffers from this systemic disease. However, renal damage might be due to the intimacy of the glomerular circulation with renal function, thus rendering renal issues more easily seen.

DR. HARRINGTON: Is the higher capillary pressure in the glomerulus, as compared to non-renal capillaries, the cause of the more severe involvement of the kidney?

DR. LAFAYETTE: I don't know whether the kidney is more susceptible than other organs to the endothelial injury of preeclampsia, but it certainly is the classic organ involvement described. Whether or not it is the uniquely high capillary pressure in the glomerulus that leads to organ specificity is of interest, but I have no data supporting that notion. We do know that our cardiology colleagues blame turbulent flow and shear stress for some of the endothelial injury that occurs in coronary and carotid vessels in patients with atherosclerosis. Although it's an attractive hypothesis, it would require further investigation.

DR. HARRINGTON: What is the cause of the increased fluid intake and polyuria in pregnant women? The fact that these women are hyponatremic and volume expanded would argue that thirst must have another stimulus.

DR. LAFAYETTE: I don't know. Basically, the theory is that the volume expansion and hyperfiltration of pregnancy produce active glomerulotubular balance and lead to a diuresis. However, because of the physiologic adjustment in neuronal and hormonal tone in pregnancy, the expanded volume is maintained and gradually increased throughout pregnancy by net sodium retention. The mechanism of the increased thirst has not been well studied. It is clear that angiotensin II levels, a major stimulus for thirst, are increased in pregnancy, but there is resistance to their pressor effect [43], so it is unclear whether angiotension II effectively stimulates thirst. Also, antidiuretic hormone (ADH) is reset in pregnancy, allowing for hyponatremia, and also becoming more responsive to any reduction in volume.

DR. JANE TAN (*Assistant Professor of Medicine, Stanford University*): What is remarkable to me about preeclampsia is its rapid reversibility, a characteristic rarely seen in other glomerular diseases. For example, after delivery, the patient's blood pressure, GFR, and urinary protein pretty much returned to baseline conditions. The representative renal biopsy in preeclampsia that you showed earlier looked horrendous (Fig. 1), with its severe swollen basement membrane and mesangial interposition. In most other glomerular diseases, that degree of histologic damage would result in significant residual impairment even after treatment. Are there any data on renal histology following the resolution of preeclampsia? In other words, is there complete ultrastructural resolution? And if there is, what are the leading theories on how this great repair occurs?

DR. LAFAYETTE: I would point out that we are obviously selecting some best examples of the morphologic changes. Remember these are women with, on average, very mild renal dysfunction, and the renal injury is not as severe as that which we see in our typical patient with hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura (TTP). Even patients with severe acute renal failure from thrombotic microangiopathy sometimes recover. There is something about those kinds of injuries, which include swelling, edema, and inflammation that certainly should be reversible. How the kidney clears the deposition of the fibrinous material and early scar formation is harder to know, but more and more proteolytic mechanisms in the kidney have been described [44]. As the mesangial cells can invade the peripheral capillary loop under certain influences, they should be able to retract again. As an example, in mesangial proliferative diseases, in some patients, the proliferation resolves during recovery. Essentially, glomerular diseases that don't reach the level of scarring, and perhaps even some of those that are resolved early, probably can be fully reversed. Obviously, underlying the reversibility is

the idea that whatever caused the endothelial injury disappears with delivery of the baby and the placenta, and this change allows repair mechanisms to occur. Kincaid-Smith et al have done serial biopsy studies of preeclamptic patients in recovery and have readily demonstrated that these processes reverse themselves [23]. In order, first the swelling goes away, then interposition goes away, and it takes a little bit longer, not unexpectedly, for the fibrinous material to disappear. It can take months for that to fully disappear, despite the fact that the GFR improves and proteinuria resolves rapidly. One interesting point is that in Japanese biopsy studies [45] and even in our studies [18], many preeclamptic women have sclerotic injuries that look like focal segmental sclerosis. Even those patients tend to get completely better clinically and have normal GFR and protein excretion. No registry has documented what happens to a preeclamptic patient 20 to 30 years later, but most of these women successfully maintain excellent renal function, and even though there is an increased risk of them developing high blood pressure, it is not clear whether that is because they lose some nephrons during this illness or because hereditary or other factors predispose them to hypertension in the first place.

DR. JEFFREY PETERSEN (*Professor of Medicine, Stanford University*): Are there any known effects of the circulating inhibitory factors that you are describing in the studies out of the Beth Israel reported by Maynard et al [40, 41]?

DR. LAFAYETTE: Other than their direct effect on reducing the activity of VEGF and PlGF by binding them, I haven't seen anything written about them. What I would point out from these studies is that they've utilized in vitro techniques to show that these factors can inhibit vascular growth. Further, in vivo they can create an animal model that looks a lot like preeclampsia by infusing these soluble inhibitors. The animals become hypertensive and develop proteinuria, and they develop a glomerular pattern that looks similar to the human endotheliosis. Taken together with the demonstration of increased levels of these angiogenic inhibitors in preeclamptic women, it lends a lot of credence to the theory that this contributes to the development and severity of preeclampsia. In humans, it is also interesting that VEGF inhibitors in clinical practice for treatment of cancer patients have been associated with the development of hypertension and proteinuria. To date, there are no biopsy studies of these patients, but this association lends further strength to the hypothesis that there is a chronic dependence of the glomerulus on endothelial growth factors. If, indeed, endothelial growth factors are inhibited in preeclampsia, this inhibition might contribute to the renal injury.

DR. TIMOTHY MEYER (*Professor of Medicine, Stanford University*): My question is related to Dr. Harrington's. It is thought that VEGF is produced in the podocytes and causes the endothelial cells of the glomerulus to be fenestrated. The inhibitor therefore might remove the tonic VEGF effect, which is necessary to preserve normal glomerular endothelial structure. Do you see endothelial pathology in the kidney outside the glomerulus when you perform biopsies?

DR. LAFAYETTE: Generally, morphologic changes outside the glomerulus have not been investigated. I am sure you are aware that work suggested that VEGF has important effects in the peritubular capillaries allowing for fluid and electrolyte flux in the distal tubule [46]. As we had focused our attention on glomerular dynamics, we did not carefully examine the interstitium. However, there doesn't tend to be upstream arteriolar damage, and we haven't seen obvious evidence in peritubular capillaries of congestion or of injury. There isn't a lot of tubular damage and so, in terms of other non-glomerular endothelium within the kidney, we did not notice anything obvious in terms of fibrin thrombi, even in the larger vessels. But we did not look systematically.

DR. MAURICE DRUZIN (*Professor of Obstetrics, Stanford University*): The renal lesion is a unique component of this disease. Patients with chronic renal disease of any type, whether it is vasculitis, chronic hypertension, or any condition that has renal involvement, have an almost universal incidence of developing preeclampsia or superimposed preeclampsia. There is definitely something special about the kidney that seems to be the focus of endothelial cell damage.

DR. LAFAYETTE: I think there are two parts to that. The injured kidney is vulnerable in pregnancy because of the physiologic changes that occur, and probably because of unsuccessful adaptation to hyperfiltration. It is often very difficult to know whether the underlying kidney disease occurs because of hemodynamic changes or other hormonal changes, or whether the patient is really developing preeclampsia. If you have a patient with an elevated baseline serum creatinine level, proteinuria, and hypertension, and who becomes more hypertensive and proteinuric during her pregnancy, is that preeclampsia, or is it that the renal disease has worsened? Nephrologists would very infrequently biopsy a patient like this, because we don't have any special therapy in either case. So, it is interesting to know whether renal insufficiency or renal disease itself would amplify the response to pregnancy that causes preeclampsia, or whether we're just seeing another consequence of pregnancy in an abnormal, unhealthy kidney.

DR. DRUZIN: There is also an increased risk among women with very mild kidney disease or even simple diabetes or hypertension.

DR. LAFAYETTE: Yes, and one would wonder whether this is because those underlying problems have already initiated a very early pattern of endothelial dysfunction that predisposes these women to preeclampsia.

DR. HARRINGTON: You mentioned the genetics of preeclampsia and some familial risks. Do we know anything about specific genes or gene clusters using microarrays that we have these days to investigate that?

DR. LAFAYETTE: There has been a long-term interest in the genetics of preeclampsia, because there is familial clustering in preeclampsia. If you have a first-degree relative who has had preeclampsia, your risk increases substantially. Investigators have looked for genetic patterns in these affected families, searching for genes that underlie hormonal abnormalities, hypertension abnormalities, renin-angiotensin system changes, as well as clotting system abnormalities. But there is no support for a monogenic abnormality underlying the disease. Some links to HLA types have been noted, but these links have not proven to be predictive of disease. So investigators moved to a genomic approach to see what genes are differentially expressed in preeclampsia. In fact, that is how they found the defect in the regulation of placental growth factor. Further data from Boston's Beth Israel Hospital have followed up from clues found from gene chip technology and genomics [47]. Researchers are looking at other factors, such as genes that regulate insulin resistance, because there has been a separate clinical correlate that women with preeclampsia seem to be more prone to insulin resistance [48]. This, of course, is another state of endothelial dysfunction, and some genetic correlates have been noted, at least in terms of RNA and protein production, that reflect insulin resistance. But in terms of trying to lay the burden for this disease on a single gene defect, investigators have not been successful.

DR. TAN: What about the flip side? You've elaborated on the genetic variances in pregnant women that could render some more susceptible to preeclampsia. Is there any evidence of increased susceptibility to preeclampsia with specific fetus and mother pairs? In other words, are there examples of a change in the risk of preeclampsia after a woman changes partners?

DR. LAFAYETTE: Among the many theories was a very strong belief that an immune response to the placental-fetal unit leads to a cytokine-fueled inflammatory reaction that then produces endothelial damage. The main evidence for this is that there is an inflammatory milieu in preeclampsia with elevated levels of many cytokines. Furthermore, epidemiologic evidence indicates that women who have never had preeclampsia and then change partners have a similar risk of preeclampsia as primigravidas. Also, some women who have recurrent preeclampsia and change partners have normal pregnancies [49]. This suggested that immune determinants passed to the fetus from the father were involved in the pathogenesis. Furthermore, epidemiologic evidence indicates that women who change partners and who have never had preeclampsia might get preeclampsia. Some women who had preeclampsia and changed partners didn't get preeclampsia again, and that was the level of evidence. We do not have much data about the role of ABO or HLA incompatibility and the risk for pregnancy complications. Recently, a large, long-term epidemiologic trial examined the effect of changing partners on the incidence of preeclampsia, and suggested that other factors involved in subsequent pregnancies determined the outcome rather than the changing of the male partner [50]. Thus, this immunologic concept is more in doubt. Still, many laboratories are looking at cytokines in preeclampsia, and looking at cytokine inhibitory strategies. Most of the data suggest that a lot of cytokine and hormonal changes are very much downstream events, and while you might have some ability to change some of the clinical features of the disease by manipulating the response, you are unlikely to prevent it. We desperately need to have a better understanding of the upstream events. Even for the disturbances of angiogenesis, it is unclear how much of the changes are cause or consequence. We await clinical trials of endothelial growth factors to assess their impact on this disorder.

DR. IVONA BERSKA (*Community pediatrician*): Does the preeclampsia increase in women who have subsequent pregnancies?

DR. LAFAYETTE: I will ask Dr. Druzin to help me with this. My understanding has been that once you have preeclampsia, your risk of disease in subsequent pregnancies is very much increased. The relative risk increases substantially, I believe 4- to 5-fold. The severity of preeclampsia, however, seems to be consistent with what it was before, and it doesn't tend to accelerate to the point that you can predict increasing severity in subsequent pregnancies. Thus, it does not appear to be an issue of "sensitization" to pregnancy with conditioning from prior pregnancies. Instead, if you have numerous risk factors, such as hypertension, diabetes, renal disease, then your risk for severity does increase.

DR. DRUZIN: On the question of some type of genetic or immunologic basis, we used to think that if a woman has preeclampsia during her first pregnancy, she is protected subsequently. In fact, mild preeclampsia, which is late-onset disease, is probably a different disease than what we are looking at, which is severe and early preeclampsia. The presentation of mild preeclampsia is very different compared to the severe form of the disease. There is no growth restriction of the fetus, there is clearly not a lot of placental disease, and all you see is some hypertension and proteinuria. The earlier and more severe disease, which is usually defined as occurring at gestational ages of less than 34 weeks, has a distinctly different presentation compared to mild preeclampsia. In patients with previous mild preeclampsia, the recurrence risk is 2% to 5% with the same partner. If the patient has a new partner, it used to be thought that the risk reverts to that of a first pregnancy, which is doubled at 6% to 8%. The Norwegian

epidemiologic study did not find this to be true after following patients for a prolonged period [50]. With severe preeclampsia at 34 weeks or less, HELLP syndrome, or eclampsia, the recurrence risk is between 20% and 25% of developing repetitive severe disease, and about 40% to 50% of developing some type of hypertensive disorder of pregnancy [51]. This is the current state-of-the-art thinking. The renal biopsies of mild and severe disease are similar, with severe disease having more pronounced histologic changes. However, in both disease states, the kidney almost always returns to normal. It is unclear why there is such a large difference in clinical presentation and recurrence between mild and severe disease.

DR. HARRINGTON: Is there any evidence that the fetus itself might contribute to the toxins released that produce preeclampsia, or is it the assumption that virtually all the evil humors come from the placenta?

DR. LAFAYETTE: I would have to say there is no great evidence that the fetus directly contributes. Some observations suggest that when the fetus is unwell or growing poorly, it produces signals that initiate or worsen preeclampsia [52]. Analysis of the different fetus type in terms of HLA type, gender, or developmental stage has not revealed evidence implicating the fetus, per se. It is more likely that the placental-uterine component determines disease, as suggested by the fact that molar pregnancies can cause preeclampsia in the absence of a fetus [53].

DR. DRUZIN: In terms of fetal contribution to this disease, may we discuss the thrombophilias, both inherited and acquired? Inherited thrombophilias such as Factor V Leiden, prothrombin gene mutation, and hyperhomocysteinemia are risk factors for preeclampsia and placental insufficiency. In our preliminary studies, we showed that, in fact, mother/fetus pairs expressing the same gene disorder have a higher prevalence of severe disease [54]. That has not really held up in larger studies, but different outcomes used in different studies have led to controversy concerning the contribution of thrombophilias in these patients. If mother and fetus both have long-chain fatty acid deficiency, acute fatty liver of pregnancy is very common. This disease presents in a very similar fashion to HELLP syndrome and preeclampsia and can be difficult to diagnose. There is therefore evidence of a fetal contribution to this clinical entity. The mechanism is unclear, but the placenta is a fetal organ attached to the pregnant patient. We do not have a complete understanding of this interface at the present time.

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