PAPERS AND ORIGINALS

Immunofluorescent Studies in Renal Biopsies in Pre-eclampsia

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Summary

Renal biopsies were performed on 11 patients considered clinically and histologically to have pre-eclampsia. Immunofluorescent studies with fluorescein-labelled anti-IgG, IgA, IgM, and IgE complement, albumin, fibrin, and fibrinogen were carried out on the tissue obtained. Significant correlation was obtained between the clinical severity of the disease and the density and pattern of IgM and IgG deposition. Complement was found in glomeruli in severe cases, while complement deposition in the walls of afferent and efferent arterioles was a constant finding. These findings support the concept that an immunological mechanism may be responsible for the renal lesions in pre-eclampsia. If immunity does play a part in the pathogenesis of pre-eclampsia, possible mechanisms include the involvement of histocompatibility antigens and cross-reactivity of fetal and maternal tissues. Renal fibrin deposition in pre-eclampsia may be secondary to an immune process, an occurrence well-described in other forms of glomerulonephritis in man and other species.

Introduction

The light- and electron-microscopical changes occurring in the glomerulus in pre-eclampsia have been well described (Pollak *et al.*, 1956; Spargo *et al.*, 1959; Pollak and Nettles, 1960; Thomson *et al.*, 1972). Immunofluorescent studies have shown

fibrin deposition within glomeruli, but have failed to show significant antibody or complement deposition (Vassalli *et al.*, 1963; Morris *et al.*, 1964). Partly on this basis there has been little support for the hypothesis that an immunological mechanism may be responsible for the renal lesion of pre-eclampsia.

The present study was undertaken to re-investigate the possibility that glomerular immunoglobulin and complement deposition occurs in pre-eclampsia.

Patients and Methods

PRE-ECLAMPSIA GROUP

Renal biopsies were performed on 11 patients considered clinically to have pre-eclampsia. A diagnosis of pre-eclampsia was made when persistent proteinuria and raised blood pressure occurred in previously normotensive patients after 20 completed weeks of pregnancy. Patients who developed pre-eclampsia in conjunction with either a urinary tract infection, vaginal discharge, underlying renal disease, or essential hypertension were excluded from the study.

Three patients were biopsied antepartum, seven within 72 hours of delivery, and one patient seven days postpartum. Follow-up biopsies were performed three months postpartum in four of the cases studied.

RENAL DISEASE GROUP

Renal biopsies were also performed on three pregnant women in whom a clinical diagnosis of essential hypertension (case 12), medullary sponge kidney (case 13), and idiopathic nephrotic syndrome (case 14) was made before or during pregnancy. Histological studies confirmed the clinical diagnosis in case 12, while in case 13 the patient proved to have chronic pyelonephritis. The other woman (case 14) was diagnosed histologically to have minimal lesion glomerulonephritis.

Antepartum biopsies were performed in cases 1 and 2 for the purpose of diagnosis and management. Both patients developed severe hypertension and proteinuria in the second trimester before fetal viability, necessitating accurate diagnosis before termination of pregnancy was carried out. In case 11 the patient

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had persistent proteinuria and oedema from 31 weeks; however, she was only mildly hypertensive. In view of the atypical presentation antepartum biopsy was performed at 34 weeks and this enabled a diagnosis of glomerulonephritis to be made histologically. Renal function was not deteriorating, and conservative therapy was maintained until 37 weeks.

In case 13 the patient had antepartum biopsy performed at nine weeks' gestation while being evaluated for termination of pregnancy. Case 14 presented with gross oedema, proteinuria, and hypoalbuminaemia. For the purpose of diagnosis, implementation of therapy, and to exclude superadded pre-eclampsia, renal biopsy was performed at 35 weeks gestation.

A Franklin-modified Vim-Silverman needle was used and, where possible, patients were biopsied under direct x-ray fluoroscopic control with minimal radiation exposure. Shielding was used in antepartum patients to protect lower abdominal areas. All patients were volunteers to whom the nature of the procedure was fully explained. Two biopsy core specimens were apportioned for histological, electron-microscopical, and immunofluorescent antibody studies.

Tissue for immunofluorescent study was snap-frozen on dry ice, embedded in gelatine, and stored at -20°C until examined, usually within a few days. Sections 4 µm in thickness were cut using a cryostat, fixed in acetone for 10 minutes, and stained with fluorescein-labelled anti-IgG, IgA, IgM, and IgE complements (β₁c, β₂a globulin), albumin, fibrin, and fibrinogen. The sections were then incubated at 37°C for 30 minutes, washed with Coon's buffer, reincubated for a further 30 minutes, re-washed, dried, and finally mounted. A Leitz fluorescent microscope was used to view the slides. Fluorescein-conjugated antisera were obtained from Hyland laboratories and checked for specificity by immunoelectrophoresis and by "blocking" studies.

Tissue taken for electron microscopy was fixed in 2% phosphate-buffered glutaraldehyde and postfixed in 1% osmium tetroxide in phosphate buffer. Ultra-thin sections were stained in uranyl acetate and lead citrate and examined with an AEI 801 electron microscope.

Results

Clinical, histological, and immunofluorescent antibody studies. are correlated in the table.

Of the 11 cases diagnosed clinically as pre-eclamptic nine showed histological changes compatible with pre-eclampsia.

The most prominent features were endothelial cell swelling, narrowing of capillary lumina, proliferation of mesangial matrix, increase in mesangial cells, and the presence of basement membrane deposits. The most common electron microscopical features of pre-eclampsia observed were endothelial swelling and proliferation of mesangial basement membrane-like material (fig. 1). Cases 10 and 11 showed proliferative mesangial changes more suggestive of glomerulonephritis than pre-eclampsia. These latter cases had persistent proteinuria three months after delivery, whereas the pre-eclamptic group were protein-free. In the three patients known to have renal disease but who did not develop pre-eclampsia (cases 12 to 14), histological diagnoses were

FIG. 1—Endothelial swelling (A) and proliferation of mesangial basement membrane-like material (arrowed) typical of pre-eclampsia. (Electron micrograph. × 2,133.)

Correlation of Clinical, Histological, and Immunofluorescent Antibody Studies in the Two Groups of Patients

Clinical Findings						Histological and Electron Microscopical Findings	Immunofluorescent Staining Pattern					
Case No.	Parity	B.P.	Proteinuria	Biopsy Time	Clinical Diagnosis		IgG	IgA	IgM	IgE	Complement	Fibrin
Pre-eclampsia Group												
1	0	160/105	+++	A.P. 28 weeks	Pre-eclampsia	Severe pre-eclampsia	++	0	+++	0	+ Glom.	+++
2	0	150/105	+++	A.P. 24 weeks	Pre-eclampsia	Severe pre-eclampsia	+	0	+++	0	+ Art.	+++
3	0	150/100	++	P.P. 7 days	Pre-eclampsia	Moderate pre-eclampsia	+	0	++	0	+ Glom.	+
4	0	155/105	++	P.P. 1 day	Pre-eclampsia	Moderate pre-eclampsia	+ Art.	0	0	0	+ + Art.	+
5	0	140/100	++	P.P. 1 day	Pre-eclampsia	Moderate pre-eclampsia	++	0	+++	0	+ Art.	+++
											+ Art.	
											+ + Art.	
6	0	145/100	+	P.P. 1½ days	Pre-eclampsia	Moderate pre-eclampsia	+	0	++	0	+ Art.	++
7	0	140/95	+	P.P. 3 days	Pre-eclampsia	Mild pre-eclampsia	-	0	+	0	+ + Art.	+
8	3	145/90	+	P.P. 2 days	Pre-eclampsia	Mild pre-eclampsia	-	0	+	0	+ + Art.	+
9	0	150/95	+	P.P. 2 days	Pre-eclampsia	Mild pre-eclampsia	+	0	-	0	+ + Art.	+
10	3	130/90	+++	P.P. 3 days	Pre-eclampsia	Glomerulonephritis	±	0	0	0	0	+
11	0	125/90	++	A.P. 34 weeks	Pre-eclampsia	Glomerulonephritis	±	0	0	0	0	+
Primary Renal Disease Group												
12	1	160/110	+	P.P. 5 days	Essential hypertension	Essential hypertension	0	0	0	0	0	0
13	0	120/80	+++	A.P. 4 weeks	Medullary sponge kidney	Chronic pyelonephritis	±	0	±	0	0	0
14	0	130/80	+++	A.P. 35 weeks	Idiopathic nephrotic syndrome	Minimal lesion glomerulonephritis	0	0	0	0	0	0

A.P. = Antepartum. P.P. = Postpartum. Glom. = Glomerular. Art. = Arteriol.

essential hypertension, chronic pyelonephritis, and minimal lesion glomerulonephritis. The immunofluorescent findings in these three patients without pre-eclampsia are included for comparison of results.

In all the biopsies three to four glomeruli were identified for immunofluorescent antibody examination. Fluorescent staining in the areas of immunoglobulin complement or fibrin deposition was graded from 0 to 3 plus. Clinical and histological severity of pre-eclampsia showed good correlation with the density and pattern of immunoglobulin deposition. In the histologically severe cases (1 and 2) quite dense IgM deposition and less dense IgG deposition were found. Patchy areas of fluorescence were seen on capillary loops and also within mesangial areas (figs. 2 and 3). The deposits on capillary loops were probably within endothelial cells. In a similar manner fibrin and fibrinogen were densely deposited in mesangial areas and in capillary loops. Only slight complement deposition was observed within capillary loops and in mesangial areas. However, complement was very strongly deposited within the walls of afferent and efferent arterioles (fig. 4).

FIG. 2—Glomerular IgM deposition in pre-eclampsia, mesangial deposits, and capillary loop deposits. ($\times 24$.)

FIG. 3—Single glomerulus showing dense mesangial deposits and less dense loop deposits of IgG. ($\times 61$.)

In the four cases (3, 4, 5, and 6) classified as moderate the density and presence of IgM deposition was variable, being found in three cases and not in one. In only two of these cases was IgG found. Once again, fibrin deposition comparable to the

density of IgM deposited was seen within mesangial areas and on capillary loops. Prominent complement deposition within afferent and efferent arterioles was again noted as well as arteriolar IgG in one case.

In the three cases classified as mild (7, 8, and 9) mild IgM deposition was seen in two, while afferent and efferent arteriolar complement was again noted in all three cases. In no case was IgA, IgE, or albumin found.

FIG. 4—Complement deposition in pre-eclampsia within afferent and efferent arterioles. ($\times 36$.)

In both cases classified as mild proliferative glomerulonephritis only slight mesangial fibrin deposition was observed. In one of these minimal IgG and IgM deposition was seen. However, complement was not seen in either case. In the three patients known to have underlying renal disease deposition of immunoglobulin, complement, and fibrin could not be found.

Repeat biopsies performed three months postpartum in four cases of pre-eclampsia failed to show immunoglobulin, complement, or fibrin deposition.

Discussion

Results of this study indicate that in pre-eclampsia not only fibrin but also immunoglobulin and complement are deposited in the glomeruli. IgM deposition was usually observed with a lesser amount of IgG and no IgA or IgE. The distribution was seen mainly as patchy deposits on capillary loops probably within endothelial cells, but also within the mesangium. The degree of IgG and IgM deposition was proportional to the severity of the renal histological changes. Complement deposition was found particularly within afferent and efferent arterioles, but in severe cases was also found within the glomerulus. The degree of complement deposition within arterioles did not correlate with the clinical or histological severity of the disease. The presence of complement in glomerular arterioles was a constant finding and has not been described previously in pre-eclampsia to our knowledge. Fibrin deposition was observed especially within mesangial areas but was also seen within capillary loops where its density correlated with the severity of the disease process. The lack of linear immunoglobulin deposition suggests that the lesion is not due to antglomerular basement membrane disease.

These findings are in contrast to those in studies by Vassalli *et al.* (1963) and Morris *et al.* (1964) in which constant antibody or complement deposition within glomeruli in patients with pre-eclampsia was not seen. The discrepancy between the present study and previous published work is difficult to explain. One possible factor may be the type of antisera used. In

the studies quoted the antiserum produced was active against total gammaglobulin. It is possible that this antiserum had predominant specificity for IgG, with relatively little activity against IgM.

It is unlikely that the present findings were due to trapping of immunoglobulin by fibrin because in other renal diseases where fibrin deposition is abundant immunoglobulin deposition is not a constant feature (Churg *et al.*, 1969). The possibility that immunoglobulin deposition was secondary to non-specific fixation of these plasma proteins in damaged glomeruli cannot be excluded, and complement deposition without immunoglobulins in arteries and arterioles has been described in hypertension. However, the constant finding of IgM, IgG, and complement deposition together suggests that it may well be due to an antigen antibody reaction in the glomerulus and its vascular supply. In addition the disappearance of all deposits in the follow-up biopsy suggests reversal of an immunological process.

Numerous theories have been suggested to explain the aetiology of pre-eclampsia. However, the pathogenesis of this syndrome remains unknown. Environmental causes (Brewer, 1966), familial factors (Chesley *et al.*, 1968), and placental ischaemia initiating a uterorenal vascular reflex (Sophian, 1972) are thought to play a part. Alterations in the renin-angiotensin system occurring in early pregnancy (Gordon *et al.*, 1969) have also been implicated. However, in other studies renin activity was found to be within the normal range for pregnancy in pre-eclamptic patients (Brown *et al.*, 1966). Low grade disseminated intravascular coagulation occurs in pre-eclampsia (McKay *et al.*, 1953, 1964; McKay, 1965; McCluskey 1971; Bowman *et al.*, 1971; Howie *et al.*, 1971). It has been suggested that secondary to placental separation or damage, thromboplastin is released into the maternal circulation initiating disseminated intravascular coagulation (Brown and Stalken, 1969). Histological evidence of placental damage is, however, not a constant feature of this syndrome. A primary vascular disorder associated with damage to endothelium would explain the occurrence of disseminated intravascular coagulation, fibrin deposition, and the secondary microangiopathic haemolytic anaemia known to occur in severe pre-eclampsia (Brain *et al.*, 1967).

Vascular changes occurring in vessels apart from the kidney have been described in pre-eclampsia (Hertig, 1945; Zeek and Assali, 1950; Dixon and Robertson, 1961).

These studies have shown that a characteristic morphological change described as an "acute atherosclerosis" occurs in the basal arteries of the maternal placental bed. The main features of this lesion are a fibrinoid necrosis of the vessel wall and infiltration by lipophages and mononuclear cells.

Because of the similarity between the morphological changes in placental bed vessels and those found in vessels of rejected transplanted kidneys, Robertson and his co-workers (1967) suggested that the maternal vascular lesion may be secondary to a fetus-mediated rejection phenomenon.

In acute renal transplant rejection deposition of immunoglobulin in glomeruli and arterioles has been described, and it is also known that acute allograft rejection involves the humoral antibody system (Busch *et al.*, 1970).

From several viewpoints renal allograft rejection may be compared to failure of the fetus to tolerate maternal tissue. Breakdown of the protective mechanism operating at the placental level could result in damage to maternal tissues and, in particular, to deposition of complexes containing transplantation antigens in the glomerulus. The passage of fetal white blood cells across the placental barrier stimulates the formation of HL-A antibodies in the mother (Terasaki *et al.*, 1970). Swinburne (1971) put forward the hypothesis that the antigen-antibody complexes formed may be responsible for fibrin deposition between placental villi, and in placental vessels, and maternal glomeruli. Further evidence in support of an immunological basis for pre-eclampsia has come from several other studies. Seegal and Loeb (1946) suggested that the aetiology of

pre-eclampsia may include the formation of anti-placental antibodies, while Steblay (1962) found that antibodies produced against human placenta are active against human glomerular-basement membrane. Shirai and Sims (1970), in an unpublished study, found that the injection of anti-kidney gammaglobulin into pregnant rats could produce renal lesions resembling those of pre-eclampsia, especially endothelial cell swelling and narrowing of the lumen of capillary loops, while in the non-pregnant animals a typical Masugi nephritis resulted. Anti-placental antibodies were found in 40% of 406 normal pregnant women, while antibodies against liver and kidney antigens were present in 24%, with no increase in titre occurring in successive pregnancies. A significant increase in these percentages was observed in patients with pre-eclampsia (Wilner *et al.*, 1964).

The above evidence suggests that immunological processes may play a part in pre-eclampsia. The finding of immunoglobulin and complement in glomeruli in the present study lends some support to this concept, though this finding by itself does not necessarily indicate that an immunological mechanism is responsible for the glomerular disease.

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