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Glomerular endotheliosis in normal pregnancy and pre-eclampsia

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Objective To investigate the proportion of women with findings characteristic for pre-eclampsia, as opposed to renal disease, in a controlled study of hypertensive pregnant women undergoing antepartum renal biopsy.

Design An observational prospective controlled study.

Setting University Hospital of Lund, Sweden.

Sample Thirty-six previously healthy women with hypertensive disease in pregnancy, consecutively admitted to the antenatal ward at onset of disease during a 20 month period and giving informed consent, as well as 12 voluntary healthy pregnant controls.

Methods Renal biopsy samples were obtained from all participants and evaluated by light microscopy, electron microscopy and immunofluorescence techniques.

Main outcome measures Presence and degree of glomerular endotheliosis.

Results Glomerular endotheliosis was present in all women with pre-eclampsia and gestational hypertension, and in 5 of the 12 controls, although significant differences in the degree of endotheliosis were found between the groups. Clinically undetected renal disease was not diagnosed in any of the women.

Conclusion Glomerular endotheliosis was found in women with normal pregnancy as well as in both non-proteinuric and proteinuric hypertension and is consequently not, as earlier believed, pathognomonic for pre-eclampsia. The transition between normal term pregnancy, gestational hypertension and pre-eclampsia appears to be a continuous process, perhaps of increasing adaptation to pregnancy. Pre-eclampsia may be the extreme of the adaptational process, rather than a separate abnormal condition. Clinically undetected renal disease could be a rare cause of hypertension in pregnancy.

INTRODUCTION

Glomerular endotheliosis is a characteristic lesion found in renal biopsies from pre-eclamptic women, and has been considered pathognomonic for the condition[1,2]. It comprises endothelial cell swelling, obliteration of endothelial fenestrae and encroachment of the capillary space area. Pre-eclampsia, in turn, is a serious complication in pregnancy associated with significant fetal and maternal morbidity and mortality. Correlations between the presence of proteinuria[3] as well as the degree of hyperuricaemia[4] and increased perinatal mortality have been reported. However, hypertension and proteinuria, being the cardinal signs of pre-eclampsia, may be present in other conditions such as clinically undetected underlying renal disease or nephrosclerosis, which has been reported in 20–40% of pregnant women with hypertension and proteinuria[1,5].

The aim of this prospective study was to investigate the proportion of women with hypertensive disease in pregnancy, with and without proteinuria, having the characteristic morphologic findings of pre-eclampsia, as opposed to other renal disease. For this purpose, renal biopsy material was obtained antepartum in unselected women close in time to the onset of symptoms of hypertensive disease. The morphological changes in renal tissue in women with hypertensive disease were compared with those of healthy pregnant women, who served as controls.

This work has previously been abstracted in the *American Journal of Obstetrics and Gynecology* in association with its presentation at the Annual Meeting of the Society of Maternal Fetal Medicine in New Orleans, January 2002.

METHODS

The study was performed during a 20 month period from November 1999 to June 2001 at the University Hospital in Lund. The inclusion criteria required participants to be

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healthy prior to pregnancy with no history of essential hypertension, diabetes or known renal dysfunction. Additionally, a diastolic blood pressure measuring no more than 105 mmHg and a blood platelet count of no less than 100×10^9/L were requirements at the time of biopsy, although not necessarily when recruited. The control material came from healthy pregnant women recruited during the study period from maternal health care centres in the catchment area of the hospital.

Gestational hypertension was defined as a diastolic blood pressure >90 mmHg on two or more consecutive occasions >4 hours apart arising after 20 weeks of gestation. Pre-eclampsia was defined as gestational hypertension with proteinuria >300 mg/L in two random clean-catch midstream urine specimens collected 4 hours or more apart. All pregnancies had been dated according to routine ultrasonographic measurement of the fetal biparietal diameter and femur length in the 17–18th week of gestation. Blood coagulation parameters (plasma activated partial thromboplastin time, plasma prothrombin complex, blood platelets and blood haemoglobin) were confirmed as normal within 4 hours prior to biopsy. A blood compatibility test was performed on all enrolled women as well as a dipstick test of the urine to ensure absence of bacteriuria.

One experienced radiologist, Dr Julian Willner, performed all biopsies according to a standard procedure. The sample was taken with a 1.2 mm (outside diameter) needle using a Bard biopsy device ('biopsy-pistol') under ultrasound guidance. Two biopsy samples were taken from each woman. If the quantity of cortex material was considered insufficient, which was the case on two occasions, a third biopsy was taken. Each biopsy, approximately 20 mm in length, was immediately submerged in 0.9% sodium chloride solution and processed and prepared at the Department of Pathology the University Hospital of Lund for further transport to the Department of Pathology at Herlev Hospital, University of Copenhagen, Denmark.

Biopsies were divided into three parts: one was fixed in neutral formaldehyde 4% for light microscopy, the second was fixed in 2.5% glutaraldehyde for electron microscopy and the last was snap frozen in Tissuetech for immunofluorescence, according to standard procedures. The light microscopy evaluation was performed by Professor Steen Olsen and the electron microscopy evaluation by Dr Alistair Hansen without knowledge of the clinical condition.

Each specimen for light microscopy was cut into 2 μm sections and stained with PAS-haematoxylin, haematoxylin eosin, trichrome Masson, Congo red for amyloid and silver methenamin. Changes in tubuli, interstitial tissue, vessels as well as glomeruli were registered. The degree of endotheliosis was evaluated using a semiquantitative scale: 0 (*no endotheliosis*), 1 (*<20% of the lumina obliterated*), 2 (*20–80% of the lumina obliterated*), 3 (*>80% of the lumina obliterated*). Semiquantitative grading was based upon a general evaluation of all glomeruli in a silver methenamin stained section. Thickening of arteriolar walls was also evaluated semiquantitatively with the scale: 0 (*no thickening*), 1 (*moderate thickening*), 2 (*marked thickening*).

The presence and localisation of IgA, IgG, IgM, properdine, C1q, C3, C4 and fibrin was registered. A semiquantitative scale was used: (+) (*not discernible in photographic imaging*), + (*scarce presence*), ++ (*moderate presence*), +++ (*rich presence*).

One glomerulus in each biopsy was examined by transmission electron microscopy, and the occurrence and localisation of dense depositions, mesangial cell interposition around capillary loops and endotheliosis was registered. The degree of endotheliosis was semiquantified: 0 (*no swelling of endothelial cells with preserved fenestration*), 1 (*at least one capillary loop was normal*) and 2 (*all capillary loops showed endothelial swelling*).

Blood pressure was monitored and a fetal heart trace was recorded after each biopsy. Only minor discomfort followed the procedure except in three women, who complained of loin pain. Simple analgesics such as paracetamol or diclofenac sufficed to alleviate the pain. One woman with early onset pre-eclampsia and the most pronounced glomerular lesion developed a 13×6 cm retroperitoneal haematoma below the right kidney, requiring blood transfusion. The woman was delivered by emergency caesarean section due to clinical signs of placental abruption, with full recovery of her twins and a complete remission of the haematoma two months after the delivery.

All participants were thoroughly informed concerning the biopsy procedure and the risk of complications, such as renal haematoma or haematuria, and agreed to receive blood transfusion if this should prove necessary. Informed consent was received from all participating women. The study was approved by the Ethics committee at the University of Lund. The healthy pregnant women at term who volunteered to participate in this investigation were compensated economically solely for one working day and for possible pain and discomfort experienced during the procedure. They displayed a great willingness to contribute, many having themselves had close friends affected by pre-eclampsia. The inclusion of controls in this study was believed to be necessary to establish whether glomerular endotheliosis indeed is pathognomonic for pre-eclampsia or not. If not, it would be unethical to continue to use renal biopsy for diagnostic purposes, whether for research or in the clinical situation, on precisely the women with the highest risk of complications.

RESULTS

During the 20 month period of the study, 54 eligible patients were admitted to the antenatal ward while one of the two involved obstetricians was on duty and the

Table 1. Characteristics and laboratory values, highest value at inclusion. Data are presented as *n*, mean [standard deviation], mean (range) or as median (range) for urinary albumin.

	Healthy controls ($n = 12$)	Hypertensive women ($n = 36$)	
		Non proteinuric hypertension ($n = 8$)	Proteinuric hypertension ($n = 28$)
Primigravidae (*n*)	5	7	19
Maternal age (years)	28 (21–38)	29 (21–35)	30 (22–42)
Gestational age (days)	240 (201–268)	243 (179–275)	237 (191–265)
Biopsy to delivery time (days)	41 (8–68)	6 (1–16)	7 (0–34)
Systolic blood pressure (mmHg)	114 [11]	144 [11]	150 [12]
Diastolic blood pressure (mmHg)	69 [7]	97 [5]	101 [8]
Urinary albumin (mg/L)	<300 (dipstick)	145 (49–259)	910 (316–12,112)

radiologist was available. Twelve women declined to participate in the study for fear of pain or complications, and six patients failed to meet the additional inclusion criteria relating to diastolic blood pressure and platelet count. Thirty-six women with hypertension in pregnancy were recruited. The 12 healthy women, who served as controls, had uneventful pregnancies and deliveries and none developed subsequent signs of hypertension during the pregnancy. In the 36 women with hypertension, the onset of disease occurred in the third trimester in all cases but one, where onset occurred at 179 days of gestation. Eight women had hypertension without proteinuria at the time of the biopsy. Twenty-eight women were classified as having pre-eclampsia with significant proteinuria, three of these in twin pregnancies. The relevant clinical data are given in Table 1.

One woman with pre-eclampsia had a mild form of cystic kidney disease noticeable at the ultrasound examination preceding the renal biopsy. One woman had remaining proteinuria four months postpartum and a re-biopsy was then performed, showing moderate mesangial cell proliferation. No immunofluorescence and no deposits in electron microscopy were present, however, and no sign of renal disease could be seen in the antepartum biopsy. The findings were therefore interpreted as a healing stage of the renal lesion. Proteinuria, although persisting nine months postpartum, resolved within a year.

Endotheliosis was present in women with pre-eclampsia, in women with gestational hypertension without pre-eclampsia and in 7 of the 12 controls. In four controls, endotheliosis was demonstrated either by light microscopy or by electron microscopy, but not by both techniques.

Light microscopy

Hypertensive patients ($n = 35$). One biopsy was excluded from evaluation due to lack of glomeruli. The average number of glomeruli in the remaining biopsies was 12 (range 2–27). Endotheliosis was present in all patients with proteinuric hypertension and all except one had this lesion to a severe or moderate degree (Table 2). Usually all glomeruli in a section showed endotheliosis but the severity varied from one glomerulus to another. All patients with non-proteinuric hypertension had endotheliosis, but to a milder degree. The endothelial cytoplasm was often finely vacuolated, with a gradual transition towards true foam cells, which were found in some of the biopsies with severe endotheliosis.

Fine PAS positive deposits in or between the endothelial cells were occasionally observed, but they were sometimes lacking even in glomeruli with marked endotheliosis. PAS positive hyaline globules or small droplets, usually in the podocytes, were also occasional findings. Peripheral mesangial interposition, seen as double contours of the peripheral capillary walls in silver stained sections, was present in some glomerular sections, but usually affecting only a few of the capillaries in a glomerulus. Mesangial proliferation and matrix expansion were not features of the glomerular lesion and were present only in the one re-biopsy.

Table 2. Light microscopy; degree of endotheliosis. Fischer's exact test reveals significant differences between women with proteinuric hypertension and controls ($P < 0.00001$), women with non-proteinuric hypertension and controls ($P = 0.02$) and between the two hypertensive groups ($P = 0.01$).

Semiquantified score	Healthy controls ($n = 12$)	Hypertensive women ($n = 35$)	
		Non-proteinuric hypertension ($n = 8$)	Proteinuric hypertension ($n = 27$)
0	7	0	0
1	4	4	1
2	1	2	12
3	0	2	14

Table 3. Electron microscopy; degree of endotheliosis. Fischer's exact test reveals significant differences between women with proteinuric hypertension and controls ($P < 0.00001$), women with non-proteinuric hypertension and controls ($P = 0.035$), but not between the two hypertensive groups ($P = 0.067$).

Semiquantified score	Healthy controls ($n = 12$)	Hypertensive women ($n = 33$)	
		Non-proteinuric hypertension ($n = 8$)	Proteinuric hypertension ($n = 25$)
0	7	1	0
1	5	4	6
2	0	3	19

The histological picture was markedly uniform in all biopsies. There were no lesions suggesting primary glomerulonephritis. Focal segmental glomerulosclerosis or hyalinosis was not seen in any biopsy. Global glomerular sclerosis was rare or absent, without any difference between the groups. A semiquantitative analysis showed significantly thickened arteriolar walls in hypertensive patients compared with controls (Fischer's exact test, $P < 0.001$). There was no hyperplasia of the juxtaglomerular cells.

Controls ($n = 12$). Glomeruli, averaging 11 (range 4–23) in each biopsy, showed endotheliosis in five of the women. The degree was slight or moderate (Table 2).

Immunofluorescence

Hypertensive patients ($n = 32$). Sufficient glomeruli for evaluation were obtained in 32 specimens. Scarce presence of IgM, IgA and C3 was found in few glomeruli along the capillary loops and in the mesangium, but never in all glomeruli belonging to one biopsy. In one case only was IgA nephritis suspected initially, due to IgA deposition in mesangial areas of all the glomeruli. The electron microscopic evaluation did not reveal deposits in the mesangium, however, and follow up was normal.

Controls ($n = 12$) did not show any fluorescence.

Electron microscopy

Hypertensive patients ($n = 33$) revealed endotheliosis in all biopsies but one (Table 3). Similarly, mesangial cell interposition, with double contours and electron dense depositions (primarily subendothelial), was demonstrated in hypertension both with and without proteinuria.

Controls ($n = 12$) showed endotheliosis in five cases (Table 3) but only to a slight degree. Mesangial cell interposition and electron dense depositions were not seen.

DISCUSSION

This study is the first of its kind where full histopathological evaluation of renal biopsies was systematically performed, during a limited time period, in women with hypertensive disease in pregnancy at the time of diagnosis, and in healthy pregnant controls for comparison. The investigation showed endotheliosis to be present not only in pre-eclampsia, but also in gestational hypertension without proteinuria, and similarly in normal healthy pregnancies. Electron dense deposits and mesangial cell interposition were found only in hypertensive women, who also presented a more pronounced degree of endotheliosis. Finally, clinically undetected renal disease was not diagnosed in any of the women.

Earlier renal biopsy studies have avoided the use of healthy pregnant controls, except for occasional specimens, which were evaluated as normal by light microscopy alone[6]. During normal pregnancy, the renal blood flow and the glomerular filtration rate increases by at least 40%[7–9] and the kidney volume increases by up to 30%[10], which suggests that physiological renal morphologic changes might be expected. Autopsy material of kidneys from non-pregnant women has been used as controls, with obvious problems of interpretation. In many investigations, renal biopsies have been deferred to the postpartum period, when a pre-eclamptic lesion may already have resolved or findings may be determined by the healing stage of the lesion. Regression of some glomerular lesions in pre-eclampsia can be seen as early as the first week postpartum[11–15] and complete resolution has been reported within four weeks[12]. Renal function is usually restored within only a few days after delivery.

In the past, glomerular endotheliosis has been regarded as pathognomonic for pre-eclampsia, and has been used to confirm the diagnosis, both in the clinical context and in research. As endotheliosis could be found in both gestational hypertension and in normal healthy pregnant controls, the mere presence of glomerular endotheliosis cannot verify the diagnosis of pre-eclampsia as a distinct abnormality. Rather, women with endotheliosis in gestational hypertension and normal pregnancy could be seen as the 'missing link' in the supposed continuum between normal pregnancy and pre-eclampsia[16]. Thus, pre-eclampsia, as such, would constitute the extreme of maternal reactions to pregnancy.

Endotheliosis was found in only a mild or moderate degree in normal pregnancies, and because the phenomenon varied from glomerulus to glomerulus, it is not surprising that the semiquantitative assessments by light

microscopy differed from those by electron microscopy. Other general histopathological findings in women with hypertensive pregnancy were similar to the typical findings described in pre-eclampsia in previous studies[1,6,12,17–22].

Previous renal biopsy studies have shown the diagnosis of pre-eclampsia by clinical criteria alone to be uncertain. Investigators from Chicago[1,23] found biopsy-proven glomerular capillary endotheliosis in only 84% of nulliparae with a clinical diagnosis of pre-eclampsia, and in only 38% of multiparae. Unsuspected nephrosclerosis, renal disease or both was found in 24% of nulliparae and 65% of multiparae. Consequently, in multiparae the clinical diagnosis of pre-eclampsia is less accurate[5,6]. It has therefore been suggested that recurrent hypertension with proteinuria in multiparae represents an early manifestation of chronic renal disease, rather than pre-eclampsia. No cases of clinically undetected renal disease were found in primiparae or in multiparae in our study, both groups displaying the same morphologic changes characteristic for pre-eclampsia. Thus, undetected renal disease seems to be a rare cause of hypertensive disease during pregnancy in our population. The explanation could be improved health among fertile women as well as early detection of chronic renal disease in fertile women, before the childbearing period, together with better prenatal counselling.

The necessity and the safety of renal biopsy during pregnancy have been widely debated[5,24,25]. Strict indications, such as sudden renal failure or the nephrotic syndrome before the final two months of pregnancy, have been recommended[26]. The rate of complications, such as retroperitoneal or perirenal haematoma and haematuria[27], has not been considered to be more common in pregnancy than in the non-pregnant state[5,6,24]. Modern techniques, with thinner needles, and the use of biopsy devices with ultrasound guidance, in the hands of highly experienced physicians, have reduced the risks considerably. In this study, the healthiest women experienced very little discomfort from the procedure, whereas serious complications arose in the most severe case of pre-eclampsia. Whether the subsequent placental abruption was related to the complications of the procedure or to the patient's disease cannot be surmised, but recommendations to avoid renal biopsy in cases of severe pre-eclampsia seem appropriate. Renal biopsy on clinical indications should in pregnancy be reserved for cases where the outcome could affect patient management, as in suspected progressive glomerulonephritis with sudden renal failure, or the nephrotic syndrome early in pregnancy, when delivery cannot be safely performed.

In conclusion, underlying renal disease was not found to be a cause of hypertension in pregnancy in our population. Glomerular endotheliosis is found in women with normal pregnancy and non-proteinuric gestational hypertension as well as in pre-eclampsia and is consequently not, as earlier believed, pathognomonic for pre-eclampsia.

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