Pre-eclampsia

Current Perspectives on Management

Edited by
Philip N. Baker
John C. P. Kingdom

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PRE-ECLAMPSIA

Dedication

To my son Tom (PB)

To Hannah, Emily and Theo (JK)

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Edited by

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and

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Preface

Pre-eclampsia remains a massive cause of perinatal and maternal morbidity and mortality. Recent estimates suggest that 50–100000 women die from the condition each year, and that pre-eclampsia is responsible for approximately 300000 perinatal deaths. There are many more mothers and babies who suffer significant morbidity consequent upon the disease.

After decades when there was minimal progress in our understanding and management of the condition—such that Zwiefel's quote of pre-eclampsia as 'the disease of theories', remained pertinent—there have been a plethora of recent advances. These advances relate to increased understanding of the etiology and pathogenesis of the disease, and lead to both hope and expectation of translation into improvements in prevention and treatment.

This book provides a multifaceted update on the complex syndrome of pre-eclampsia—and we hope that it will be of value to all those involved in caring for pregnant women.

Philip N.Baker
John C.P.Kingdom

Definition and classification

R. Hayman and J. Myers

Pre-eclampsia is much more than just 'pregnancy-induced hypertension'. The extremely variable clinical presentations reflect the complexity of the underlying pathology and it is likely that pre-eclampsia is not a single entity, but the final common pathway that reflects the maternal response to a pathological pregnancy.

Pre-eclampsia is a syndrome, and attempts at definition use arbitrarily selected markers that may not reflect changes of pathophysiological importance. Several classification schemes have been published using different diagnostic criteria. However, the classification system originally devised by Davey and MacGillivray is the most commonly used, and is summarized below[1].

CLASSIFICATION OF THE HYPERTENSIVE DISORDERS OF PREGNANCY

A Gestational hypertension and/or proteinuria

Hypertension and/or proteinuria developing during pregnancy or labor, or in the puerperium, in a previously normotensive, non-proteinuric woman is subdivided into:

(1) Gestational hypertension (without proteinuria);

(2) Gestational proteinuria (without hypertension);

(3) Pre-eclampsia (gestational proteinuric hypertension).

B Gestational hypertension and chronic renal disease

Hypertension and/or proteinuria in pregnancy in a woman with chronic hypertension or chronic renal disease diagnosed before, during or after pregnancy is subdivided into:

(1) Chronic hypertension (without proteinuria);
(2) Chronic renal disease (proteinuria with or without hypertension);
(3) Chronic hypertension with superimposed pre-eclampsia.

C Unclassified hypertension and proteinuria

This is hypertension and/or proteinuria which is found either:

(1) At first examination after the 20th week of pregnancy in a woman without chronic hypertension or renal disease; or
(2) During pregnancy or labor, or in the puerperium, where information is insufficient to permit classification.

PRE-ECLAMPSIA

For the purposes of research, strict diagnostic criteria need to be adhered to, as overdiagnosis can weaken research studies. In clinical practice, the purpose of defining pre-eclampsia must be to allow identification of a group of patients at risk of potentially serious maternal and fetal complications. The clinical definition, therefore, should be as safe as is practicable and is likely to include a number of false positives.

The accepted working definitions are pregnancy-induced/gestational hypertension: new hypertension with a blood pressure of 140/90 mmHg on two separate occasions, arising *de novo* after the 20th week of pregnancy. This group will not necessarily develop pre-eclampsia but warrant increased antenatal surveillance. Second, pre-eclampsia: proteinuria (≥300 mg over 24 h or ++ on two voided urine samples) in addition to pregnancy-induced/gestational hypertension.

Pregnancy-induced/gestational hypertension not associated with proteinuria, *but in the presence of* abnormal hematological or biochemical markers or associated symptomatology, is *also likely* to be pre-eclampsia[2]. For an appropriate diagnosis of pre-eclampsia to be confirmed, there must be evidence that the woman was normotensive prior to the 20th week of pregnancy and that the hypertension and proteinuria has resolved by 6 weeks postpartum.

The third group of women includes those with chronic/pre-existing hypertension and or renal disease: hypertension with or without proteinuria in a patient with pre-existing disease diagnosed prior to, during or after pregnancy.

Pre-eclampsia is a syndrome, a specific collection of signs and symptoms, a disease unique to human pregnancy. The definitions detailed above are only some possible manifestations of a complex pathophysiology, and many other clinical end-points can occur. Consequently there are a multitude of ways in which patients with pre-eclampsia can present. Although some women present with general malaise, abdominal pain or convulsions, the vast majority of patients with pre-eclampsia are asymptomatic, and any clinician caring for such women must possess a high index of suspicion when the 'pattern' does not quite seem to fit the classical appearance.

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2

Pre-eclampsia: a historical perspective

S. Ong

THE RECOGNITION OF ECLAMPSIA/PRE-ECLAMPSIA

For obvious reasons, the first descriptions of pre-eclampsia relate to the advanced stages of this illness: eclampsia. Although eclampsia is dramatic, it is perhaps not surprising that there are so few references to it in older manuscripts, as the ancient writings covered the whole field of medicine. Furthermore, obstetrics was largely in the hands of midwives, and eclampsia had not been differentiated from epilepsy[1]. It is notable that relatively modern textbooks of obstetrics such as those by Burton and Exton make no mention of convulsions in pregnancy[2,3], and it is only in the later editions of books by Mauriceau that convulsions are mentioned[4].

One of the first possible references to eclampsia comes from ancient Egypt. Bernhart quoted the writings from the *Kahun* (Petrie) papyrus dating from about 2200 BC[5]. His source appears to be from Menascha, who in turn had quoted Griffith. Griffith had translated Prescription no. 33 on the third page of the papyrus, which was subsequently edited in German by Menascha as: 'To prevent a woman from biting her tongue auit pound…upon her jaws the day of birth. It is a cure of biting excellent truly millions of times.' Menascha suggested that the word 'auit' meant 'small wooden stick'. It is possible that the ancient scribe had eclampsia in mind, but the interpretation is tenuous at best[6,7].

Bernhart also wrote that the Indian *Atharva-Veda* of old but unknown dates mention eclampsia. He said that the *Atharva-Veda* described an amulet to ward off convulsions in childbirth[5]. However,

Chesley maintains that, although the *Atharva-Veda* do mention an amulet, there is no mention of convulsions in the translation by Whitney[8].

Bernhart also suggested that the ancient Chinese recognized eclampsia. The source of this suggestion was Wang Dui Me, whose work was translated into German by Lo[9]. The work was originally published in AD 1832, and was thought to be free of any Western influence. But other workers have questioned this, and suggest that the work contains material that would suggest contemporary 19th century views[1].

It does, however, appear that the Greeks recognized pre-eclampsia/eclampsia before the time of Hippocrates. In the *Coan Prognosis XXXI,* no. 507, we find: 'In pregnancy, drowsiness with headache accompanied by heaviness and convulsions, is generally bad.' Later, in no. 523, we find: 'In pregnancy, the onset of drowsy headaches with heaviness is bad; such cases are liable to some sort of fits at the same time'[10]. Hippocrates, in the fourth century BC, wrote in his *Aphorisms* (Section VI, no. 30): 'It proves fatal to a woman in a state of pregnancy, if she be seized with any of the acute diseases.' Much later, Galen in the second century AD commented that epilepsy, apoplexy, convulsions and tetanus are especially lethal[11]. However, Chesley states that epilepsy and eclampsia were not to be differentiated for another 1600 years[1].

In the first century, Celsus also mentions fatal convulsions with the extraction of a dead fetus[12], while Aetios in the sixth century AD wrote: 'Those who are seriously ill are oppressed by a stuperous condition…some are subject to convulsions…'[13].

There is a possible reference to eclampsia in Rösslin's *Der Swangern Frawen und Hebammen Rosengarten,* a book that was the standard text of midwifery in Europe and England for almost two centuries. (This book was later translated into English in 1540 as *The Byrth of Mankinde.*) In a section discussing maternal prognosis after fetal death in labor, Rösslin listed unconsciousness and convulsions as ominous signs[14].

Gaebelkhouern in 1596 distinguished four sorts of epilepsy in relation to the seats of their causes, which he placed in the head, the stomach, the chilled extremities and the uterus[15]. He also wrote about the biting and gnawing in the uterus and diaphragm leading pregnant women to think that something was gnawing at their heart. Chesley suggests that this description might be that of epigastric pain in pre-eclampsia.

Apparently, the word 'eclampsia' first appears in 1619 in Varandaeus' treatise on gynecology. In 1694, Peu, the celebrated accoucheur of Paris, in his *Pratique des Accouchemens,* makes clear references to generalized seizures in pregnancy[16].

In 1694, in the later edition of his book, Mauriceau provides four aphorisms dealing with eclampsia: no. 228, the mortal danger to the mother and fetus is greater when the mother does not recover consciousness between convulsions; no. 229, primigravidas are at far greater risk of convulsions than are multiparas; no. 230, convulsions during pregnancy are more dangerous than those beginning after delivery; no. 231, convulsions are more dangerous if the fetus is dead. Mauriceau also observed that convulsions often cease with delivery, and he recommended prompt treatment by the termination of pregnancy[4].

Like Mauriceau, de la Motte in 1722 recognized the beneficial effect of delivery on convulsions. He wrote: '…parce que la convulsion ne peut cesser que par l'accouchement…Je delivrai la mère, et les convulsions cessèrent aussitôt'[17].

Puzos in 1759 described eclampsia and also the prodromal symptoms such as headache in great detail. He stated that if the convulsions were weak and well spaced out there was hope for the woman. If the convulsions were frequent and lasted longer the prognosis was poor, especially if she became unconscious[18].

Madame le Bousier du Coudray, the chief midwife of Paris, wrote in 1773 about convulsions in labor. Like Mauriceau, she appeared to appreciate the importance of delivery. She wrote that if the cervical os was open and labor pains were coming regularly, there was hope for the woman, especially if there was a good presentation. If the woman were to become unconscious, the case was virtually hopeless and the only way the woman could be saved would be to deliver the baby[19].

Professor Alexander Hamilton, from the University of Edinburgh, wrote in 1781 *A Treatise on Midwifery,* in which he described: 'When the fits are slight and of short duration, recur at distant periods and the woman is sensible during the interval, there is less danger. But when they come on steadily, when the face is frightfully distorted with foamings, when the fit continues long, or recurs often, leaving a total stupor behind, the most unhappy event is to be dreaded.' He also recommended repeated laxative clysters and keeping the woman in a cool quiet room. Like Mauriceau and Madame le Bousier, he appreciated the importance of hastening delivery, and said that if there were any symptoms of labor, the membranes should be broken[20].

Denman wrote in 1821 that if the cervix was closed, he would resort to phlebotomy, gentle clysters and emolient fomentation applied to the cervix. He attributed the convulsions to an excess of heated blood arising from the uterus. He also believed that a dead fetus *in utero* would give rise to malignant vapors to cause convulsions[21]. Chesley suggests that despite his misguided understanding of the illness, Denman was possibly the first person to distinguish eclampsia from epilepsy by assigning convulsions to specific causes[1].

THE RECOGNITION OF PROTEINURIA

It took a further two decades after Denman for clinicians to recognize the significance of proteinuria. John Charles Weaver Lever published a paper in 1843 in Guy's Hospital. He was the first to describe swelling of the ankles and puffiness around the eyes and the finding of albumin in the urine[22]. He reported: '…I further have investigated the condition of the urine in upwards of fifty women from whom the secretion has been drawn during labour…and the result has been that in no cases have I detected albumin, except in those in which there have been convulsions, or in which symptoms have presented themselves, and which are readily recognized as the precursors of puerperal fits.'

Unfortunately, many of Lever's less astute colleagues seized upon his findings as evidence that eclampsia was a manifestation of chronic nephritis. Lever observed that the proteinuria of eclampsia abated and disappeared after delivery, and he therefore concluded that eclampsia was not nephritis. Nevertheless, the misguided view that eclampsia equated with nephritis was so popular that it persisted for another 100 years. In fact, by the 1940s, the term 'nephritic toxemia' was still in common use[23].

THE RECOGNITION OF EDEMA

In 1794, Demanet wrote that all six of his patients with eclampsia had anasarca, but wrongly suggested that edema should be added to the list of causes of convulsions in pregnancy. In 1843, Johns of Dublin stated that if during the last months of pregnancy headaches and edema of the upper half of the body occurred, this might be a warning that fits would be a later manifestation[24].

THE RECOGNITION OF HYPERTENSION

It seems likely that the hard bounding pulse of eclamptic women had been noticed by physicians of old. Aetius in the sixth century AD mentioned that in pregnant women with convulsions, 'the pulse is strong and swollen'[13]. But it was only with the invention of the sphygmograph, centuries later, that arterial pressure was measured. Ballantyne made sphygmographic tracings from dying eclamptic women, which inferred vascular collapse in these women. Although the discovery of eclamptic hypertension is generally credited to Vaquez and Nobécourt in 1897, Vinnay had used a primitive sphygmomanometer to document hypertension of 180–200 mmHg in pregnant proteinuric women, 3 years

earlier. However, in line with his other misguided colleagues, Vinnay thought that his patients had nephritis.

To confuse the issue further, in 1896, Allbutt observed that middle-aged patients often developed hypertension with no evidence of renal disease. He called this disorder 'senile plethora' (now called essential hypertension). Herrick from Sloane Hospital in New York suggested between 1926 and 1936 that essential hypertension was the usual form of hypertension in pregnancy. Thankfully, many other obstetricians led by H.J.Stander strongly opposed his views, and insisted that hypertension in young women was either pre-eclampsia/eclampsia or—nephritis[23].

EVOLUTION OF THE MANAGEMENT OF PRE-ECLAMPSIA/ECLAMPSIA

Hippocrates wrote of convulsions in general: 'Convulsions take place from either repletion or depletion' (Section VI, no. 29). Phlebotomy and purgation were probably of late origin, because Hippocrates also wrote that these measures were contraindicated in pregnancy. Nevertheless, phlebotomy and purgation were the sheet anchors of physicians' treatment of eclampsia in the 17th, 18th and 19th centuries. Certainly there is evidence that prominent figures such as Mauriceau in 1694, Madame le Bousier du Coudray in 1773, Alexander Hamilton in 1781 and Denman in 1821 all practiced phlebotomy.

Mauriceau, Madame le Bousier du Coudray and Alexander Hamilton among many others had observed that delivery seemed to resolve the frightening specter of convulsions in pregnancy. Over time, artificial termination of pregnancy was deemed by many others to be a logical solution. For what could be more logical than the removal, as quickly as possible, of the *sine qua non* of the disease? The shocking mortality associated with forceful and traumatic delivery led Denman in the 18th century to protest: 'Some writers have recommended the speedy delivery of the patient, as the most eligible, and only effectual method of removing puerperal convulsions…From the histories of all cases of puerperal convulsions which we have recorded, it appears, that a greater number have died of those who were delivered by art, than when the labours were resigned to nature'[25]. The plea was repeated in 1823 by Dewees: '[Should we find] that there is no change made in the os tincae, nor any evidence of uterine contraction—in a word, not a symptom of labour—then, should we attempt delivery by forcing the mouth of the uterus, as some direct, we should inevitably destroy our patient; delivery in this case should not be thought of…, it were madness, nay, I had like to have said, murderous to attempt it…'[26].

Denman in England and Dewees in America were two of the most highly respected figures in obstetrics at that time, and their teachings carried great influence. However, with the birth of obstetric anesthesia in the mid-19th century, obstetric intervention assumed new license, conservative practices were promptly forgotten and *accouchement forcé* began to flourish. By the late 19th century, Halbertsma in the Netherlands advocated prompt abdominal delivery, and Dührssen in Germany practiced 'vaginal Cesarean section' by using cervical incisions that bear his name (although Van Swieten had used similar incisions for the same purpose a century before Dührssen). In Germany especially, the radical approach flourished. In fact, the Germans came to differentiate Schnellentbindung (quick delivery) from Frühentbindung (early delivery). Although quick delivery carried a higher maternal mortality, German clinics continued with its use presumably because late hospitalization prohibited waiting, coupled with the gratitude from early cases.

Fortunately, at about the same time, in direct contrast to Halberstma and Dührssen, Tweedy in Ireland and Stroganov in Russia advocated a conservative approach. In 1896, from the Rotunda in Dublin, Tweedy pleaded: 'It is an unfortunate fact that surgical treatment is growing in favour day by day, and this is not surprising, for not only has it the sanction of great names, but it also affords the medical attendant much satisfaction, substituting as it does an active mode of treatment for inactivity with apparent impotence. I cannot but think, however, that it is unsound in theory and disastrous in practice'[27].

Tweedy's program of sedation (although unsound in modern obstetrics) is the basis of modern conservative management: 'Let me now turn to the method of treating eclampsia... I allude to the hypodermic injection of large doses of morphine. Beginning with the injection of 1/2 grain, this followed in two hours by 1/4 grain, and so on gradually, until either the symptoms are alleviated or until 2 grains have been given in 24 hours. If in spite of this treatment, labour sets in, forceps are applied to hasten delivery so soon as the os will safely admit their application; but it is held that manual dilatation of the cervix, which in reality means bursting of that structure, is not a justifiable proceeding.'

Stroganov, in 1899, gave a preliminary account of 45 cases treated by his new conservative method, and the following year reported 92 cases of eclampsia with a maternal mortality of 5.4%. This contrasted sharply with a maternal mortality of 20–30% in most clinics at that time. The 'Stroganov regimen' was quickly adopted throughout the world. Stroganov wrote: 'The most important objective in the treatment of eclampsia is elimination of the convulsions… The oftener the attacks occur, the more disastrous their effects. After nine or ten attacks a

woman, previously in the bloom of health, may be at the brink of the grave. The best results in eclampsia depend on the prevention or reduction in frequency of these convulsions. For this purpose I regard the systematic, prophylactic use of morphine and chloral hydrate as a comparatively innocuous measure. Experience has shown that two sedatives, acting concurrently, are more effective than one, even if given in larger doses. In eclampsia, moreover, an effect is necessary both on the sensory centers and the convulsive center. The best agent for the former is morphine, for the latter, chloral hydrate… In practice I employ the following schedule of treatment for eclampsia of moderate severity: Immediately after the first attack or upon arrival of the patient in hospital I inject 0.015 gm of morphine hydrochloride subcutaneously. An hour later, or sooner if the patient is restless, the injection is repeated, even in the absence of another attack. Two hours after the second injection, or earlier if an attack threatens, I administer 2 to 3 gm of chloral hydrate per rectum. I repeat the instillation four hours later, either in the same dosage or, if the patient has quieted, in a slightly smaller one… I repeat this after six and again after eight hours… Medical treatment, stopping convulsions and improving the condition of the patient…eliminated the need for forceful methods, which are dangerous for both mother and child'[28].

Tweedy and Stroganov reported marked reductions in maternal mortality with their respective regimens, and slowly gained followers. However, it was not until the 1920s that two publications convinced most obstetricians that conservative management was better. Eden in 1922 analyzed hundreds of cases of eclampsia, and reported that in severe eclampsia, the maternal mortality for spontaneous or induced labor, Cesarean section and 'forced delivery' was 34, 46 and 63%, respectively[29]. Plass in 1927 reported 10000 cases of eclampsia, and showed that maternal mortality was 11.1 and 21.7% for conservative management and operative delivery, respectively.

Ironically, these reports had an initial detrimental impact on the management of eclampsia. Chesley recounts, with horror, how the pendulum swung the opposite way to ultraconservatism and how the rate of stillbirths soared. He writes: 'For a time, they awaited spontaneous onset of labor, and 50 years ago many pregnancies were carried for weeks after the onset of convulsions… I remember one patient who went on for 12 weeks with severe hypertension, gross proteinuria, and anasarca. She had a second set of convulsions, abruptio placentae, and a stillbirth…in the 1930s significant numbers had recurrence of convulsions, sometimes with fatal outcome'[23]. As a result, the usual practice came to be termination of pregnancy at some arbitrary time after the control of convulsions. That interval has been shortened progressively from several days to a few hours in modern obstetrics.

OTHER 'RATIONAL' TREATMENTS OF ECLAMPSIA

Currently, the concept of endothelial cell involvement in the pathogenesis of pre-eclampsia has gained widespread acceptance. This concept was first raised by Weiss and Dexter in 1941, but was promptly forgotten and remained so until resurrected by Roberts and colleagues nearly half a century later[30,31]. Since then, the search for a circulating factor at which to direct treatment has been a priority at major research centers (see Chapter 6).

An interesting but horrifying account of other treatments of eclampsia can be found in the articles by Chesley[1,23], although much of what is written is difficult to verify.

When physicians decided that eclampsia was caused by repletion, the logical answer was depletion. Hence phlebotomy, purgation and the induction of diaphoresis and diuresis seemed rational. When physicians came to regard eclampsia as toxemia, those means seemed to be logical ways by which to remove toxins.

Meat had a bad name for centuries, particularly red meat. The 'toxic split products' of protein had been postulated as the cause of eclampsia. Other variations were that amino acids were decarboxylated but not deaminated, and that amines were the toxic agents or that autointoxication was the cause. Accordingly, low-protein diets were the vogue and were advocated by the 9th edition of *Williams Obstetrics* as late as 1946.

The bulky uterus was thought to compress the kidneys, ureters and renal veins. 'Logical' treatments included keeping the woman lying prone with her belly in a hole in the mattress. Ventral suspension of the uterus, ureteral catheterization, transplantation of the ureters to the gut and decapsulation of the kidneys were other methods used.

Chesley mentions a Scandinavian physician who found large lutein cysts in a woman with hydatidiform mole and severe pre-eclampsia. According to Chesley, this physician subsequently treated eclampsia by oophorectomy.

Chesley also describes a time when bovine parturient paresis was mistaken for eclampsia in cows. He writes: '…An efficacious treatment of parturient paresis was inflation of the udder with air. Selheim tried it, found the breasts resistant to inflation, and lopped them off.'

Perhaps realizing how arrogant present-day obstetricians can be, Chesley writes rather poignantly: 'In time, many of our present practices will seem bizarre as those just rehearsed, with the difference being that the former were rational in the light of hypotheses as to the cause and nature of eclampsia; whereas ours are empiric, too often symptomatic, and in some respects, based upon imitative magic.'

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3

The epidemiology of pre-eclampsia

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BACKGROUND

The multisystem disorder of pre-eclampsia continues to be a leading cause of maternal and perinatal morbidity and mortality. On a worldwide basis, the World Health Organization estimates that over 160000 women die from pre-eclampsia each year, and the condition has been the most important cause of maternal death over recent decades. Recent reports from the Confidential Enquiry into Stillbirths and Deaths in Infancy (CESDI) cite that one in six stillbirths and one in six sudden infant deaths occur in pregnancies complicated by maternal hypertension, and the condition is responsible for the occupancy of approximately 20% of special care baby unit cots. A summary of the risk factors for developing pre-eclampsia is provided in Table 1.

INCIDENCE

Despite extensive study into the incidence of pre-eclampsia worldwide, only a small amount of the data available can be used to estimate the incidence of hypertensive disorders of pregnancy with any degree of reliability, and even fewer studies can be used for comparisons. There are several reasons for this; first, a number of different disease definitions and classification systems have been utilized and even when one definition has been standardized and agreed by clinicians, case reporting can vary widely. Second, in any epidemiological study of incidence it is essential to use a geographically based population rather than one comprising all

Table 1 Summary of risk factors for the development of pre-eclampsia

Factor	*Risk ratio*
Nulliparity	3 : 1
Age>40 years	3 : 1
African-American race	1.5 : 1
Family history of pregnancy-induced hypertension	5 : 1
Chronic hypertension	10 : 1
Chronic renal disease	20 : 1
Diabetes mellitus	2 : 1
Twin gestation	4 : 1

births in a hospital or group of hospitals. Often more subtle forms of bias also occur, for example, the more often blood pressure and urine samples are tested, the more likely it is that hypertensive disorders will be detected. In addition, the longer the gestation the more likely it is that pre-eclampsia will appear.

The incidence of eclampsia has declined dramatically during the 20th century. Eden observed a 20-fold decrease in the incidence of eclampsia in the UK by 1922[1], which coincided with the provision of universal prenatal care, and in New Zealand the incidence fell from 3.2/1000 between 1928 and 1933 to 0.8/1000 between 1956 and 1958[2]. Similar findings were reported in the USA where cases of eclampsia decreased from 0.4% in 1931 to 0.03% 20 years later[3]. In a more recent British survey in 1992, Douglas and Redman observed the incidence of eclampsia to be 0.49/1000 pregnancies[4]. Most of the seizures occurred despite prenatal care (70%) and even after admission to hospital (77%).

The reported occurrence of pre-eclampsia varies from rates as low as 0.51% to as high as 38.4%. For the reasons discussed above it is impossible to make direct comparisons between these studies as very few are population-based and a wide variety of disease definitions have been utilized. A Norwegian study incorporated all births in a defined geographical region between January 1993 and December 1995[5]. During the study period there were 12804 deliveries, and the risk of developing pre-eclampsia was 2.5% in this unselected population. An extensive statistical survey published in the USSR covered a population of 78311 pregnant women from urban and rural areas of the Ukraine between 1962 and 1964[6]. Hypertension and proteinuria was observed in 6.9% of pregnancies. There are numerous other studies which have

reported disparate incidence rates in women from many different countries. The reported incidence was 2.8% among 5878 women in Israel[7], 5.8% in a population-based study in Aberdeen, Scotland[8], 9.7% among 2434 women in Australia[9] and 2.9% in a very large study in Canada (140773 pregnancies)[10].

A number of studies have estimated the rate of pre-eclampsia as part of drug trials for the prevention of pre-eclampsia. For example, the Maternal-Fetal Medicine Network trial recruited 1500 nulliparous women in the control arm of their aspirin trial and reported an incidence of pre-eclampsia of 5.3%[11].

RACE AND ETHNIC GROUP

Although many studies have been reported in various parts of the world, different racial and ethnic groups tend to receive different standards of health care, even in the same country, and this dramatically affects the reported incidence of hypertensive disorders of pregnancy in different racial groups. Davies and colleagues investigated the relationship between pre-eclampsia and ethnic group in a study involving pregnant women in Jerusalem between 1964 and 1966[12]. This study showed that the incidence of pre-eclampsia was significantly higher in the Muslim Arabs and in the Jews born in the Islamic Republic of Iran than in Jews born in North Africa or Israel. Other studies have provided conflicting reports; for example, some studies have reported a higher incidence of pregnancy-induced hypertension for African-American women than for white women. A study of an American military population showed that nulliparous black women had a 20% higher risk of developing pre-eclampsia compared to nulliparous white women[13]; conversely, a study of a different population in the same area of the USA concluded that there was no increased incidence amongst the African-American population[14]. In a European prospective cohort study of 2413 women, the relative risk of developing pre-eclampsia was 2.4 (95% confidence interval (CI) 1.1–5.6) for black women, 2.1 (CI 0.7–6.3) for Asian women and 1.9 (CI 0.6–5.5) for Mediterranean women compared with white women[15]. In another Asian population, however, the incidence of pre-eclampsia was reported to be very low at 1.4%[16].

Many of the differences in the incidence of pre-eclampsia observed in different ethnic groups may be attributable to genetic factors, in particular genetic factors which convey an increased risk of developing chronic hypertension. Samadi and co-workers conducted a large epidemiological study in the USA involving hospital records from over 86000 deliveries between 1988 and 1992[17]. They reported significantly

elevated rates of maternal hypertension in black women compared with women from other ethnic groups; however, after exclusion of women with a diagnosis of chronic hypertension, there was no increase in the incidence of pre-eclampsia in black women.

Another reported explanation for the differences in incidence in different racial or ethnic groups is the bias of other demographic variables that can influence the risk of developing pre-eclampsia. Most epidemiological studies control for parity; however, other factors such as age, body mass, nutritional factors, environmental factors, use of contraception and smoking are not always accounted for and, therefore, further confuse the data available. In addition, demographic variables such as age may be more important in some populations than in others. For example, Knuist and colleagues reported that higher age at booking conferred considerably more risk of developing pre-eclampsia in black women than in white women[15].

MATERNAL AGE

Most studies have shown that there is a 'J-shaped' curve for the relationship between maternal age and the incidence of pre-eclampsia. For example, Saftlas and co-workers reported this observation in their nationally representative cross-sectional study of the US population[3]. The risk for a woman over 35 is about three- to four-fold that for a younger woman. This effect is probably independent of misdiagnosed chronic hypertension in older women and may reflect the normal vascular changes that occur with aging. Many studies that have not used strict diagnostic criteria for the selection of patients with pre-eclampsia will be biased by older women with undiagnosed chronic hypertension and will report falsely high rates of pre-eclampsia in this age group. The incidence of pre-eclampsia is significantly elevated in teenage girls, particularly in those under 15 years of age. It remains uncertain whether this is truly an increase in the incidence or whether it is a reflection of the greater tendency to social neglect among this group, including poor antenatal care, improper nutrition and an increased incidence of concealed pregnancies.

PARITY

Pre-eclampsia is largely a disease of first pregnancies and according to Chesley, approximately 75% of women with pre-eclampsia are nulliparous[18]. MacGillivray noted in 1958 that pre-eclampsia occurred in 5.6% of primiparas and only in 0.3% multiparas among a well-

characterized population in Scotland[19]. Other authors have confirmed that nulliparous women are five to ten times more likely to have pre-eclampsia than multiparous women[20]. Interestingly, few studies have investigated the association of nulliparity with gestational hypertension separately from its association with pre-eclampsia. Misra and Kiely studied 4500 pregnancies and concluded that, allowing for confounding variables such as age, weight and race, nulliparity conferred a significant increased risk of developing pre-eclampsia (odds ratio 2.94 for white women and 2.86 for black women) but there was no such increase in the rate of non-proteinuric pregnancy-induced hypertension[21]. This highlights the difference in etiology of the two conditions and the importance of separating these conditions in epidemiological analyses. It is proposed that the normal fetal-maternal transfusion that occurs in pregnancy, particularly during delivery, exposes the mother to products of the fetal genome, protecting her in subsequent pregnancies. It has been suggested that in later pregnancies there is the development of protective mechanisms against paternal antigens.

In a large population-based study in Norway spanning 30 years, the strong effect of parity was confirmed but, in addition, the power of this study allowed the effect of changing paternity on the risk of developing pre-eclampsia to be assessed. The risk was 1.7% in women who had their second pregnancy with the same partner and 1.9% among second pregnancies in mothers who had changed their partner[22]. This small tendency was highly significant even after allowing for other confounding variables. Other studies have also investigated the effect of changing paternity and concluded that the risk in a second pregnancy with a new partner is the same as that of nulliparous women. Further analysis of the Medical Birth Registry in Norway concluded that the risk of developing pre-eclampsia in the second or third pregnancy was directly related to the time that had elapsed since the preceding delivery, and when the inter-birth interval was greater than 10 years, the risk approximated to that among nulliparous women[22]. After adjustment for maternal age and the presence or absence of a change in partner, the odds ratio for each 1-year increase in the birth interval was 1.12[22].

Sibai and colleagues found that women who had had one previous pregnancy ending before 20 weeks' gestation had a reduced incidence of pre-eclampsia and that this was further reduced in women who had a history of two or more previous pregnancies[11]. This finding has been confirmed in other populations.

A number of studies have suggested that length of exposure to paternal antigens in sperm reduces the risk of developing pre-eclampsia[23]. During a protracted sexual relationship, women develop an immune response to paternal antigens expressed on spermatozoa or in seminal fluid. Epidemiological data relating to the use of

contraceptives have also demonstrated that prolonged use of oral contraceptives is protective against the risk of pre-eclampsia and that barrier methods are associated with a moderately increased risk. Women inseminated with sperm that are not from their husband have an increased risk of developing pre-eclampsia, and women who conceive following embryo donation have an even greater risk, although none of the relevant studies has adjusted for potential confounding variables such as older maternal age and more frequent multiple births.

GENETICS

The occurrence per confinement and geographical distribution of pre-eclampsia have been summarized by Chesley[18] and Davies and colleagues[12]. These two massive compilations of information suggest that eclampsia and pre-eclampsia can occur under all environmental conditions; thus, purely environmental hypotheses are implausible. Genetic analysis is rendered very difficult by the nature of a condition that occurs only in women who have reproduced. The greatly decreased incidence in second and later pregnancies makes analysis of the fetal contribution very problematical. Although, eclampsia is an unambiguous phenotype, pre-eclampsia is easily confused with other hypertensive disorders of pregnancy. Lack of data in men and uncertainty of diagnosis has blunted the power of conventional genetic analysis to reveal the mode of inheritance.

Most of the family data suggest that the maternal genotype is responsible for conveying most to the susceptibility, with only a minor contribution from the fetal genotype. There is, however, a striking discordance between identical twins, although pre-eclampsia in a first-degree relative confers a three- to four-fold increased risk of developing the condition. Interestingly, both men and women who were the product of a pre-eclamptic pregnancy are more likely to have a child from a pregnancy complicated by the condition[24]. In addition to this, a woman who becomes pregnant by a man who has already fathered a child with another woman who suffered from pre-eclampsia, is twice as likely to have a pregnancy complicated by the disease.

For a more detailed discussion on the inheritance of pre-eclampsia, see Chapter 4.

OBSTETRIC HISTORY

Mothers with a history of pre-eclampsia in their first pregnancy have a significantly higher risk of developing the condition in their second pregnancy. In the Norwegian study, the risk in the second pregnancy

was 13.1% if a woman had her second pregnancy with the same partner, and 11.8% if she changed partner[5]. This translates to a 12-times increased risk of developing pre-eclampsia in a second pregnancy compared to women who had an uncomplicated first pregnancy. Zhang and co-workers concluded that the recurrence of pre-eclampsia was 32% for women whose first pregnancy was complicated by pre-eclampsia and up to 46% for those women who developed pre-eclampsia superimposed on chronic hypertension[25]. Interestingly both Zhang and colleagues and others have shown that a history of low birth weight, adjusted for gestational age, is associated with the subsequent occurrence, as well as recurrence, of pre-eclampsia.

MULTIPLE PREGNANCY

The incidence of pre-eclampsia in primigravidae women with twins has been shown to be four to five times that of singleton pregnancies[26]. Pre-eclampsia has been reported as being more common in women expecting dizygotic twins than women expecting monozygotic twins. However, several recent studies that have determined chorionicity within the first trimester have demonstrated that there is no difference in the incidence of pre-eclampsia between the two different types of twin pregnancy. It has also been demonstrated that this risk is increased further in women with triplet pregnancies.

PRE-EXISTING MEDICAL CONDITIONS

Hypertension

A recent study revealed that, compared with normotensive women, both black and white women who have chronic hypertension have an 11-fold higher risk of developing pre-eclampsia during pregnancy[27]. This confirms several previous studies regarding chronic hypertension and the risks of developing pre-eclampsia. However, it also demonstrated that black women had double the rate of chronic hypertension compared with white women (1.5 vs. 0.7%, respectively), and that chronic hypertension intervention programs would provide significantly greater prevention benefits and a lower disease burden among black women[27]. As well as being at an increased risk of pre-eclampsia, women with chronic hypertension also made up a higher proportion of women who developed early onset or recurrent disease. These studies have also demonstrated that those women who were at greatest risk of developing superimposed pre-eclampsia were those

with the disease present for more than 4 years, and evidence of severe underlying blood pressure elevation prior to pregnancy.

Several studies have demonstrated that higher blood pressure prior to 20–27 weeks' gestation is associated with the development of pre-eclampsia. Longitudinal data in women destined to develop pre-eclampsia have shown that normotensive women who subsequently develop pre-eclampsia have higher blood pressures in the first trimester[5].

Diabetes

Patients with diabetes mellitus have been shown to be at increased risk of developing pre-eclampsia[28]. Garner and co-workers studied 334 pregnant diabetic women and compared them to a non-diabetic control group; they demonstrated that the incidence of pre-eclampsia was 9.9% in the diabetic group compared to 4% in non-diabetics[29]. This rate increased to 30% in women with insulin-dependent diabetes with nephropathy or pre-existing hypertension. The evidence regarding the incidence of pre-eclampsia in women with abnormal glucose tolerance and gestational diabetes is contradictory. This probably reflects the plethora of definitions used when defining these conditions; however, it is likely that the development of gestational diabetes does confer a small increase in risk.

Hematological

Inherited and acquired thrombophilias have been associated with an increased risk of developing pre-eclampsia, although much of the evidence is contradictory. For example, one study reported factor V Leiden in 20% of women with pre-eclampsia, placental abruption, fetal growth restriction or stillbirth, compared to only 6% of women without these complications, translating to an odds ratio of 3.7[30]. Additional case-control studies have also reported a higher prevalence of factor V Leiden in women with pre-eclampsia (up to 26%) compared to women with normal pregnancies (2–6%) with odds ratios ranging from 2 to 5. Conversely, other studies have concluded that the presence of factor V Leiden did not increase the risk of pre-eclampsia[31].

The evidence to support increased rates of antiphospholipid antibodies in women with pre-eclampsia is also incongruous. The prevalence in the general population of acquired thrombophilias is around 2–4%; of these patients, half (50%) of them have the primary antiphospholipid antibody syndrome. Despite numerous reports that the presence of these antibodies was associated with an increased risk of developing pre-eclampsia, several groups have been unable to demonstrate any association. In the largest, unselected study of

pregnant women to date, there was no difference in the observed rates of adverse pregnancy outcomes between the women who tested positive and those who tested negative for antiphospholipid antibodies[32].

A recent study that evaluated the recurrence risk of pre-eclampsia in women with a history of previous pre-eclampsia was able to demonstrate that only severe early-onset disease was associated with antiphosphatidylserine antibodies[33]. They did show that other antiphospholipid antibodies were associated with intrauterine growth restriction. Therefore, the predictive value of routine testing for both acquired and inherited thrombophilias in low- and high-risk women remains to be proven.

ADDITIONAL FACTORS

As previously discussed, many of the variations in the incidence of pre-eclampsia between different populations can be attributed to disparate demographic features. For example, the age at first delivery and the prevalence of conditions such as diabetes and chronic hypertension will vary considerably across different populations and ethnic groups. In addition to these important variables, however, a number of other factors may influence the incidence of pre-eclampsia; these include nutrition and environmental conditions.

Obesity

Several studies have highlighted the relationship between food intake and pre-eclampsia as reported by MacGillivray[34]. The best of these studies examined the effect of severe food rationing upon the Dutch population during the Second World War. This showed that there was a decrease in the incidence of pre-eclampsia during the period of rationing. However, similar studies performed during the First World War provided conflicting reports. Although these studies provide some evidence that nutritional status affects the incidence of pre-eclampsia, they fail to account for the changes in reproductive patterns during wartime.

Although there is no universal definition of obesity within the literature, the majority of the data supports the view that obese women are at greater risk of pre-eclampsia. In a case-control study, Eskenazi and colleagues[20] examined pre-pregnancy body mass index (BMI) data obtained from medical records and compared them to controls. They demonstrated that a BMI of greater than 25.8 kg/m^2 was associated with a 2.7-fold increase in risk of pre-eclampsia. More recently, Sibai and co-workers confirmed the association between increased BMI and pre-eclampsia; however, there appears to be no relationship to height

as a single variant[11]. Several recent studies have also examined whether body-fat distribution is a risk factor for pre-eclampsia. Some investigators have demonstrated that upper-body-fat distribution might be associated with pre-eclampsia[35]. They utilized dual energy X-ray absorptiometry (DEXA) scans to measure body-fat distribution of postnatal women who had been diagnosed with pre-eclampsia and compared these to a control population. However, their results may be biased by altered fluid distribution, which is involved in the pathophysiology of pre-eclampsia. Two other studies have examined body-fat distribution and the risk of pre-eclampsia. The best of these studies demonstrated that a waist circumference of greater than 80 cm at 16 weeks was associated with a 2.7-fold increase in pre-eclampsia[36].

Epidemiological data have suggested that a pre-pregnancy BMI of less than 19 is a significant protective factor against the development of pre-eclampsia. There are no studies that have demonstrated that women with a very low BMI have a reduced risk of developing the condition; however, this may be due to reduced pregnancy rates in severely malnourished women.

Calcium

Epidemiological data suggest an inverse relationship between dietary calcium intake and maternal blood pressure and the incidence of pre-eclampsia[37]. Two meta-analyses, which investigated the use of calcium supplementation, have demonstrated that calcium causes a reduction in both systolic and diastolic blood pressure. It has been postulated that the positive effect of calcium on blood pressure is mediated through alterations in renin and parathyroid hormone. Several well-conducted studies have been unable to demonstrate that calcium supplementation in women with a normal calcium intake reduces the risk of pre-eclampsia; therefore, the beneficial effect of calcium on maternal and fetal morbidity remains to be substantiated. Studies conducted in South American women who have lower serum calcium levels have shown a reduction in the risk of developing pre-eclampsia and a significant improvement in pregnancy outcome[38]. This highlights the importance of considering the demographic and nutritional features of a study population when interpreting clinical trial data.

Magnesium and zinc

There are several studies that have demonstrated that magnesium and zinc deficiency increases the risk of pre-eclampsia[39]. However, routine supplementation has failed to show any benefit on fetal or maternal outcomes.

Vitamins

Women who are deficient in vitamin B_2 are more likely to develop pre-eclampsia than normal women. These results were observed in countries where vitamin deficiencies are more common than in the Western World.

Altitude

Geographical location has been noted to affect the incidence of pre-eclampsia and some observations have suggested that altitude may influence the risk of developing the condition. Several studies from the same geographical area of Colorado have demonstrated that women living at higher altitude have an increased risk of developing pre-eclampsia[40]. This was shown to be highest (16%) in a group of women living at altitudes above 3100 m above sea level, whereas the incidence below this was 3–4%. This effect has not been confirmed in any other geographical region as there are relatively few communities worldwide that live permanently above 2500 m, and those that do (South America and the Himalayas) are complicated by the fact that a significant proportion of the women are not delivered in hospital; thus, there is a different standard of diagnosis of the disease.

Smoking

Although smoking in pregnancy has long been associated with many adverse fetal outcomes including low birth weight and abruptio placentae, there are now many studies that demonstrate that women who smoke are protected from pre-eclampsia. A recent systematic review has confirmed this observation and has demonstrated that women who smoked were overall 32% less likely to develop pre-eclampsia than non-smoking controls[41]. In addition, they showed that this was a dose-related phenomenon with increased consumption lowering the risk further. Interestingly, those women who did develop pre-eclampsia in the smoking group had significantly higher rates of low birth weight, perinatal loss and abrutio placentae than did the non-smoking group with pre-eclampsia.

Periodontal disease

A recent study has demonstrated that there is an association with active periodontal disease and pre-eclampsia[42]. This association was independent of the effect of maternal age, race and smoking.

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4

Genetics

C. Tower

INTRODUCTION

There have been considerable advances within the field of human genetics, not least with the recent completion of the Human Genome Project, in which 97% of the human genome has now been mapped. The search is continuing to describe the genetic basis of human disease, and it is hoped that the sequence of the human genome will provide a point of reference for this search. It is likely that many common disorders caused by a single defective gene have now been described. Hence, interest is turning to diseases or traits that are caused by several factors, both genetic and environmental, working together. These are multifactorial or complex disorders, and pre-eclampsia is a likely example. A complex trait is any phenotype that does not exhibit classic Mendelian recessive or dominant inheritance at a single gene locus. Typically, the degree of risk to relatives reduces by more than one-half with each degree of relationship. The predisposing genes will have a small relative effect on a background of substantial genetic and environmental variation. Often, the genes involved are variants of the forms involved in normal human health and development, and, although the important genes may have a small individual effect, they may be relatively common within a population and therefore have considerable public health significance.

Broadly speaking there are two approaches to the investigation of possible genetic cause of disease: a candidate gene of interest can be studied, or the genome can be searched for an unknown region or gene important for the development of the disease. In the

first approach, the gene is already known and described, in the second it is not. In addition, there are studies defining function and roles in disease.

PROBLEMS FOR INVESTIGATING THE GENETIC BASIS OF COMPLEX TRAITS

Pre-eclampsia is a multifactorial disorder; therefore, it is unlikely that there is a single gene at fault. Despite research into possible genetic factors important in this life-threatening disorder spanning the past 20 years, the answers have remained elusive. This is largely due to problems encountered in the investigation of complex disorders with multifactorial origins. These include the following:

Definition of phenotype

It is vitally important to define carefully a phenotype for pre-eclampsia, to ensure reproducibility and meaningful comparison between studies. Definition of pre-eclampsia is particularly difficult as it involves variables that are continuous, often inaccurately measured (blood pressure and proteinuria) and develop late in the disease process. There are no known reliable markers or predictors of disease in early pregnancy or in non-pregnant women. Also, women developing pre-eclampsia in their first pregnancy may be a group distinct from those developing it for the first time in their second or subsequent pregnancy. Parous women developing pre-eclampsia for the first time are far more likely than nulliparas to suffer ischemic heart disease in later life, and are less likely to show diagnostic glomerular endotheliosis on renal biopsy. Also, a definition suitable for one ethnic group may not be suitable for another.

Other problems with phenotype

Some individuals who carry the disease allele may not demonstrate the disease phenotype, known as incomplete penetrance. Also, some individuals may have the disease phenotype but not carry the disease allele, known as phenocopy. In this case, the disease occurs because of random causes or environmental factors. In other words, although the genotype influences the probability of the disease, it does not fully determine the outcome. Environmental factors, age, sex and possibly other genes are important in the expression of the disease phenotype. Certain genes may be associated with multiple outcomes (pleiotropy). This is possible with pre-eclampsia since it embraces a broad spectrum of disease, for example mild and severe cases, growth

restriction and HELLP syndrome (hemolysis, elevated liver enzymes and low platelets).

Genetic or locus heterogeneity

This describes different genetic mutations resulting in the same phenotype, for example different mutations affecting a biochemical pathway which result in the same end-point. This is distinct from allelic heterogeneity in which there are multiple disease-causing alleles at a particular locus. Genetic heterogeneity complicates genetic studies, as a disease may segregate with a particular chromosome in some families but not in others.

Polygenic disorders

Some complex disorders may be the effect of several genes acting together, known as polygenic disorders. Indeed, some genes may become pathogenic only when in the presence of another (epistasis). Polygenic inheritance is likely to be a common phenomenon and has been demonstrated in other diseases, and is possible in pre-eclampsia. Polygenic inheritance has considerable implications for genetic studies as no single gene is associated with disease.

High frequency of disease-causing alleles

Mapping of both simple and complex disorders can be complicated if the disease-causing alleles occur at a high frequency in the population. First, multiple independent copies of a disease-causing allele may be segregating within a pedigree under study. Second, when dealing with a common allele, there will be a higher frequency of homozygotes within a population. Homozygotes are uninformative because either homolog can be transmitted to an affected offspring. This prevents linkage to a nearby genetic marker being observed.

Additional modes of inheritance

Further to the above problems, additional modes of genetic inheritance exist which complicate analysis. These include mitochondrial inheritance, imprinting and anticipation. Mitochondrial inheritance involves transmission entirely through the maternal germline. Imprinting is the differential expression of a gene dependent on the parent it originates from. This involves methylation, which silences expression of either the maternal or the paternal copy of the gene. Both mitochondrial inheritance and imprinting have been suggested in pre-

eclampsia. Anticipation is the increase in severity of a disorder with successive generations, usually by expansion of trinucleotide repeats.

FAMILIAL PREDISPOSITION IN PRE-ECLAMPSIA

Genetic epidemiology is the study of the patterns of disease incidence in families and populations to establish whether a genetic cause is plausible. The three main approaches are twin studies, calculation of relative risk and segregation analysis.

Twin studies

Twins share both genetic and environmental factors. Monozygotic twins share all their genes, whereas dizygotic twins are believed to share 50%. Hence, twin studies can provide useful information on heritability, which is a measure of the extent to which phenotypic variation in the population can be explained by genetic variation. Twin studies of monozygotic siblings give a direct estimate of penetrance of a condition for a given environment.

Although initial twin studies failed to identify concordance for pre-eclampsia, later studies, using strict diagnostic criteria, refuted this. The earlier studies relied on anthropological data collected by retrospective questionnaire to confirm monozygosity and diagnosis. In contrast, later studies identified monozygotic twins concordant for pre-eclampsia using DNA fingerprinting. It therefore seems that concordant twins for pre-eclampsia do exist, although retrospective questionnaire studies may not detect them.

Relative risk

Relative risk is an important epidemiological parameter. It is defined as the recurrence risk for a relative of an affected individual divided by the risk for the general population. Since it is related to the degree of concordant inheritance for genetic determinants, relative risk reflects how easy the disease or trait will be to map. In other words, the higher is the relative risk the easier genetic mapping will be.

Several studies have documented increased risk of pre-eclampsia in relatives of index cases. An Icelandic study of 107 women with eclampsia or pre-eclampsia found that daughters of these women had a 23% chance of developing pre-eclampsia, compared with 10% in daughters-in-law[1]. Subsequent studies have supported these findings in different populations.

Epidemiological studies have also suggested fetal and paternal contributions. A large Norwegian study found that men who fathered

one pre-eclamptic pregnancy were nearly twice as likely to father a pre-eclamptic pregnancy with a different partner[2]. Again, subsequent work has supported these findings. These epidemiological studies suggest a role for inherited components from both parents (and thus fetal genotype) in the pathogenesis of pre-eclampsia.

Segregation analysis

Segregation analysis involves fitting a model of inheritance to that observed in a pedigree affected by a certain disease. Bias can occur if there is overinclusion of affected individuals, causing penetrance to be overestimated, and it has little ability to distinguish between many possible modes of inheritance or many distinct genes in complex disorders.

Epidemiological studies have suggested several patterns of inheritance for pre-eclampsia. A study from Iceland suggested either a single recessive gene or a dominant gene with incomplete penetrance[1]. This retrospective study included women and their families with eclampsia or severe pre-eclampsia delivering between 1931 and 1947. However, some of the women did not have proteinuria, which would now be considered a diagnostic criterion. A later review of previously published family studies suggested that homozygosity for a single recessive gene shared between mother and fetus provided the best fit[3]. The variation between these studies may be explained by the differences in phenotypic definition and population.

IDENTIFYING CHROMOSOMAL REGIONS OF POTENTIAL INTEREST

Genetic mapping builds on epidemiological data that have established a potential inheritance pattern in order to identify which genes may be important. Essentially, genetic mapping of any disease or trait (simple or complex) is the observation that certain chromosomal regions tend to be shared among affected relatives and tend to differ between affected and unaffected individuals.

The three main methods of mapping are functional cloning, candidate gene strategy and positional cloning. Functional cloning is used when the underlying biological basis of the disease is known, and therefore is very rarely applicable to complex traits, for example the isolation of the ß-globin gene in sickle cell anemia. The candidate gene approach involves the investigation of a gene with a known or proposed function in the pathogenesis of the disease. Positional cloning is the process of identifying a gene by locating its position on the chromosome without any previous knowledge of its function. The region of interest is located by using

markers distributed evenly across the genome (a genome-wide screen) before determination of the gene function and role in disease.

Genetic markers

Markers are simply identifiable changes within a chromosome that can be tested for, and a pattern of inheritance followed. Protein and blood group loci were originally popular markers, but these limited progress owing to a lack of variation. These were then superseded by restriction fragment length polymorphisms (RFLPs). A restriction enzyme cuts DNA at a particular restriction site. DNA polymorphisms at restriction sites then produce DNA segments of varying lengths. More recently, microsatellites have been used as markers. Microsatellites are DNA sequences containing between ten and 50 copies of the same repeating sequence, occurring in tandem. Each repeating sequence contains 1–6 nucleotide base pairs. Microsatellite loci show high variability and occur commonly and frequently in the human genome. For these reasons, they are more frequently used as markers than are RFLPs. Variable number tandem repeats (VNTRs) are larger versions of microsatellites. They are also highly polymorphic but, in contrast to microsatellites, are less common, and do not occur as frequently or evenly across the genome. Single nucleotide polymorphisms (SNPs) are the most common type of variation observed in human DNA. They occur on average once every 1000 base pairs. A polymorphism, as opposed to a rare variant, is a single base change within the DNA occurring at a frequency of at least 1% in a population. SNPs can also be used as markers, as they are more common and more stable in comparison with microsatellites. However, they are biallelic (only two common alleles at the locus), and tend to be less informative. However, this biallelic quality can allow easier genotyping, occasionally by automation.

Linkage

Markers are used to identify a chromosomal region of interest based on the principle that genes closely placed on a chromosome will tend to be inherited together. This is the principle of genetic linkage. The recombination fraction ? is a measure of the distance between two loci, so is an indication of the likelihood of recombination between two loci. On average, unlinked loci will be inherited together 50% of the time, hence $\theta=0.5$. Linkage is considered to occur if $\theta=0.05$, i.e. the alleles segregate 19 times out of 20 and a recombination event occurs in only 5% of meioses.

Linkage analysis is the methodology used to map gene loci. The segregation of disease within a family is studied using markers and,

eventually, a marker is identified which is inherited with the disease more often than would be expected by chance. The mathematical analyses employed to define whether or not loci are linked are complex but are essentially based on likelihood ratios, the logarithm of which is known as the LOD score. In brief, the likelihood ratio for any given value of θ is the ratio of the likelihood of the observed data, if the loci are linked, to the likelihood of the observed data if the loci are not linked (θ=0.5). The logarithm to the base 10 of this ratio is the LOD score. It is generally agreed that a LOD score of +3 is adequate evidence of linkage.

Although linkage analysis has been used with much success in monogenic disorders and in some complex disorders such as adult polycystic kidney disease and early-onset Alzheimer's, the same success has not been enjoyed in the investigation of pre-eclampsia. A significant problem is that, although epidemiological studies have documented a familial tendency, a clear pattern of inheritance has not been defined. The major limitation of linkage is that it is model based. Calculation of the LOD score depends on an assumed model for both the trait and marker phenotype. Choice of the correct model requires knowledge of several genetic parameters obtained from epidemiological data, such as pattern of inheritance, number of loci, number and frequency of alleles at each loci and penetrance. Hence, the choice of a suitable model is particularly difficult for complex disorders. If the correct model is chosen, linkage can be a powerful investigative tool, but use of the incorrect model will produce inaccurate analysis. In general, the more complex is a trait or disease, the more difficult it is to use linkage analysis.

Linkage studies and pre-eclampsia

These methods have located regions of potential interest in pre-eclampsia. Family studies have suggested linkage to regions of chromosomes 1, 3, 4, 9 and 18. However, it should be remembered that these studies have assumed a pattern of inheritance that may not be accurate in all populations. The effect of genetic parameters on LOD score estimation was demonstrated in a study of linkage between the eNOS region (a candidate gene important for nitric oxide production) and pre-eclampsia[4]. The maximum LOD score varied between 2.54 and 4.03, depending on which previously published model of inheritance was used.

Allele-sharing methods

Chromosomal loci of potential interest can also be identified by the allele-sharing methods. This involves the study of affected relatives within a pedigree to show that a particular chromosomal region is inherited by affected individuals more often than would be expected

by chance. In other words, inheritance deviates from the expected Mendelian segregation patterns. The chromosomal region is described as identical by descent (IBD) because it is inherited from a common ancestor within the pedigree. The frequency of IBD sharing at a particular locus is then compared with that which would be expected by random variation. In contrast to linkage analysis, the allele-sharing method makes no assumptions about the inheritance of the trait, i.e. it is non-parametric. Therefore, IBD sharing between affected family members will be evident regardless of the presence of phenocopy, incomplete penetrance, genetic heterogeneity or high frequency of disease alleles. However, allele-sharing methods tend to be less powerful than linkage analysis. Affected sib pair analysis is the most straightforward example of this method.

Occasionally, although two relatives have the same alleles at genetic markers, it is not clear from the available pedigree that the alleles have been inherited IBD. In this case the alleles are described as identical by state (IBS). However, statistical and analytical tools are available to approximate IBS to IBD. This is more accurate when a dense collection of highly polymorphic markers has been studied. This non-parametric method of analysis is also known as the affected-pedigree-member (APM) method.

The APM method was used in a genome-wide scan of Icelandic families (343 women with pre-eclampsia). A significant locus was suggested on the short arm of chromosome 2 with an LOD score of 4.7[5]. The genome-wide screen used 440 markers evenly spaced across the genome. Although this is the first reported locus for pre-eclampsia meeting the criteria for genome-wide significance, the phenotypic definition included women without proteinuria. However, the study is large, does not presume a model of inheritance and was carried out in a homogeneous population. This finding has been supported by work in other populations, albeit with a less convincing LOD score.

A further study using the APM method as well as parametric tests suggested linkage with the locus for the endothelial nitric oxide synthase (eNOS) gene on chromosome 7[4]. This is a candidate gene for pre-eclampsia, and linkage was investigated using three markers in this region. Linkage with an LOD score of 3.36 was shown using both non-parametric and parametric tests. However, attempts to replicate these findings in other populations were unsuccessful. Several sites of potential interest have thus been identified but these have yet to be confirmed by the pinpointing of actual genes. There has been a lack of replicability between studies, which may be due to the use of inaccurate models, population differences or the limited power to detect genes of small individual effect. Therefore, unless LOD scores are very high, these studies should be regarded as pointers for future research rather than conclusive evidence.

CANDIDATE GENES AND ASSOCIATION STUDIES

Association studies compare the frequencies of a potential disease-causing gene between affected and unaffected individuals. In contrast to linkage studies and allele-sharing methods, they are not family based, but as a result require a control group. The control group is vital to the study's validity, thus must be carefully matched to the cases. The gene under investigation will usually have a proven or hypothetical function in the disease process under investigation, and be described as a candidate gene. Hence, there is an opportunity to increase biological understanding of the disease, but, at the same time, the selection of candidate genes is limited by our understanding of the disease. A further advantage of association studies compared with linkage studies is increased power to detect several genes of small effect. In addition, analysis is simple using a 2×2 contingency table.

A positive association discovered by this type of study can be interpreted in three ways. First, the allele (allele X) may be a true cause of the disease, in which case the results should be reproducible in any population unless the trait has a different cause in different population groups. Second, the disease allele X may be in linkage disequilibrium with the disease. Linkage disequilibrium occurs when two alleles at linked loci are associated more frequently than would be expected by chance. Third, the positive association may occur as a result of population admixture, for example increased representation of one ethnic group within the case group compared with the controls. For instance, allele X is simply more common in one ethnic group that is over-represented in the case group. To avoid these false-positive results, association studies should be conducted only in homogeneous populations, and stringent criteria applied to the control group. It should be remembered that case-control studies are vulnerable to bias, especially in the selection of subjects.

Once an association has been established using the case-control comparison, transmission disequilibrium testing (TDT) should be employed. This is a family-based test (non-parametric as no assumptions regarding pattern of inheritance are made) that studies distortion of transmission of alleles from heterozygous (informative) parents to affected individuals from the expected probability of 0.5. For example, a parent heterozygous for an associated allele should transmit this allele to an affected offspring more often than not. TDT has occasionally been used in the investigation of the genetic basis of pre-eclampsia. The above study identifying the eNOS region used TDT to support additional findings from parametric testing[4].

There has been an abundance of association studies investigating potential candidate genes in pre-eclampsia. This is largely due to the

relative speed and simplicity of the experimental methods and analysis. Once the samples have been collected, they can be used several times for genotyping studies. However, if this is the case, the appropriate statistical correction should be employed. Unfortunately, the vast majority of these studies have failed to generate reproducible results. The main candidate genes investigated are discussed below.

Thrombophilias

There has been much interest in the genetic causes of thrombophilia as candidate genes, since the identification of thrombotic lesions in placentas from preeclamptic pregnancies. These include factor V Leiden, prothrombin and methylenetetrahydrofolate reductase (MTHFR). The studies investigating the prothrombin mutation have yet to identify an association with pre-eclampsia. However, the findings regarding factor V Leiden and MTHFR remain unclear.

Resistance to activated protein C is the most common familial thrombophilia. Activated protein C is a natural anticoagulant that controls thrombin generation by inactivating factor Va. Substitution of adenine to guanine at position 1691 (G1691A) in the factor V gene causes the arginine residue at position 506 to be replaced with glutamine (Arg506Gln). The resulting protein is called factor V Leiden, and this is resistant to activated protein C since the mutation occurs at a cleavage site. This in turn leads to increased thrombin generation and increased clotting tendency.

Activated protein C resistance is present in 20% of women with pre-eclampsia, and the gene for factor V is located on chromosome 1, hence the interest in factor V Leiden as a candidate gene. Table 1 summarizes some of the studies to date.

MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This generates the active form of folate required for the remethylation of homocysteine to methionine. Reduced activity of this enzyme can therefore cause raised plasma levels of homocysteine, especially in response to low folate. Raised homocysteine levels have been associated with vascular disease, thrombosis and pre-eclampsia. Substitution of cytosine to thymine at position 677 (C677T) in the MTHFR gene produces a thermolabile enzyme with reduced activity, which in turn elevates homocysteine levels. Therefore, MTHFR emerged as a candidate gene for pre-eclampsia. The gene is also found on chromosome 1, one of the chromosomes suggested by linkage studies. Table 2 summarizes some of the studies investigating the association between C677T MTHFR and pre-eclampsia.

Tables 1 and 2 clearly demonstrate apparently conflicting results reported for these two candidate genes. The tables are by no means exhaustive, and studies were selected on the basis of phenotypic

Table 1 Summary of association studies between factor V Leiden and pre-eclampsia

Source	*Ethnic origin*	*Parity*	*Cases*	*Controls*	*p Value*	*OR*	*95% CI*
Dizon-Townson *et al.* (1996)[6]	USA	?	158	403	0.03	—	—
O'Shaughnessy *et al.* (1999)[7]	UK	P, M	283	200	NS	0.96	0.43–2.14
De Groot *et al.* (1999)[8]	Holland	P	163	163	—	1.07	0.51–2.25
Kupferminc *et al.* (1999)[9]	Israel	P, M	34	110	—	5.3	1.8–15.6
Kupferminc *et al.* (2000)[10]	Israel	P, M	63	126	0.001	4.6	1.8–11.6
Rigo *et al.* (2000)[11]	Hungary	P, M	120	101	< 0.001	6.17	1.9–20.0
Kim *et al.* (2001)[12]	USA	P, M	281	360	> 0.13	—	—
Currie *et al.* (2002)[13]	Australia	P, M	48	46	0.52	0.61	0.16–2.31

P, primiparous; M, multiparous; NS, not significant; *p* value not quoted; OR, odds ratio; CI, confidence interval

Table 2 Summary of association studies between C677T methylenetetrahydrofolate reductase (MTHFR) gene and pre-eclampsia

Source	*Ethnic origin*	*Parity*	*Cases*	*Controls*	*p Value*	*OR*	*95% CI*
Sohda *et al.* (1997)[14]	Japan	?	67	98	> 0.02	—	—
O'Shaughnessy *et al.* (1999)[7]	UK	P, M	283	200	NS	1.05	0.74–1.51
Kupferminc *et al.* (1999)[9]	Israel	P, M	34	110	—	2.9	1–8.5
Kupferminc *et al.* (2000)[15]	Israel	P, M	63	126	0.008	3	1.3–6.8
Kobashi *et al.* (2000)[15]	Japan	P, M	73	215	NS	—	—
Rajkovic *et al.* (2000)[16]	Zimbabwe	P, M	171	185	—	1.0	0.5–1.9
Lachmeijer *et al.* (2001)[17]	Holland	?	47	120	0.97	0.92	0.28–3.1
Prasmusinto *et al.* (2002)[18]	Germany-Croatia, Indonesia	P, M	81	99	> 0.5	—	—

P, primiparous; M, multiparous; NS, not significant; *p* value not quoted; OR, odds ratio; CI, confidence interval

definition, sample size, description of ethnic origin and application of statistical methods. There are many reasons for these varying findings; potential problems are outlined below.

Replicability

Once the initial studies suggested an association between MTHFR and factor V Leiden and pre-eclampsia, an obvious method of validating the findings is replication. Although potentially simple, for statistical results to be compared, this replication should be conducted in an independent population, which is both ethnically and epidemiologically similar. In addition, phenotypic definitions and subject selection should be similar, if not the same. True replication is rarely achieved, hence comparing results is difficult. The studies in the tables were conducted in ethnically diverse populations, and this is one reason for different findings.

Although all the studies used the basic definition of pre-eclampsia of hypertension (>140/90 mmHg) and significant proteinuria (>300 mg/24 h) occurring after 20 weeks' gestation and resolving postnatally, there were other variations. Some studies considered only 'severe pre-eclampsia', whereas others excluded HELLP syndrome.

Control groups were also difficult to define and compare between studies. Most studies included both primiparous and multiparous women in both the case and control groups, but most did not match for this variable. Predictably, the pre-eclamptic group often contained more primiparous women. In some studies, the difference in parity even reached statistical significance. One study investigated only primiparous women, and another used blood donors as controls. Three studies failed to describe parity of the participants. These are further reasons for disparate findings, especially since it has been suggested that primiparas and multiparas represent different forms of the disease.

Statistical considerations

p Values and confidence intervals are common statistics quoted in association studies, and, where available, are listed in Tables 1 and 2. Statistical tests calculate how likely it is that any difference observed between two groups could be due to chance variations. This likelihood is the *p* value, or the probability that the difference is due to chance. Convention has arisen that, if the *p* value is less than 0.05, the difference can be considered genuine, as there is only a 5% chance that the difference is due to normal population variation. The differences are then described as significant. Despite *p* values being presented as either significant or non-significant (indicating positive or negative findings),

it should be remembered that this methodology was developed to indicate the weight of the evidence, not to generate an absolute conclusion.

In addition, *p* values do not give any indication of the size of the difference, and are strongly influenced by sample size. If the difference is small, then a large sample size is needed to achieve a significant *p* value, whereas a large difference would be significant with a smaller sample size. For this reason, a relative risk or odds ratio with 95% confidence intervals should also be included, as they indicate the size of the difference. Confidence intervals are a measure of the extent to which the sample represents the whole population and allows for sampling variation. Therefore, a 95% confidence interval gives a range within which the population risk would lie, with a probability of 0.95. Therefore, the narrower is the range, the higher is the likelihood that the risk calculated for the sample represents the population it is taken from. Usually, if the null hypothesis is true and there is no difference between two groups, the 95% confidence interval will contain the value 1 and the *p* value will be greater than 0.05. Therefore, both *p* values and confidence intervals should be presented with the results of association studies, as they aid interpretation of the findings.

Unfortunately, few of the studies in Tables 1 and 2 include both *p* values and confidence intervals, and some simply describe the findings as non-significant rather than quoting actual values, making interpretation difficult. For example, one study describes an odds ratio of 2.9 attributable to the MTHFR polymorphism in pre-eclampsia (Table 2). However, because the 95% confidence interval contains 1, there may be no increased risk associated with this polymorphism. Also, some confidence intervals quoted are wide. For example, the six-fold increased risk associated with the factor V Leiden mutation for pre-eclampsia has a 95% confidence interval of 1.9–20 (Table 1). This suggests that the size of the risk could be as low as two-fold but as high as 20-fold.

Type I and type II errors and statistical power are important concepts when comparing studies of association. A type I error is the acceptance of a significant difference when such a difference does not exist in the population. Even when the null hypothesis is true, chance fluctuations mean that 5% of the time a statistically significant difference will be identified at the 0.05 level. This type of error is important in the earlier reports of an association between pre-eclampsia and factor V Leiden or MTHFR, in that there is a 5% chance of the difference not being genuine. Type II errors occur when, although there is a difference between cases and controls the statistical test does not detect it. This is a possibility when attempts at replication produce negative findings.

Power is the probability of a statistical test reaching significance at a particular level, usually 0.05 or 0.01. In other words, a study with a

power of 88% to detect a difference between cases and controls at the 0.05 level will detect that difference only 80% of the time. It is generally considered that a power of at least 80% when testing at the 0.05 level is needed if the findings are to be considered conclusive. Few of the studies listed in Tables 1 and 2 included a power statistic. It is possible that failure to reach statistical significance has occurred in some studies owing to low statistical power, secondary to small sample size.

It is clear from the above that even when there is a difference in allele frequencies between pre-eclamptics and normal women, the statistical tests will not always detect it. This is one possible reason why the two Japanese studies investigating the MTHFR polymorphism produced differing results[14,15] (Table 2). Also, statistical theory suggests that apparently conflicting results are to be expected, even when good replicability is achieved.

It is therefore difficult to arrive at meaningful conclusions regarding the role of thrombophilic mutations in pre-eclampsia. Both factor V Leiden and the MTHFR C677T appear to be over-represented in pre-eclamptic women in some populations, but not others. This may be due to differences in study design or simply population differences. These mutations may be in linkage disequilibrium with other functionally important polymorphisms (for example, unidentified thrombophilias) in some populations and not in others. In many studies, it is difficult to be confident that confounding factors such as ethnic group have been accounted for. No study has yet utilized transmission disequilibrium testing, as this involves collecting samples from fathers, mothers and babies. Different environmental factors may also have an influence. This is particularly likely for MTHFR, as increased folate and vitamin B_{12} intake can correct the hyperhomocysteinemia. Therefore, routine screening for these polymorphisms cannot be recommended.

Renin-angiotensin system

Although the renin-angiotensin system is activated in normal pregnancy, the pressor response to it is lost. This does not occur in pre-eclampsia; therefore, components of this system have been suggested as candidate genes.

A genetic variant of angiotensinogen (235Thr) was found to occur more commonly in a group of Caucasian women with pre-eclampsia, compared with controls[19]. This study of 41 affected women included only primigravidas, and applied a strict definition of ethnic group. The control group was large (478), but the ethnic group was less well validated. These findings were not confirmed by a UK study of 43 women with pre-eclampsia and 84 controls[20]. Although strict criteria were used to define phenotype, no information regarding ethnic group

was given. Population differences could explain the contrasting findings. However, neither study carried out careful examination of the controls. Despite not confirming the findings regarding the 235Thr variant, the UK study found that another variant in the angiotensinogen gene (dinucleotide repeat allele) was transmitted to the fetus in affected pregnancies more frequently than would be expected by chance, and this allele was in partial positive linkage disequilibrium with 235Thr.

Angiotensin II acts via two membrane receptors in the human, the AT_1 and AT_2 receptors. The AT_1 is responsible for most of the vasopressor effects. The AT_1 gene is on chromosome 3, one of the chromosomes suggested by linkage studies. A case-control comparison between 43 women with pre-eclampsia and 83 normotensive pregnant women at six coding polymorphisms (87, 133, 186, 573, 1062 and 1166) and a CA repeat (variable numbers of cytosine-adenine dinucleotides) in the 3 flanking region did not find any differences in allele frequency[21]. However, pre-eclamptic women homozygous at 573C had higher levels of angiotensin II platelet binding, suggesting a possible role in the disease not detected by the smaller genotyping study.

An extension of this study considered maternal-fetal transmission of the 573, 1062, 1166 and the CA repeat polymorphisms[22]. The 573T allele, which was in partial linkage disequilibrium with the CA repeat, was found to be transmitted to the fetus more commonly in pre-eclampsia, highlighting a potential role for the fetal genotype. Therefore, the renin-angiotensin system remains of interest and further work is required to confirm these findings.

Histocompatibility antigens

Pre-eclampsia is more common in first pregnancies and in women conceiving with a new partner; therefore, immunological factors have been suggested as important in the pathogenesis. Human leukocyte antigen (HLA) molecules are believed to play an important role in trophoblast invasion. Fetal trophoblast does not express classical types I and II antigens (HLA-A, -B, -DR, -DQ, -DP). However, trophoblast cells directly in contact with maternal tissue uniquely express HLA-G, a non-classical Ib molecule. Although linkage studies have excluded a direct link between HLA-G and -DR with pre-eclampsia in Caucasian populations, there has been particular interest in the sharing of HLA types between mothers, fathers and infants. A Scottish study found HLA-DR4 to be more common in women with pre-eclampsia and their babies ($p<0.005$, relative risk 3.1 and $p<0.03$, relative risk 2.6, respectively)[23]. Unfortunately, no confidence intervals were given, and this work has not been replicated. An Italian study also suggested an increase in HLA-DR homozygosity ($p<0.000001$) in pre-eclamptic

women and their partners[24]. Much of the work investigating HLA-G polymorphisms has not suggested an association.

Other potential candidate genes, usually those suggested by hypotheses regarding pathogenesis, such as tumor necrosis factor (TNF), eNOS, lipoprotein lipase and plasminogen activator-I (PAI-I), have also been investigated. Again, the results have been inconclusive, largely owing to problems described above. Future studies should ensure that these problems are addressed with adequate study design.

As the number of reported genetic variants increases, choice of not only the candidate gene, but also the variant, will become important. Polymorphisms in areas of the gene suggested to affect function should be prioritized, since these are more likely to influence disease risk. The population frequency of the variant is also important, since those with low allele frequencies need to be associated with a large relative risk to be detected by an association study. Polymorphisms with population frequencies of greater than 5% are most likely to be important.

EXPERIMENTAL CROSSES

Animal studies of experimental crosses (particularly mice and rats) provide the opportunity to study many offspring from a single set of parents, thereby avoiding genetic heterogeneity. Complex genetic interactions can then be studied with relative ease and in far more detail than in the human. The use of transgenic and knock-out mice is a technique of manipulating genes in intact animals, allowing the functional contribution of a gene to disease to be studied. To study a certain gene, its function can be increased (gain of gene function) as in transgenic mice, or ablated as in knock-out mice.

The use of this technology has increased substantially over recent years, although there have been few studies of direct relevance to pre-eclampsia to date. For example, transgenic female mice expressing angiotensinogen were mated with transgenic males expressing renin[25]. The pregnant females demonstrated a rise in blood pressure in late gestation that resolved after delivery. However, several problems are being realized. First, mice with supposedly the same genotype occasionally demonstrate different phenotypes, producing difficulty with reproducibility and standardization. This is because the phenotypes of genetically manipulated animals are affected, as in any other animals, by the other genes present.

A further problem with transgenic mice is the position effect. The new gene is incorporated randomly into the mouse genome so each contains the gene in a different location. Position of the gene can

substantially influence phenotype owing to different regulatory elements nearby. These problems can be overcome by using inbreeding to produce control mice, differing only in the transgene. However, these congenic mice (congenic strains differ in only a single region of one chromosome) are expensive and time-consuming to produce.

These experimental crosses have provided important insights into the genetic control of placental development in mice. Although there are fundamental differences between mouse and human placentas, gene expression patterns have identified potentially homologous cell types. For example, human extravillous trophoblast and rodent trophoblast giant cells both display invasive behavior, and are found at the outer edge of the placenta. They share expression of matrix metalloproteinase (MMP)-9 and $a_1ß_1$ integrin, both believed to be important for trophoblast invasion. Unfortunately, differences in hormone production have cast doubt on the degree of homology. Since humans are the only species known to develop pre-eclampsia, caution must be applied to cross-species extrapolation of results. Even so, this technology has exciting prospects for the study of placental defects in animals, which may help to focus the study of human placental disease, including pre-eclampsia.

SUMMARY

The investigation of genetic causes of pre-eclampsia is far from straightforward owing to many complicating factors. Issues such as definition and population stratification are paramount. Despite the high likelihood of a genetic contribution from epidemiological studies, there is little concrete evidence for specific genes. However, pre-eclampsia may be a polygenic disorder, and statistical theory expects some negative findings in the replication of previous studies. Future studies should be large, include internal controls and utilize transmission disequilibrium testing to minimize the effects of cryptic ethnic differences. Careful consideration should be given to candidate gene selection. Collaboration on larger-scale studies with rigorous definition of phenotype, for example the multicenter Genetics Of Pre-EClampsia (GOPEC) study, should enable more thorough investigation of candidate genes. Collection of pedigrees in a similar way may help to resolve the pattern of inheritance and improve the success of genetic mapping. Further understanding of the molecular level of the disease may help to focus the choice of candidate genes in the future.

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5

Trophoblast invasion

L. Kenny

INTRODUCTION

The development of the human hemochorial placenta and the maintenance of a successful pregnancy are dependent on the proliferation, migration and invasion of trophoblast into the maternal decidua and myometrium in early pregnancy. The process of trophoblast invasion leads to the transformation of the spiral arteries supplying the intervillous space. These small, narrow-caliber arteries are gradually converted into large sinusoidal vessels, as the endothelium and the internal elastic lamina are replaced by trophoblast. These changes transform the vascular supply to a low-pressure, high-flow system, allowing adequate blood flow to the placenta and fetus. Thirty years ago, morphological examination of placental bed biopsies from women with pre-eclampsia demonstrated shallow invasion of trophoblast and failure of vascular remodelling[1]. However, the mechanisms governing trophoblast invasion in both normal and compromised pregnancies are controversial and remain incompletely elucidated. This is partly because studies of spiral arteries have mainly involved immunohistochemical analysis of placental bed biopsies, while *in vitro* studies have been hampered by a lack of suitable models to examine cellular interactions during invasion directly. Recently, applications of contemporary bioscientific approaches have provided increased insight into the complex process of trophoblast invasion. The literature reviewed in this chapter, concerning trophoblast invasion and putative pathological mechanisms associated with the pathogenesis of compromised pregnancies, focuses mainly on this more recent work.

BACKGROUND

Within the placenta, the cytotrophoblast stem cells differentiate into two populations of cells that are functionally and morphologically distinct[2]. In the first trimester, cytotrophoblast stem cells either fuse to form the syncytiotrophoblast layer or aggregate to form columns of anchoring villous trophoblast. Cytotrophoblast cells in anchoring villi break through the syncytium at selected sites and form multilayered columns of non-polarized extravillous trophoblast cells. These columns physically connect the placenta to the uterine wall and give rise to the extravillous trophoblast cells. Extravillous trophoblast cells follow two different pathways[3]. In one pathway, the cells invade the uterine wall (interstitial invasion), and in the other its blood vessels (endovascular invasion). During normal differentiation, the trophoblast cells destined to be endovascular adopt a more endothelial cell-like phenotype and invade the uterine spiral arteries in a retrograde fashion as far as the myometrial segments[4]. This process probably starts at least as early as at 4–6 weeks of gestation. It has previously been suggested that endovascular trophoblast invasion occurs in two waves, the initial decidual phase being completed by around 10 weeks and the later myometrial phase starting 4–6 weeks later[3]. However, a recent histological study of placental bed biopsies demonstrated a progressive increase in the proportion of myometrial vessels containing endovascular trophoblast from 10–12 weeks' gestation, suggesting that rather than two distinct waves, endovascular migration into myometrial arteries is a progressive process[5]. Immunohistochemical studies of placental bed biopsies suggest that trophoblast cells and endothelial cells transiently coexist on the walls of partially modified spiral arteries, where they migrate along the luminal surface of endothelial cells, invading the vessels and partially replacing the endothelial cells and most of the musculoelastic tissue in the vessel walls. This creates a high-flow, low-resistance circulation that maximizes maternal blood flow to the placental villi at the maternal-fetal interface. There is contrasting evidence as to whether trophoblast cells themselves are important in arterial remodelling. Although it has been suggested that some changes in the decidual vessels occur independently of trophoblast cells as part of the maternal response to pregnancy, there is also strong evidence that invasive interstitial trophoblast cells prepare the decidual spiral arteries for endovascular trophoblast cell migration. The invasive interstitial trophoblast cells may play an important role in inducing further changes either by interactions or factors produced by the interstitial (perivascular) trophoblast cells, or by direct cellular interactions of the endovascular trophoblast cells with the cells of the vessel that they subsequently replace.

In normal pregnancy, invasion of the uterine wall and spiral arteries requires that the cells take on an invasive phenotype, a process analogous to tumor progression, and this is accompanied by a reduction in their proliferative capacity as well as expression of specific proteinases. Invasive trophoblast cells also have altered expression of cell adhesion molecule (CAM) phenotypes and matrix-degrading enzymes.

In pregnancies complicated by pre-eclampsia and intrauterine growth restriction (IUGR), invasion fails to proceed beyond the superficial portions of the spiral arteries. Since interstitially migrating trophoblast cells are still abundant in the placental bed, the ability of trophoblast cells to enter and transform the spiral arteries appears to be a significant difference between normal and compromised pregnancies. It is therefore important to understand the molecular processes that occur during trophoblast invasion, as differences in these mechanisms are associated with placental pathology, including the development of pre-eclampsia.

TROPHOBLAST INVASION AND CELL ADHESION MOLECULE EXPRESSION

Adhesion molecules and integrins play an important role in cell migration, and consequently have been studied extensively in the trophoblast cell.

There are four main classes of CAM: the cadherin family, the immunoglobulin family (platelet endothelial cell adhesion molecule-1 (PECAM-1), vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), -2 and -3 and neural cell adhesion molecular (NCAM)), the selectin family (e.g. E-, P- and L-selectin) and the integrin family. Integrins are made up of two subunits, α and β. To date, approximately 20 integrins made from 14 α and nine β subunits have been identified. CAMs possess a wide range of functions, including maintenance of tissue integrity, cellular signal transduction and regulation of inflammatory and immune responses.

Immumohistochemical and cell culture studies indicate that the repertoire of CAMs expressed by the trophoblast changes during differentiation and invasion. For example, the stem cell population of villous cytotrophoblast expresses E-cadherin, but this molecule is down-regulated in invasive trophoblast[6], which is characterized by the expression of VE-cadherin[4]. *In vitro* studies have reported that VE-cadherin enhances trophoblast invasion whereas E-cadherin inhibits invasion[4].

Similarly, the expression of other CAMs such as VCAM-1, PECAM-1, integrins and E-selectin alters during invasion, such that differentiating cytotrophoblast cells transform their adhesion receptor phenotype so as to resemble the endothelial cells they replace[4].

Immunocytochemical studies have shown that pre-eclampsia is associated with abnormal expression of CAMs by invasive trophoblast cells[7]. Placental bed biopsy specimens from control pregnancies and from those complicated by pre-eclampsia showed that in pre-eclampsia, differentiating/invading trophoblast failed to express integrin CAMs associated with normal pregnancy[8]. Furthermore, more recent studies have reported that in pre-eclampsia trophoblast cells do not express the same repertoire of endothelial CAMs reported for normal pregnancy[7]. Thus, there are several pieces of evidence to link abnormal CAM expression with abnormal trophoblast invasion, suggesting that a defect in trophoblast adhesion molecules may contribute to incomplete or inadequate remodelling of the spiral arteries in pre-eclampsia.

EXTRACELLULAR MATRIX DEGRADATION

The extracellular matrix (ECM) of tissues is composed of a variety of proteins and polysaccharides assembled into an organized network, which are mainly produced by cells within the matrix. To achieve successful invasion, trophoblast cells must induce the repertoire of genes involved in digestion of the ECM. This involves several proteinases, activators and their inhibitors.

The best-studied proteinases are the matrix metalloproteinases (MMPs). MMPs are regulated by their tissue inhibitors (TIMPs) and activated by plasmin, which in turn arises by activation of plasminogen by the plasminogen activators, urokinase PA (uPA) and tissue-type PA (tPA).

Many studies have examined the role of proteinases, their activators and inhibitors in trophoblast degradation of the ECM. MMP-1, MMP-2, MMP-3, MMP-7, MMP-9 and MMP-11 are closely associated with the invasive phenotype of trophoblast. In pre-eclampsia, however, enzymatic degradation of the ECM by trophoblast, is defective. Cells isolated from the placentas of women with pre-eclampsia fail properly to modulate MMP-9 expression and have reduced invasive potential. Furthermore, the enzymatic activities of uPA and plasminogen inhibitor are also altered in pre-eclampsia, suggesting a role for these molecules in the defective invasion of trophoblast in pre-eclampsia[9].

Although there is some evidence to support a role for proteinases, their activators and inhibitors in trophoblast degradation of the ECM, it is probable that there is also interaction with the maternal cellular environment. This contribution is much less clearly understood and further work, particularly in pathological pregnancies, is still required.

OXYGEN TENSION AND TROPHOBLAST PROLIFERATION

During early pregnancy, trophoblast differentiation occurs in an environment of relatively low oxygen tension. At around 10–12 weeks' gestation, when the intervillous space opens to maternal blood, there is an increase in oxygen tension. This increase correlates with the time of maximal trophoblast invasion into the maternal decidua, which allows extravillous trophoblast cells to access and remodel the maternal spiral arteries.

The placental environment of pre-eclampsia has been reported to be relatively hypoxic. The effects of altering oxygen tension have therefore been investigated on both trophoblast invasion and adhesion molecule expression. Under hypoxic conditions, trophoblast appears to enter a proliferative pathway as opposed to the normal invasive pathway of differentiation. Consequently, the trophoblast cells fail to express the molecules involved in migration, adhesion and immune response. The role of several molecules has been investigated using a variety of experimental approaches. Hypoxia-inducible factor-1 (HIF-1) is a transcription factor induced under hypoxic conditions, which activates gene transcription of several genes linked to the development of pre-eclampsia including transforming growth factor-β_3 (TGF-β_3), an inhibitor of trophoblast differentiation. HIF-1α mRNA and protein expression are abnormally elevated in pre-eclamptic placental tissue when compared with normal placental tissue. TGF-β_3 expression has also been reported to be increased in pre-eclamptic placentas, when compared with age-matched controls. This suggests that if, in early pregnancy, oxygen tension fails to increase, or trophoblast does not detect this increase, HIF-1a and TGF-β_3 expression remains high. This will result in shallow trophoblast invasion, predisposing the pregnancy to pre-eclampsia.

IMMUNOLOGICAL ASPECTS

The epidemiology of pre-eclampsia indicates an immune component in the pathogenesis of the disease (see Chapter 6). Primipaternity and pregnancy without prior cohabitation both increase the risk of developing pre-eclampsia. In addition, the increased incidence of the disease in pregnancies resulting from the use of donated gametes, multiple pregnancies and pregnancies associated with an increased placental mass indicates an association with increased fetal antigen load.

Obviously, for the hemiallogenic trophoblast to invade maternal tissue, a mechanism must have evolved for the trophoblast to avoid maternal immune detection. The discovery that human leukocyte antigen (HLA)-G, and not HLA-A or HLA-B, is expressed in extravillous trophoblast

cells led investigators to speculate on the potential role of HLA-G in protecting trophoblast from maternal-fetal immune intolerance. HLA-G is a major histocompatibility tissue-specific antigen of low polymorphism, which is subjected to alternative splicing, yielding four membrane-bound and two soluble forms[10]. The strong expression of HLA-G by invasive trophoblast may, in part, explain maintenance of the fetal semiallograft during pregnancy, with HLA-G inhibiting activation of maternal T and natural killer (NK) cells resident in the decidua[11]. The protection of trophoblast from attack by NK cells is of critical importance, since in a normal pregnancy trophoblast cells associate closely with NK cells at the implantation site. HLA-G is up-regulated as trophoblast differentiates along an invasive pathway *in vitro*[12].

A reduced level of expression of HLA-G has been observed in placental tissue from patients with pre-eclampsia[13]. There may be several possible consequences of low-level HLA-G transcription and protein expression in pre-eclampsia. First, as expression of HLA-G has been demonstrated to protect fetal trophoblast from lysis by maternal NK and T cells resident in the decidua[11], a lack of significantly reduced expression may make trophoblast from pre-eclamptic placentas more susceptible to lysis. Protection of trophoblast from attack by the maternal immune system is of vital importance in maintaining maternal-fetal tolerance. Second, recent work has suggested that the expression of HLA-G is correlated with increased invasiveness, and that HLA-G may be a necessary precondition for invasion[13]. This indicates that, in pre-eclampsia, clusters of trophoblast cells that do not express HLA-G may be unable to invade into maternal spiral arteries.

HLA-G expression on trophoblasts has also been demonstrated to alter the release of cytokines from effector mononuclear cells, with an induction of interleukin-3 (IL-3) and IL-1β and a decrease in tumor necrosis factor-α (TNF-α)[14]. This suggests a role for HLA-G in triggering maternal-fetal immune interplay and thereby maintaining pregnancy. Altered HLA-G expression in pre-eclampsia may, in part, explain the widespread alterations in circulating cytokine levels associated with the disorder.

GROWTH FACTORS, THEIR RECEPTORS AND PROTO-ONCOGENES

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a secreted growth factor composed of two identical subunits linked by disulfide bonds. VEGF action is mediated through two receptors: flt-1 (fetal liver tyrosine-like)

and KDR (kinase insert-domain-containing receptor). VEGF plays a specific role in vasculogenesis and angiogenesis. As vascular remodelling of the placental bed is a critical feature of placental development and is defective in pre-eclampsia, the role of VEGF has been the subject of intense speculation[15]. Circulating VEGF concentrations are elevated in women with pre-eclampsia, and VEGF increases microvascular endothelial cell prostacyclin production in a dose-dependent manner, analogous to the acute effects of plasma from patients with pre-eclampsia. Similarly, in myographic studies, when myometrial resistance arteries are incubated with VEGF, there are dose-dependent alterations in endothelium-dependent behavior, mirroring those found after incubation with plasma from patients with pre-eclampsia[16]. The source of the elevated circulating levels of VEGF in pregnancies complicated by pre-eclampsia is uncertain. Therefore, expression patterns of VEGF have been investigated in normal pregnancies and pregnancies complicated by pre-eclampsia. Expression patterns of VEGF throughout normal pregnancy suggest that VEGF exerts an important role within both the placental villi and the maternal decidua in relation to growth, differentiation and migration of trophoblast[17]. This action is mediated primarily through the spatial and temporal regulation of the flt receptor. Interestingly, when trophoblast cells are cultured in hypoxic conditions, VEGF production is increased[18]. However, *in vivo* studies of placental expression of VEGF are conflicting. Some studies have reported that VEGF is diminished in pre-eclampsia, whereas others have reported no difference in expression. Therefore, although defects in the expression VEGF or cross-talk between the receptors and ligands has the potential to cause definitive invasion of trophoblast and ultimately spiral artery insufficiency, definitive evidence remains to be observed.

Transforming growth factors

TGF-βs are composed of three related dimeric proteins, TGF-β_1, -β_2 and -β_3 and they exert their biological effects through binding to cell surface receptors designated ALK-I, -II and -III. The differential expression of TGF-β in the first and second trimesters is consistent with these molecules having an important role in trophoblast invasion. Specifically, immunoreactivity for TGF-β was found to be strongest at 5–9 weeks of gestation and markedly reduced by 12–13 weeks[19]. *In vitro* studies have reported that TGF-β suppresses invasion of trophoblast in an amnion invasion assay[9]. Moreover, cytometric flow analysis of integrin and an *in vitro* cell migration assay have revealed that exogenous TGF-β up-regulates integrin expression, and reduces the migratory ability of invasive trophoblast[20].

Studies of placentas from pregnancies complicated by pre-eclampsia have shown that there is strong staining of TGF-β_3 and its receptors on syncytiotrophoblast and stromal cells at 27–34 weeks' gestation, whereas at this gestation staining was absent from normal villous tissue[21]. This study indicates that pre-eclampsia may be associated with TGF-β expression by trophoblast cells that persists beyond the first trimester, suggesting that failure of migration is linked to this molecule. However, confirmation of this finding is awaited.

Insulin-like growth factor

Insulin-like growth factors (IGFs) produced by invasive trophoblast and their specific binding proteins (IGFBPs) are believed to interact to regulate trophoblast invasion. A study of transgenic mice has reported that endogenous fetal IGFBP-1 overexpression is associated with a transient impairment of fetal growth in mid-gestation. Maternal decidual IGFBP-1 excess is also associated with impaired fetal growth in mid-gestation, independent of fetal genotype, indicating placental insufficiency. This study also reported that decidual IGFBP-1 overexpression has a marked effect on placental development. Placental morphology was reported to be abnormal in transgenic females owing to altered trophoblast invasion and differentiation. This study provides compelling *in vivo* evidence that IGFBP-1 plays a role in placentation, and suggests that IGFBP-1 may have a pathological role in pre-eclampsia[22].

CONCLUSION

Migration and invasion of trophoblast into maternal spiral arteries and into the uterine tissue are pivotal events in normal placentation. These processes are controlled by a plethora of factors, including the enzymology of ECM degradation, trophoblast differentiation and transcriptional regulation, HLA-G and transforming growth factors. Pre-eclampsia is characterized by shallow trophoblast invasion and incomplete remodelling of the spiral arteries. Therefore, defective expression and activity of the molecules mediating trophoblast invasion are potential candidates for triggering pre-eclampsia. In the past decade, tremendous progress has been made in unravelling the cellular and molecular mechanisms leading to these events. With the advent of increased availability of novel gene knock-out and transgenic mice, it is expected that knowledge in this area will continue to increase exponentially.

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6

Placentation

I. Crocker

INTRODUCTION

As highlighted in Chapter 5, a successful pregnancy depends upon the transformation of decidual spiral arterioles by invading extravillous cytotrophoblast, such that uteroplacental blood flow increases to accommodate maternal and fetal exchange. A failure in this process can result in curtailed blood flow to the placenta and ultimately pre-eclampsia, either with or without associated intrauterine growth restriction.

Meeting the diffusional demands of the growing fetus requires close co-ordination of the maternal and fetoplacenta. The point of exchange in this system is the placental villous 'tree', the finger-like outgrowths of the placenta which remain continuously bathed in maternal blood throughout pregnancy. The correct development of the placental villus, under normal circumstances, is governed by several factors, many of which may be perturbed through pathological events to produce adaptive or maladaptive changes. In the face of impaired uteroplacental blood flow, these changes may be crucial to a successful pregnancy; however, in certain circumstances these adaptations may underscore pathological conditions, including that of pre-eclampsia.

DEVELOPMENT OF THE PLACENTAL VILLUS

To understand fully these placental adaptations, the natural development of the placental exchange system, i.e. components of the placental chorionic villus, must first be considered (Figure 1).

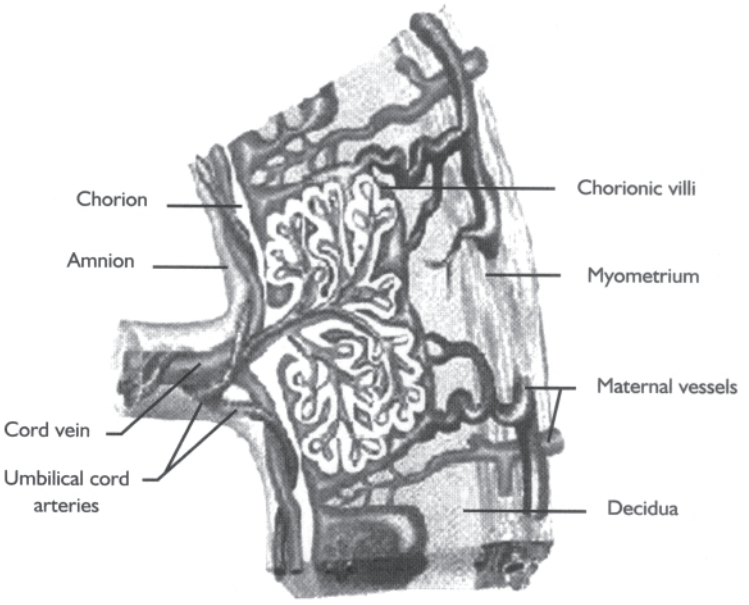

Figure 1 Diagram of maternal and fetal blood flow within the human placenta. The tree-like branches of the chorionic villi are bathed in maternal blood within the intervillous space

The successful implantation of an embryo requires an adequate blood supply in order to develop. This means that blood vessels need to be formed between ovum and decidua, thus establishing a close relationship between maternal and fetal circulations. This process is pervasive, with blood vessels growing and differentiating as a result of two mechanisms: vasculogenesis, meaning new blood vessel formation, and angiogenesis, meaning new branching from pre-existing vessels. Both actions require the interaction of numerous proteins, including cell adhesion molecules, extracellular matrix components, transcription factors and angiogenic growth factors.

At around 21 days post-conception, true vascularization within the placenta begins and capillaries are formed *de novo*. The placental villus at this stage is made up of two components, solid trophoblast-derived primary villus and loose mesenchyme-derived secondary villus. From now until the end of the first trimester, there is a surge in branching angiogenesis. The vascular bed of the placenta expands continuously,

giving rise to new villous outgrowth and new capillary networks. The 26th week of gestation until term sees non-branching angiogenesis predominate, and it is at this stage that mature intermediate villi are formed which specialize in gaseous exchange. These intermediate villi contain elongated unbranched capillaries that terminate in regions of looped and branched terminal villi. These terminal villi are focal points of diffusional activity (Figure 2a). They are coated with an outer layer of trophoblast cells, encapsulating the fetal blood vessels, enclosed by a matrix of connective tissue (Figure 2b).

In early gestation, the terminal villi consist of regions of syncytiotrophoblast (single cytoplasmic multinucleated cells) which overlie a continuous layer of undifferentiated cytotrophoblast. As pregnancy develops, regions devoid of cytotrophoblast appear, such that syncytiotrophoblast achieves direct contact with the basement membrane and lies in close proximity to the fetal blood. At term, areas of the villi contain very thin syncytiotrophoblast layers. The membranes in these areas are closely apposed to the fetal capillaries, and are consequently described as the vasculosyncytial membranes (Figure 3). To aid diffusion, the outer syncytial surface (in contact with the maternal blood) contains numerous and densely packed microvilli. The formation of terminal villi occurs exponentially in the third trimester, so much so that by term the total area for passive or active exchange has reached an astonishing 13 m^2.

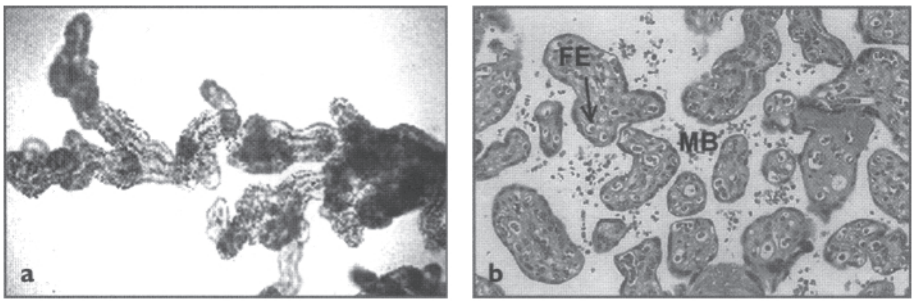

Figure 2 The morphology and capillary structure of the intermediate and terminal villous. (a) A dissected chorionic villus showing intermediate and terminal regions. The ends of the villi are barely larger than a vascular loop, (b) Cross-sectional micrograph of placental villi at term. The mature placenta has small and highly vascularized chorionic villi to support blood gas and nutrient exchange. Fetal erythrocytes (FE) are evident within the villous capillaries and maternal blood (MB) can be seen within the intervillous space

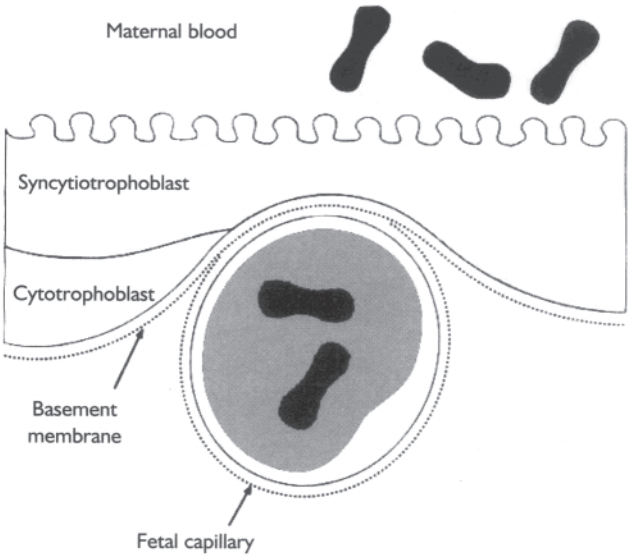

Figure 3 A schematic cross-section through a mature placental villus. In late gestation, cytotrophoblast does not intervene between fetal blood capillaries, syncytiotrophoblast and the intervillous space. This provides one layer of division between fetal and maternal circulations

ANGIOGENESIS AND PLACENTAL DEVELOPMENT

Placental villus development, particularly angiogenesis, is orchestrated by the uteroplacental milieu, under the influence of both maternal and fetal factors. The main participants are growth factors, oxygen concentrations and the maternal immune response[1].

Growth factors in placental development

Within the human placenta, a number of growth factors and their receptors are known to have angiogenic activity, the most studied being the vascular endothelial growth factor (VEGF) family. VEGF itself is expressed in placental trophoblast and circulating levels are detected in maternal plasma as early as at 6 weeks' gestation, rising to a peak at the end of the first trimester, in parallel with circulating human chorionic gonadotropin (hCG). Secreted VEGF mediates its actions via two receptors, VEGF-R1 and VEGF-R2. Binding to VEGF-R2 induces

endothelial cell proliferation, while binding to VEGF-R1 stimulates endothelial tube formation, the combination of which results in branching angiogenesis essential for villous 'tree' formation.

During the third trimester, VEGF decreases while, by contrast, placenta-like growth factor (PlGF) begins to rise. PlGF shares homology with VEGF, and is synthesized locally in villous and extravillous cytotrophoblast. PlGF binds to VEGF-R1 and is believed to contribute to a change in angiogenesis from a branching to a non-branching phenotype, thus controlling the expansion of the total capillary network.

Oxygen and placental development

A second factor implicated in placental angiogenesis is oxygen exposure[2]. The early placenta within the first trimester is established in relative hypoxia, which stimulates cytotrophoblast proliferation and inhibits trophoblast invasion. Only at around 12 weeks' gestation and towards the beginning of the second trimester does maternal flow start and the partial pressure of oxygen begin to rise. It is believed that this increase in oxygen may be the trigger for trophoblast to change and differentiate, thus promoting additional invasion and furthering placental development. A link between attenuated villous morphology and oxygenation has been demonstrated widely, both *in vitro* using explant cultures, and in animal models in response to oxygen inspiration. Locally acting growth factors, such as VEGF and PlGF, which co-ordinate vasculogenesis and angiogenesis, are themselves regulated by variations in oxygen partial pressure. Under normal circumstances the maternal supply and the fetal extraction of oxygen are carefully balanced. However, in response to 'placental hypoxia' the terminal villi become more branched, with excessive capillarization and extensive trophoblast proliferation (Figure 4), possibly to optimize oxygen delivery across the villus.

Maternal immunity and placental development

By definition, half the genes of the conceptus are paternally derived; therefore, the fetus and placenta are antigenically 'foreign'. As such, the reason why the fetus survives *in utero* is largely unknown. Several theories have been proposed to explain this fetal acceptance[3]. Originally it was assumed that the mother was immunologically incompetent; that the uterus was an immunologically privileged site; or that the placenta acted as a barrier to prevent a maternal immune response. Now it is considered that the uterus is not immune-privileged at all, and that the development of the villus takes place in a typically hostile environment. Although placental trophoblast cells are not impervious to maternal immunity they are still resistant to cytotoxic attack. The

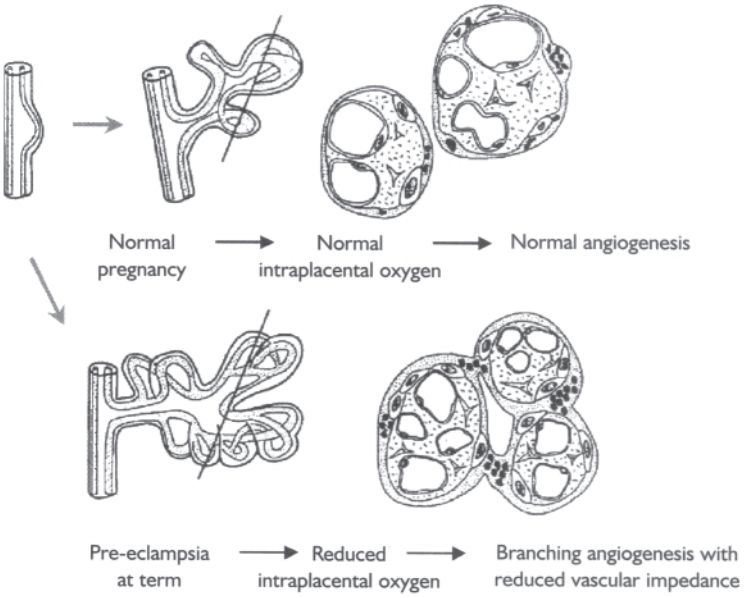

Figure 4 The effects of placental hypoxia on terminal villus development and vascular impedance. Angiogenesis in the placental villus is influenced by intraplacental oxygen. Placental hypoxia induces predominantly branching angiogenesis and reduces vascular impedance

most likely explanation for this evasion is the differential expression of certain histocompatibility antigens, essentially cellular markers that distinguish self from non-self. Placental cells are devoid of histocompatibility (human leukocyte) antigens HLA-A, -B and -C, but preferentially express HLA-G, an antigen which fails to facilitate an immune response. Consequently, a full maternal reaction is never engaged and placental development is allowed to continue unabated.

OXYGEN, GROWTH FACTORS AND THE IMMUNE RESPONSE IN PRE-ECLAMPSIA

For many pathological pregnancy conditions, the histological features in the placenta are uniformly distributed. By contrast, the findings in pre-eclampsia appear to be much more diverse. Many of the structural findings

correspond to those described for maternal anemia and intrauterine growth restriction, including excessive branching angiogenesis, increased capillary volume and number, an increase in villous cytotrophoblast and syncytial aggregates and increased numbers of macrophages[4]. The focal nature of these changes and the inherently varied nature of poor villous development may have prohibited the identification of further parameters in pre-eclampsia, but many of these classical alterations, already established, can be explained by placental adaptations to an irregular maternal environment. These changes are considered adaptive responses, first to increase fetal syncytial surface area for diffusional exchange, and second to optimize fetal blood flow within the uteroplacental unit.

Oxygen in the pre-eclamptic placenta

Pregnancies complicated by early-onset pre-eclampsia typically show reduced uterine artery blood flow. Radioisotope studies in the early 1980s demonstrated a 30–50% reduction in uteroplacental blood flow, compared with normal pregnancies, and more recently color Doppler imaging of proximal uterine arteries and magnetic resonance imaging of the placenta have confirmed these findings. There is therefore considerable evidence to support the idea of reduced blood flow (and thus oxygen delivery) to the pre-eclamptic placenta[5].

As indicated above, a typical response to prolonged oxygen depletion during critical stages in villus development is an increase in capillary numbers to maximize blood flow. Clinical situations other than pre-eclampsia that also result in impaired oxygen delivery likewise culminate in excessive capillarization and similar structural changes within the villus (Figure 4). These include pregnancy at high altitudes, maternal smoking and maternal anemia. Stereological measurements have further indicated an increase in development of the peripheral villous tree in pre-eclampsia, and have also suggested an association with accelerated placental maturation.

Growth factors and the pre-eclamptic placenta

The above-mentioned VEGF may have a pivotal role in the pathogenesis of pre-eclampsia as it has the ability to induce vascular permeability and promote local and systemic coagulation[6] (see below). Maternal VEGF is reportedly increased in pre-eclampsia, and this increase correlates with the severity of disease. In line with our pre-eclamptic model, the expression of VEGF in the whole placenta and in isolated trophoblast is rapidly and reversibly induced by hypoxia, both *in vitro* and *in vivo.* However, while evidence exists to implicate VEGF in the maternal syndrome, there is insufficient evidence to hold it responsible for abnormalities in placental

development. The source of increased levels in pre-eclampsia remains a matter of speculation. Although the placenta expresses VEGF, it is known that this expression decreases, not increases, with advancing gestational age, both in normal and in pre-eclamptic pregnancies.

Like VEGF, PlGF is also regulated by oxygen, and has therefore been implicated in the structural alterations observed in the pre-eclamptic placenta. While early studies have shown that hypoxia has little effect on PlGF expression, recent evidence suggests that hypoxia will down-regulate tissue expression. A further point of regulation may be at the receptor level. Hypoxia up-regulates VEGF-R1 expression, but not that of VEGF-R2. Moreover, soluble receptors, generated by differential splicing, have also been shown to regulate angiogenesis in the placenta. Soluble VEGF-R1 is the natural antagonist of VEGF, and as such reduces the active protein and may have a regulatory role in the angiogenic process.

In addition to VEGF and PlGF, transforming growth factor β_1 (TGF-β_1) can also alter the pathways of trophoblast differentiation. Other growth factors implicated in vessel transformations are the angiopoietin and angiostatin families. Currently no data exist regarding their activities in the developing placenta, in either normal or attenuated placental development.

Immunity and the pre-eclamptic placenta

Classically, pre-eclampsia occurs in either primigravid women or in multigravid women with a new sexual partner; this tends to indicate an immunological origin for this disorder. Recent reports suggest that the clinical features of pre-eclampsia could be explained by an excessive maternal inflammatory response, a mild reaction in the case of normal pregnancy and an over-reaction in the case of pre-eclampsia, one akin to that of toxemia or sepsis[7]. Therefore, pre-eclampsia may be considered as an unwanted maternal insult on the placenta or fetus. Certainly, chronic inflammation has been demonstrated in the decidua and placenta. This activation, attributed to a priming of the innate immune system, probably occurs because of direct interactions between trophoblast and maternal blood. Complement activation, which is also part of this innate system, can also be activated by foreign antigens, and may lead to a strong and sometimes fatal inflammatory outcome. Infection has been highlighted as a possible cause of pre-eclampsia, since a single intravenous dose of endotoxin can induce hypertension and proteinuria in the pregnant mouse. It is therefore possible that infectious agents may be a trigger for pre-eclampsia at certain critical stages in placentation; however, more research needs to be carried out to confirm or deny this.

PLACENTAL PATHOLOGY IN PRE-ECLAMPSIA

The maternal inflammatory response, in the framework of placentation, may be of fundamental importance in the pathogenesis of pre-eclampsia; however, a useful approach to understanding pre-eclamptic pathology is to separate the pathways that prevent full attainment of placental function from those that initiate the maternal syndrome. Whatever is the pathway to villus maldevelopment, optimal function of the placenta may be impaired by several mechanisms, including hypertension, oxidative stress, infarction and cytokine damage.

Hypertension

In the face of poor uteroplacental perfusion, mild to moderate hypertension during the third trimester of pregnancy may be an adaptive response by the mother to maintain flow-dependent transfer to the fetus. Women with established hypertension before pregnancy have an increased risk of developing pre-eclampsia when pregnant. In these circumstances, the myometrial segments of the uteroplacental arteries undergo severe hyperplasia and arteriosclerosis, against the backdrop of high peripheral resistance and high peripheral pressure. With the onset of pre-eclampsia, when hypertension worsens and proteinuria appears, acute atherosis is superimposed upon these hyperplastic arteries, yielding highly severe vascular lesions, the worst witnessed in any hypertensive disorder (Figure 5).

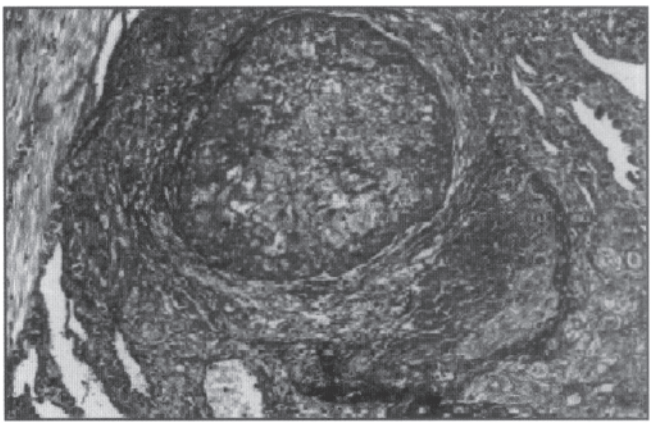

Figure 5 Myometrial segment in pre-eclampsia with superimposed essential hypertension. The uteroplacental spiral arteries show marked hyperplasia, damage by severe acute atherosis and complete obliteration of the lumen (magnification×115)

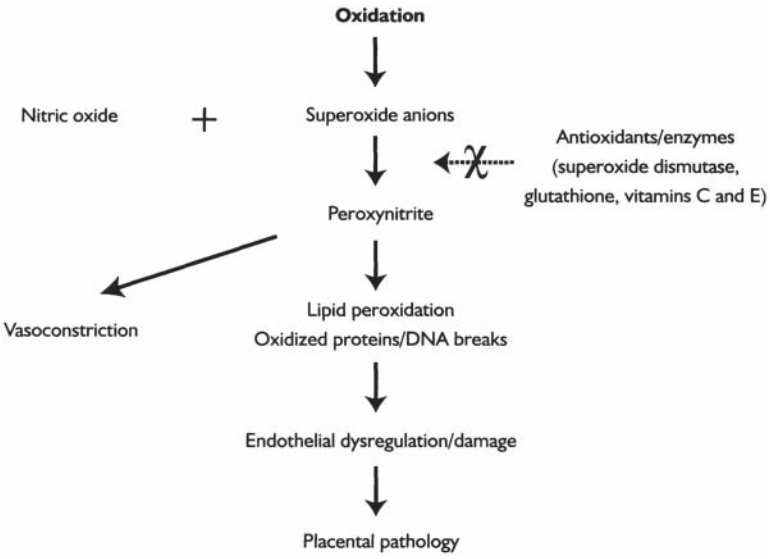

Figure 6 Diagrammatic representation of oxidative stress in the pre-eclamptic placenta

Oxidative stress

Oxidative stress is described as an imbalance between the production of prooxidant forces (reactive oxygen species) and the ability of antioxidant defenses to scavenge these toxic oxygen species. There is ample evidence that increased levels of lipid peroxidation, a hallmark of excessive oxidative attack, are found in the pre-eclamptic placenta[8]. These increased levels could arise from increased production of oxidants, but may also signify a decrease in antioxidant activities. In fact, oxygen free radicals, including superoxide anions, are shown to be elevated in the pre-eclamptic placenta, while the activities of the antioxidants superoxide dismutase, glutathione peroxidase and vitamin E are all reduced.

Blood flow in the placenta is also regulated by reactive oxygen in both an autocrine and paracrine way. Within this system, nitric oxide maintains low resistance and attenuates the actions of vasoconstrictors

(see Chapter 7). Normally, superoxide is scavenged by the superoxide dismutase, which is usually present in excess. However, when superoxide and nitric oxide are increased, nitric oxide effectively competes with superoxide dismutase for superoxide, resulting in peroxynitrite anion formation (Figure 6). This is a powerful, long-lasting oxidant that can alter cellular functions in many ways. It can interact with and cause damage to mitochondria; it can initiate peroxidation; it can cause endothelial dysfunction; and it can also disrupt signal-transduction pathways. The formation of nitrotyrosine, *in vivo,* is thought to be a specific marker for peroxynitrite interaction with tissue. The presence of nitrotyrosine residues in the fetal-placental vasculature and in the placental villus in pre-eclampsia strongly suggests that peroxynitrite formation and action may be involved in the pre-eclamptic process. As well as direct activation of tissue necrosis and damage to endothelial cells, oxidative stress can compromise fetoplacental blood flow through luminal thrombosis, through luminal obstruction by the swelling of endothelial cells and by impairment of endothelial-dependent vasoregulation (see Chapter 7).

Infarction and lesions

In pre-eclampsia, thrombosis of poorly transformed spiral arteries may result in focal areas of placental ischemia and infarction (Figure 7a). A small degree, less than 5%, is considered within normal limits, but where excessive this can overwhelm the diffusion capacity of the placenta, especially if the placenta is vulnerable through villus maldevelopment. Placental infarcts are also features of normal 'aging'

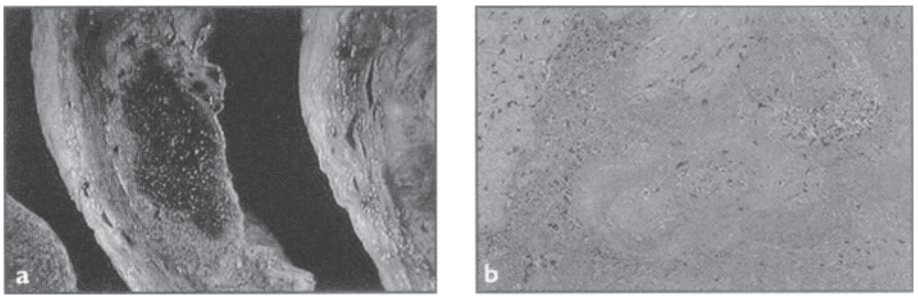

Figure 7 (a) A placenta in cross-section showing infarction as a pale and demarcated region, (b) Fibrin-laden placental villi. Microscopically this region shows coagulative necrosis with loss of trophoblast integrity. The villi are seen as ghost-like circles embedded in the intervillous fibrin

and are found in approximately 25% of uncomplicated pregnancies at term. Certain maternal diseases, such as severe pre-eclampsia, can lead to extensive placental infarcts resulting in excessive fibrinoid deposition (Figure 7b). The importance of fibrin in pre-eclampsia is unknown. However, immunopathological findings suggest that fibrin generation may have a functional connection with immunological and other coagulational responses.

Cytokine damage

Recent data indicate that the placenta may be damaged in pre-eclampsia by the release of proinflammatory cytokines, especially interleukins-6 and -8 and tumor necrosis factor-a (TNF-α)[9]. One theory suggests that these intracellular mediators of inflammation are derived through the activation of leukocytes in the intervillous space, possibly from uncontrolled lipid peroxidation. The initial source of cytokines is probably placental trophoblast or fetoplacental monocytes; however, with rising concentrations, more immune cells from both the maternal and fetal sides are recruited, especially peripheral blood neutrophils and macrophages. The activated leukocytes are believed to serve as circulating mediators that link the increased oxidative stress of the placenta with a widespread increase in oxidative insult and endothelial dysfunction. TGF-β, another multifunctional cytokine, is abundant in the human placenta, and may play a role in tissue repair. In some diseases, excessive TGF-β can compromise organ function by contributing to a pathological excess of tissue fibrosis. It is at present unknown whether this occurs in the pre-eclamptic placenta.

CIRCULATING FACTORS AND THE PATHOGENESIS OF PRE-ECLAMPSIA

Delivery of the placenta causes the resolution of pre-eclampsia, thereby implicating the placenta as the source of a circulating pathogenic factor(s). As yet, no chain of cause-and-effect has been established between the morphological changes in the placental bed (such as inadequate trophoblast invasion) and the generalized endothelial damage and dysfunction that predominate in the preeclamptic mother. A number of circulating factors are currently under investigation that may explain the maternal systemic response. Many of these are derived from the placenta, and can be linked to the adaptive mechanisms already suggested above. These include VEGF, neurokinin-B, activated platelets and neutrophils, TNF-α and products of lipid peroxidation and syncytial membrane degeneration[10].

Vascular endothelial growth factor and the pathogenesis of pre-eclampsia

VEGF is a proposed candidate for the circulating factor in pre-eclampsia, as it is effective in altering vascular permeability and endothelial function. Although the placenta is unlikely to be the primary source, serum levels are elevated and these levels have been correlated with disease intensity. In experimental studies, VEGF has been shown to promote vasoconstriction of resistance vessels in a dose-, time- and endothelium-dependent manner, possibly through the regulation of endothelial prostacyclin or nitric oxide (NO) (see Chapter 7). Vessel incubations with VEGF mirror the vasoactive outcomes seen using serum from pre-eclamptic women, and these serum-induced changes are abolished in the presence of anti-VEGF and anti-VEGF receptor antibodies, confirming VEGF as an important regulator of the maternal vascular system[11]. Further research is ongoing to establish the importance of soluble receptors and binding proteins of VEGF in vascular responsiveness.

Neurokinin-B and the pathogenesis of pre-eclampsia

Neurokinin-B is a neuropeptide, which is expressed in placental tissue and can cause hypertension by preferentially binding tachykinin NK3 receptors. In healthy pregnancy it is thought to contribute to vasoconstriction, as a way of optimizing blood flow within the placenta. In pre-eclampsia, levels are increased further, thereby promoting vasoconstriction on a general systemic level. Unlike VEGF, neurokinin B shows no regulatory effect on vascular reactivity in isolated blood vessel experiments[12].

Neutrophils and the pathogenesis of pre-eclampsia

Neutrophils may contribute to vascular dysfunction as part of a maternal inflammatory response by releasing damaging enzymes from intracellular stores. Toxic oxygen radicals are also released, and these can encourage lipid peroxidation, lysis of endothelial cells and increased vascular permeability and reactivity. In addition to promoting endothelial damage, neutrophils also interact with platelets and coagulation and complement systems, all elements integral to the pre-eclamptic syndrome. The generalized priming and activation of neutrophils has been widely accepted as a feature of pre-eclampsia, possibly in response to proinflammatory cytokines, such as TNF-a[13]. Before these primed and activated cells can mediate vascular damage, they must first undergo adhesion to the endothelial cells. The major adhesion molecules necessary for this recruitment are selectins and integrins. As expression of adhesion molecules is associated with diseases of leukocyte activation,

the possibility arises that receptors may also be involved in the pathogenesis of pre-eclampsia. In support of this proposal, circulating concentrations of both selectins and integrins are higher in women with pre-eclampsia than in those with normal pregnancy[14].

Tumor necrosis factor-α and the pathogenesis of pre-eclampsia

As well as enhancing adhesion molecule expression, TNF-α encourages the formation of vasoactive substances. One such substance is endothelin, a highly potent vasoconstrictor that can release NO and prostacyclin from the endothelium. In addition, TNF-α is shown to induce oxidative stress directly by destabilizing electron flow in the mitochondria, resulting in the release of damaging free radicals and the formation of lipid peroxides. A role for TNF-α is supported in pre-eclampsia by findings that plasma levels are significantly elevated[15]. Pregnant rats, infused with TNF-α, demonstrate elevated arterial pressures and a significant reduction in renal hemodynamics. Whether TNF-α activates the endothelium leading to higher blood pressure, proteinuria and other features of pre-eclampsia is as yet unknown.

Platelets and the pathogenesis of pre-eclampsia

Most investigations agree that low-grade chronic intravascular coagulation in the uteroplacenta is part of a physiological response of all women to pregnancy, and is necessary to maintain adequate uteroplacental blood flow. However, it is now believed that platelet activation has an integral role in pre-eclampsia, promoting vascular obstruction, tissue ischemia and further endothelial malfunction. The reduction in circulating platelets is believed to reflect a reduction in platelet life span in pre-eclampsia, while the concomitant increase in platelet-specific B-thromboglobulin, a marker for platelet activation, has also been correlated with proteinuria and is suggestive of platelet hyperactivation.

Lipid peroxidation and the pathogenesis of pre-eclampsia

In pregnancies with established pre-eclampsia, generalized lipid peroxidation is found in the maternal vascular tissues, in a similar way to that seen in the placenta. In this respect, a recent cross-sectional study found a strong correlation between products of lipid peroxidation and the mean arterial pressures in the mother. In normal pregnancy, combined serum measurements of antioxidants are increased; however, in pre-eclampsia antioxidant levels are diminished, thereby leaving the maternal vasculature open to oxidative attack.

Within blood vessels, the balance between peroxide-generating and peroxide-removing mechanisms controls the rate of prostaglandin generation. Prostacyclin (PGI_2), an endothelial-derived prostaglandin, is an extremely potent vasodilator, an inhibitor of platelet aggregation and a stimulator of renin secretion. Within the body its actions are delicately balanced by thromboxane-A_2, a potent, locally acting vasoconstrictor and proaggregatory agent. Lipid peroxides can inhibit prostacyclin generation but do not influence thromboxane-A_2, thus favoring vasoconstriction of vessels and increased arterial pressures. Early in a normal pregnancy, the excretion of prostacyclin increases, but this does not occur in women who subsequently develop pre-eclampsia. This deficiency is likely to result in increased angiotensin II sensitivity and a subsequent amplification of the vasconstrictor response.

Syncytial fragments and the pathogenesis of pre-eclampsia

Growth is a function of cell replication and destruction. Therefore, apoptosis, or physiological cell death, can be considered a natural process and one that regulates the normal development of the human placenta. In other cell systems, apoptosis follows a precisely orchestrated cascade, culminating in controlled breakdown of the cell and its recognition and removal by phagocytes. In trophoblast, this process is much more complex, as throughout pregnancy villous cytotrophoblast cells divide and fuse with the overlying syncytiotrophoblast to form a multinucleated layer called the syncytium (Figure 8). Within this process, cytotrophoblast recruitment is balanced by syncytiotrophoblast apoptosis, predominantly in areas of segregated nuclei, termed syncytial knots. Under normal circumstances syncytial material, from necrosis or apoptosis, is expelled into the maternal circulation and removed by phagocytes within the lungs. However, in pre-eclampsia, there is growing evidence that this sequence is exaggerated and that these deported placental fragments are the cause of endothelial disturbances and an undesirable maternal response.

As opposed to normal pregnancy, the pre-eclamptic placenta shows an increase in the incidence of apoptosis in the absence of proliferative change[16]. As such, the placental environment contributes to accelerated cytotrophoblast and syncytiotrophoblast turnover. *In vitro* studies using isolated trophoblast and placental fragments have demonstrated that conditions pertinent to pre-eclampsia, such as TNF-α, hypoxia and reperfusion, do indeed induce or encourage placental apoptosis, reinforcing their potential role in pre-eclamptic pathogenesis. Further studies have shown that trophoblast cells are intrinsically susceptible to apoptosis in pre-eclampsia, through the influence of either genetic or environmental factors. One result of exaggerated cell turnover is

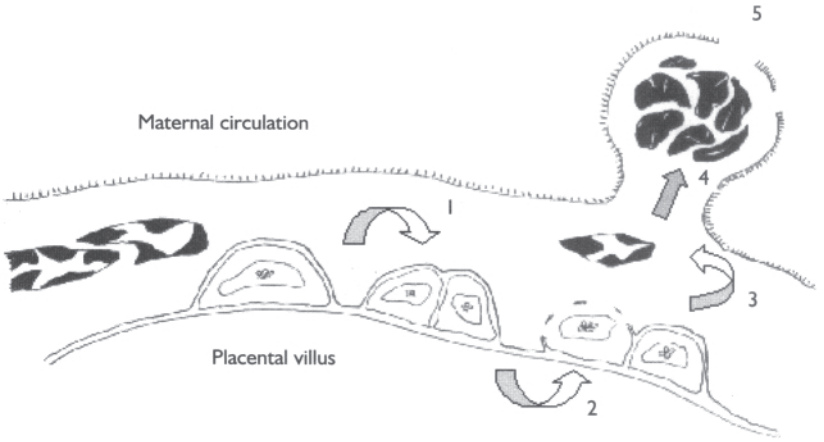

Figure 8 Diagram showing the relationship between cytotrophoblast and syncytium. (1) Cytotrophoblast proliferation; (2) a cytotrophoblast fusing with the syncytiotrophoblast layer; (3) degeneration within the syncytium proceeds; (4) trophoblast nuclei cluster into syncytial knots; (5) syncytial knots protrude into the intervillious space and are eventually expelled into the maternal circulation

the disproportionate deposition of fibrin within the placental villus; a second more crucial outcome is the excessive release of syncytial debris into the maternal circulation. Trophoblast cells of both syncytial and cytotrophoblastic origin are found in uterine veins of normal pregnancy, with higher levels in pre-eclampsia. In laboratory studies, microvillus fragments, similar to those expelled from the syncytium *in vivo,* cause endothelial cell death and dysfunction and stimulate the activation of peripheral blood leukocytes. It is therefore possible that pre-eclampsia occurs when the burden of placental debris is abnormally high, or when the woman's response to this process exceeds that of normal pregnancy[17].

OVERVIEW

Overall, the net functional capacity of the placenta in pre-eclampsia can be considered to be a balance between the responses of the villous tree to ischemia (adaptive versus maladaptive angiogenesis), the degree

of coexistent hypertension and the severity and timing of secondary pathologies. The placenta continues to grow as pregnancy advances, and thus the regenerative capacity of the remaining functional villus may be crucial for sustained fetal health. The alterations in maternal physiology and metabolism seen in pre-eclampsia are likely to be a maternal, fetal or placental response to increase nutrient delivery to the fetus, in effect, an attempt to compensate for an inadequate blood supply within the placenta. Some women will tolerate these modifications and not develop complications; others may partially tolerate these adaptations, resulting in pre-eclampsia, while others will fail to tolerate these changes and proceed to develop severe pre-eclampsia at some stage in their pregnancy.

Pre-eclampsia is undoubtedly a complex condition and cannot be attributed to any single cause. The starting point may be insufficient invasion by trophoblast in early pregnancy; however, the disorder still takes several months to develop. In the absence of further scientific evidence, a plausible hypothesis for its pathogenesis is that reduced placental perfusion, as a result of shallow invasion, leads to increased lipid peroxidation and the release of oxygen radicals without counter-regulation by antioxidants. This, in addition to other factors, leads to the activation of neutrophils and macrophages, which promotes cytokine production, and further encourages maternal endothelium dysfunction. Syncytiotrophoblast fragments elevated through excessive placental apoptosis perpetuate this endothelial response and encourage maternal inflammation. The equilibrium between clotting and anticlotting mechanisms is affected, progressing to a hypercoagulatory state, leading to decreased production of prostacyclin and a simultaneous increase in thromboxane-A_2. The intensification of vasoconstrictor responses to agents such as angiotensin II is further evidence for a disturbance in the maternal endothelial equilibrium, a cornerstone in pre-eclamptic pathogenesis.

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7

Endothelium

M. Wareing

INTRODUCTION

The circulatory system (pulmonary and systemic circulations) allows the pumping action of the heart to create a rapid flow of blood throughout the body. Via the systemic circuit, all the tissues of the body are supplied with nutrients, and waste products are removed to the lungs, kidneys and liver for disposal. The supply of blood to the tissues through the arterial side of the system (arteries, arterioles and into the capillary microcirculation) is governed by physical principles describing fluid flow, hydrostatic pressure and resistance.

The mean arterial blood pressure (MABP) of the cardiovascular system is related to the flow of blood produced by the cardiac output (CO) and total peripheral resistance (TPR) generated by the vasculature, by the equation:

$$MABP = CO \times TPR$$

The control of total peripheral resistance is thought to occur at the level of the vascular tree that includes the small arteries and arterioles. Blood flow within these vessels is related directly, and resistance inversely, to the radius. Thus, *in vivo* blood flow can be markedly altered by small alterations in the caliber of the vessel by a combination of mechanisms under both central and local control.

The walls of arteries are made up of a number of distinct layers which act in concert to control the diameter of the vessel and, hence, the flow of blood within the arterial lumen. Of these layers

the vascular endothelium, originally thought to be an inert monolayer lining the lumen of blood vessels, is thought to play an integral role in the control of organ blood flow and the generation/control of peripheral resistance by its actions on vascular smooth muscle tone.

This chapter examines how the vascular endothelium is thought to interact with both local and central inputs to control arterial diameter via alterations in smooth muscle tone, and hence influence mean arterial blood pressure. The discussion focuses on how the cardiovascular system is thought to adapt to altered demands placed on the mother during normal pregnancy and how current research is suggesting that, in pre-eclampsia, endothelial cell dysfunction is central to the increased peripheral resistance associated with this major obstetric complication.

THE STRUCTURE OF ARTERIES

Structure

The walls of arteries are made up of a number of different layers/cell types (Figure 1), and it is thought that interactions between these anatomically distinct layers control not only the vessel diameter and, hence, the resistance to blood flow through the vessel lumen, but also the vessel permeability characteristics. The vascular endothelium, which forms a semipermeable lining to the vessel, is the innermost layer of the arterial wall directly in contact with the blood flowing through the vessel lumen. As a whole, the endothelial cells form a homogeneous monolayer present throughout the vascular system. The next layer surrounding the endothelial cell layer is known as the internal elastic lamina, the thickness of which varies depending on the level of the vascular tree from which the artery is selected; normally, the thickness of this layer varies proportionally with the diameter of the artery.

The internal elastic lamina separates the endothelial cells from the vascular smooth muscle. Similarly to the internal elastic lamina, the thickness of the smooth muscle layer varies with the level of the vascular tree from which the artery is taken. Vessels adjacent to the capillary beds (arterioles) characteristically have a single layer of smooth muscle cells. This layering of smooth muscle increases proportionally with artery diameter, so that a large conduit vessel, such as the aorta, will commonly have 20–30 layers of smooth muscle cells.

The adventitia forms the outer sheath of the arterial wall, a heterogeneous layer of cells including nerves and connective tissue. The thickness of the adventitia is again dependent on the vessel diameter/location.

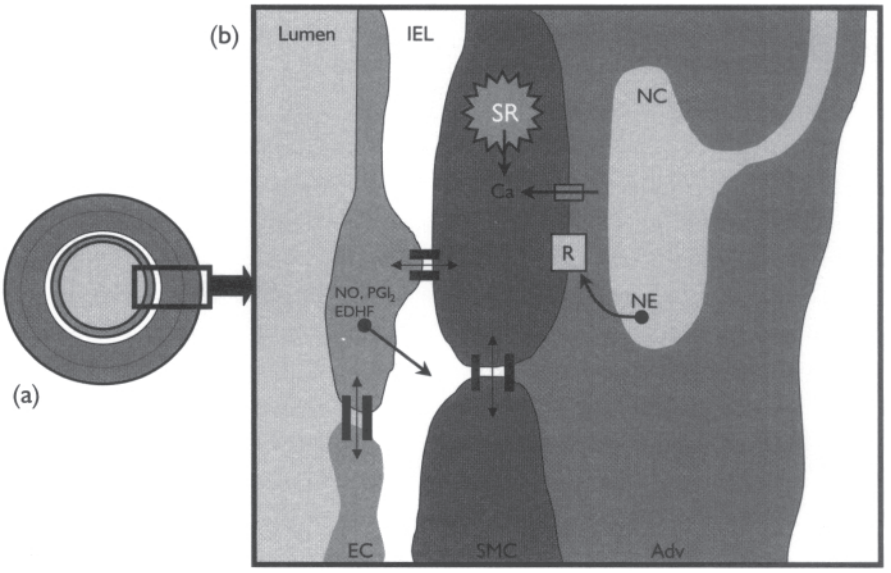

Figure 1 The structure of arteries, (a) Cross-section of an artery, showing the different layers in relation to the arterial lumen, (b) Cartoon of an arterial wall. Release of norepinephrine (NE) from sympathetic neurons (NC) in the adventitia (Adv) stimulates receptors (R) leading to calcium (Ca) entry into smooth muscle cells (SMC). Calcium entry across the cell membrane allied with release of calcium from intracellular stores (SR) promotes smooth muscle contraction. Smooth muscle contraction can be modified by release of vasodilators such as nitric oxide (NO), prostacyclin (PGI_2) and endothelium-derived hyperpolarizing factor (EDHF) from endothelial cells (EC). The presence of gap junctions allows for intra- and interlayer communication between individual cells. IEL, internal elastic lamina. Adapted from reference 1

Communication between layers and control of vessel diameter

There is evidence for communication/cross-talk between individual cells within their own subtype, and also between the different layers of cells within arteries. The presence of gap junctions between endothelial cells permits the spread of electrical current and small molecules over quite large distances laterally, a process that is aided by the longitudinal arrangement of endothelial cells within the artery[1,2]. This may be important in cases where there is localized vascular insult. For example, the presence of an ischemic pocket within a vascular bed may be counteracted by the propagation of signals to more remote

sites upstream of the affected area, leading to vasodilatation and subsequent reperfusion of the affected area.

In endothelial cells, gap junctions are made up of a number of closely related proteins called connexins. At each gap junction between two endothelial cells the arrangement of these connexins varies markedly. By altering the expression of connexin subtypes, there exists the possibility for subtle adjustment of gap junction behavior, allowing for modification of communication between adjacent cells.

There is also evidence using current transduction and dye injection experiments of similar gap junctions between smooth muscle cells. Thus, localized stimuli can spread along the vessel leading to co-ordinated smooth muscle activity within the artery. Indeed, current spread following experimental injection of current into individual vascular smooth muscle cells has been detected several millimeters from the stimulus site. There is also some evidence to suggest that calcium or small molecules from signalling cascades may also pass from smooth muscle cells to adjacent cells via gap junctions.

Interestingly, studies indicate that there is also interlayer communication, via myoendothelial gap junctions between endothelial cells and the underlying smooth muscle cells. Dye and current transfer experiments have suggested the existence of these direct connections between the two cell subtypes. As yet, there have been few reports of myoendothelial gap junctions using electron microscopy. This type of connection has been suggested as a mechanism for the promotion of smooth muscle relaxation by endothelial cells (see section below on 'Endothelium-derived hyperpolarizing factor').

Apart from these direct methods of communication via physical contact between the layers present within arteries, there is of course the possibility for communication by release of factors into the extracellular fluid. Both endothelial cells and smooth muscle cells express a wide range of receptors on the plasma membrane, coupled directly and indirectly to intracellular second-messenger cascades. Receptors on smooth muscle are sensitive to the presence of neurotransmitters, mainly norepinephrine, which acts via adrenergic receptors to produce arterial vasoconstriction; this system is tonically active, producing an intrinsic level of basal tone in the vessel. Neurotransmitters can similarly act on adrenergic and purinergic receptors present on endothelial cells. This leads to the release of vasodilators. Interestingly, neuronal release of norepinephrine can therefore produce opposite effects on vessel tone by direct actions on the endothelium and vascular smooth muscle.

On vascular smooth muscle cells there are also receptors to circulating hormones such as angiotensin and arterial naturetic peptide, as well as local autocoids such as endothelin-1 and

prostacyclin (released from the endothelium; see below), all of which are involved in the maintenance of vascular tone.

Similarly, endothelial cells have receptors to a whole range of circulating hormones and local mediators. Some of these are involved in the regulation of vascular tone. For example, endothelial cells possess receptors for acetylcholine (muscarinic receptors), histamine, arginine vasopressin, bradykinin, adenosine triposphate (ATP), endothelin and angiotensin II. It can clearly be seen that there is scope for subtle control of physiological function of endothelial cells by the interactions between both these local and circulating factors. This leads to the release of, for example, endothelin-1 or prostacyclin from the endothelium, local factors which act on the vascular smooth muscle, providing minute-by-minute control of peripheral resistance (see below). Interestingly, thrombin, through its action on specific endothelial cell receptors, has been shown to increase endothelial permeability to solute and water, and also to be involved in the secretion of von Willebrand factor, suggesting that alteration in the function of endothelial cells is not solely confined to the regulation of vascular tone.

In summary, human arteries are made up of four main layers, the vascular endothelium, the internal elastic lamina, the vascular smooth muscle and the adventitia. Two of the main roles of the arterial wall are the control of peripheral resistance and control of vascular permeability. Communication between cells within each layer and between the different layers within the arterial wall is achieved by gap junctions and via release factors into the extracellular fluid. Endothelial cells and smooth muscle cells have a variety of receptors to hormonal and local mediators.

THE VASCULAR ENDOTHELIUM

Structure

The vascular endothelium forms a continuous monolayer that lines the blood vessels of the entire vascular system. It is continually exposed to physical 'shear' stresses due to continuous blood flow and wall distension caused by the pulse pressure of the vascular system. As noted above, the endothelial cell layer was originally thought to be an inert barrier between the blood and the extracellular fluid, but recent studies have altered this view. It is now suggested that the endothelium plays an active role in the control of blood flow, vessel permeability to solutes and water, hemostasis and thrombosis[3,4]. Indeed, endothelial cells have been demonstrated to express, produce and release a range

of chemical mediators in response to neuronal, chemical and physical factors.

Many of the properties of the endothelium, such as the control of vascular permeability and the modification of blood flow, depend on the maintenance of a functional layer of cells. Thus, endothelial cells must be able to respond/adapt to alterations in the physical stresses (e.g. flow, oxygen tension) under normal physiological conditions and also in pathological situations (e.g. hypoxia, ischemia). Some of these functions require active alterations in cellular shape and/or maintenance of internal structure to counteract external pressure fluctuations on the cell.

Endothelial cells achieve this ability to alter shape in response to physical forces, like muscle cells, by containing all the major components of the contractile apparatus (e.g. actin, myosin II). The amounts and ratios of these proteins to one another in endothelial cells are greater than those in non-muscle cells (lying between those of skeletal and smooth muscle). Endothelial cells also contain several other actin-binding proteins that are involved in modifying and linking the cytoskeleton and cell membrane. The structure of the endothelial cell cytoskeleton has been the focus of much study. Microscopy data suggest that there is a cortical actin filament web, a junction-associated actin filament system and a network of stress fibers, i.e. a well-defined system of interlinked actin filaments (Figure 2). How do these different

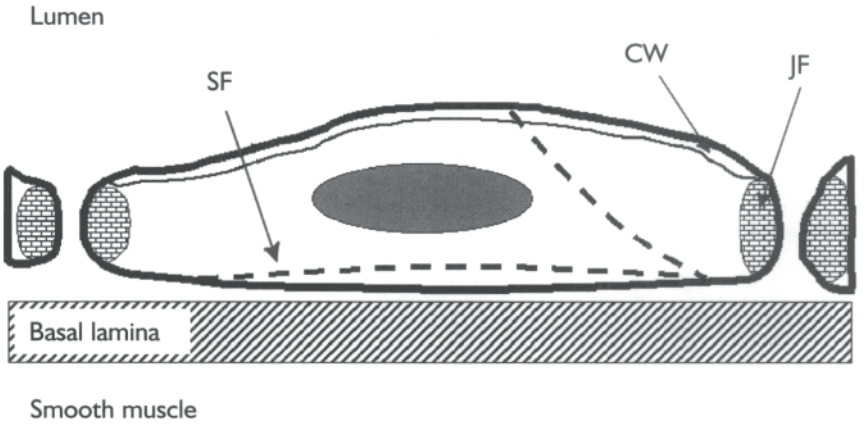

Figure 2 The endothelial cell cytoskeleton. Simplified version of the endothelial cell actin filament system. CW, cortical actin filament web; JF, junction-associated actin filament system; SF, stress fibers. Adapted from reference 4

parts of the cytoskeleton influence endothelial cell function? The cortical web may be involved in the control of vascular permeability since it is thought to be involved in remodelling the plasma membrane, holding integral membrane proteins in position and controlling endo- and exocytosis. Similarly, the junction-associated actin filament system is thought to be involved in regulation of the paracellular pathway and, hence, permeability of the endothelium by controlling the flux of solutes across the endothelium to/from the extracellular compartment. The stress fibers are thought to be involved in the response of the endothelial cell to physical forces. This conclusion stems from a number of observations, including:

(1) Stress fibers are present in all endothelial cells (mainly located on the basal membrane, with their ends attached to areas of the plasma membrane termed focal adhesion plaques);

(2) Stress fibers are more prevalent in arteries, compared with veins (especially in those that come from areas of the vasculature exposed to high levels of fluid shear stress);

(3) In small arteries and arterioles, the endothelial cells are elongated in the direction of luminal blood flow, and here the stress fibers are aligned along the long axis of the cell;

(4) Using monolayers of cultured endothelial cells, the number of stress fibers has also been shown to be increased with increasing flow/shear stress across the monolayer (an effect that is dependent on the duration and amplitude of the stress applied).

The control of this stress fiber modification is thought to involve increases in intracellular calcium, since calcium chelators inhibit the induction of new stress fibers.

From these data it appears that stress fiber modification is integral in the response of the endothelial cell to alteration of physical stress placed upon the cell, although other mechanisms involving G-protein-coupled receptors have also been suggested to have a role in mechanotransduction. Increases in endothelial cell intracellular calcium have also been suggested to be responsible for the release of vasoactive substances, which are thought to be involved in the control of smooth muscle tone (see below).

It can be seen, therefore, that individual endothelial cells are able to respond to physical stresses by modification of the interactions of contractile proteins within the cell milieu, and that stress fiber formation/modification may play an integral role in this response.

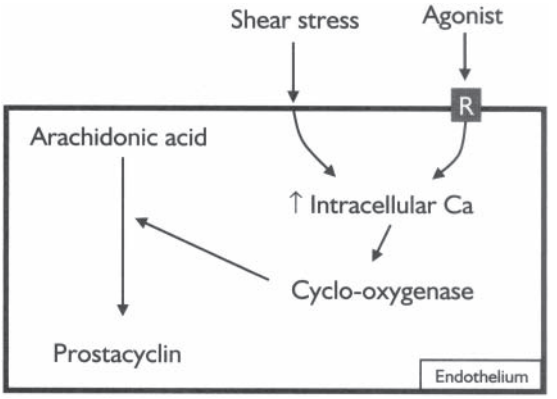

Figure 3 Prostacyclin production in endothelial cells. Physical 'shear' stress- or receptor (R)-induced increases in intracellular calcium lead to production of prostacyclin

Effects of vascular endothelium on smooth muscle tone

The endothelium is able to adapt and respond to the physical stresses placed upon it, and this plays a role in the control of vascular permeability. The detection of subtle alterations in the physical forces surrounding the endothelial cell also forms part of the mechanisms leading to the release factors that influence arterial diameter, and hence affect vascular resistance. These factors include endothelin-1, which induces vasoconstriction, and relaxing factors such as prostacyclin and nitric oxide.

Prostacyclin

The first endothelium-derived factor to be identified was the eicosanoid prostacyclin. Prostacyclin is released from the endothelium in response to rising intracellular calcium caused by the actions of a range of chemical mediators via specific receptors (e.g. thrombin, ATP) or via complex mechanotransduction pathways activated by physical forces such as shear stress (Figure 3). Prostacyclin is a potent vasodilator of smooth muscle, acting on specific receptors on the smooth muscle cells, stimulating the formation of cyclic adenosine monophosphate (AMP) by adenylate cyclase (Figure 4). The increased intracellular pool of cyclic AMP promotes relaxation of the contractile apparatus via actions on two intracellular second messengers, protein kinase A and

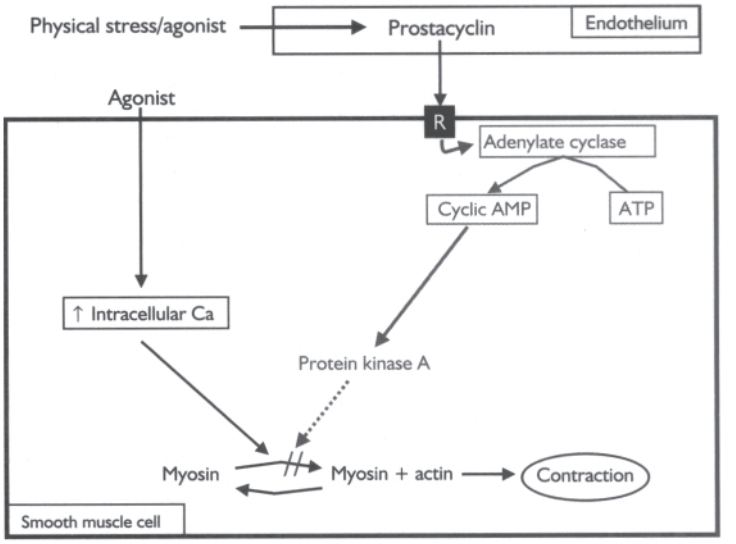

Figure 4 Promotion of vascular smooth muscle relaxation by prostacyclin. Prostacyclin release from the endothelium acting via receptors (R) on vascular smooth muscle cells stimulates cyclic adenosine monophosphate (AMP) production by adenylate cyclase. Smooth muscle contractility is reduced via actions of the intracellular second messenger protein kinase A. ATP, adenosine triphosphate

myosin light-chain kinase. In addition to its actions as a vasodilator of smooth muscle, prostacyclin has also been shown to have inhibitory actions on platelet aggregation.

Nitric oxide

Nitric oxide, originally identified as endothelium-derived relaxing factor, is also a potent vasodilator of vascular smooth muscle. In endothelial cells it is synthesized from L-arginine and oxygen (Figure 5). Similarly to prostacyclin, increased levels of endothelial cell intracellular calcium (stimulated by shear stress and agonists such as acetylcholine and bradykinin) promote nitric oxide synthesis and release from the endothelium (although the increase in calcium for nitric oxide release is thought to be less than that required for prostacyclin). Diffusion of nitric oxide into the vascular smooth muscle cell activates the

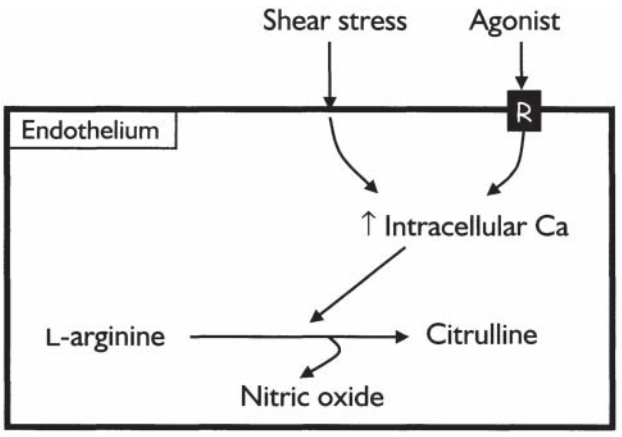

Figure 5 Nitric oxide production in endothelial cells. Physical 'shear' stress-or receptor (R)-induced increases in intracellular calcium lead to production of nitric oxide from L-arginine

production of cyclic guanosine monophosphate (GMP) by the soluble form of guanylate cyclase. This induces relaxation of the smooth muscle cell by a variety of actions on intracellular processes (Figure 6). The production of a range of inhibitors of nitric oxide synthesis has allowed the physiological significance of nitric oxide in the control of vascular tone to be elucidated. Increases in blood pressure and localized vasoconstriction in vascular beds exposed to these inhibitors suggest that the nitric oxide release is continuous, and that nitric oxide acts in concert with constrictive factors in the homeostatic regulation of vascular tone.

Nitric oxide has also been shown to have inhibitory effects on platelet aggregation, and its effects are synergistic to those of prostacylin.

Endothelium-derived hyperpolarizing factor

In many vessels, pharmacological inhibition of prostacyclin and nitric oxide does not lead to an ablation of endothelium-dependent relaxation in response to chemical or physical effectors, suggesting that there is another factor present which can induce relaxation of vascular smooth muscle. Data suggest that a putative factor promotes relaxation of the vascular smooth muscle cell via cellular hyperpolarization, and has led to this mediator being termed endothelium-derived hyperpolarizing factor. At present, the identity of this factor is unknown, but a number

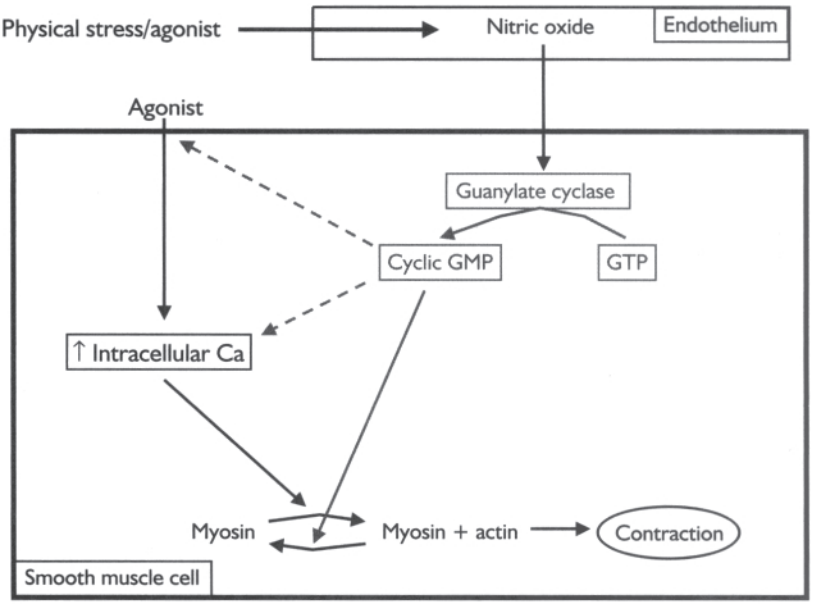

Figure 6 Promotion of vascular smooth muscle relaxation by nitric oxide. Nitric oxide released from the endothelium diffuses into vascular smooth muscle cells where it stimulates cyclic guanosine monophosphate (GMP) production by the soluble form of guanylate cyclase. Smooth muscle contractility is inhibited via a reduction in intracellular calcium and by promoting separation of the actin-myosin complex formed during contraction. GTP, guanosine triphosphate

of candidates have been put forward to account for this phenomenon, including products of arachidonic acid metabolism, endogenous cannabinoids and increased extracellular potassium. Recent evidence suggests that the vascular smooth muscle hyperpolarization may be due to the spread of current between the endothelial cells and the underlying vascular smooth muscle cells via myoendothelial gap junctions. This may negate the requirement for a true chemical factor to promote vascular smooth muscle hyperpolarization. Whatever the identity of this hyperpolarizing/relaxing factor, it is clear that at least some blood vessels have the ability to relax in response to either chemical or physical stressors.

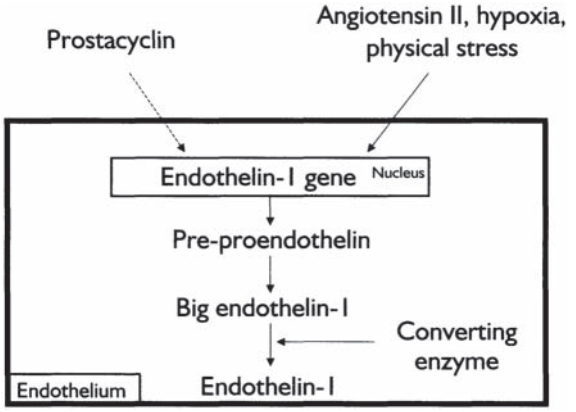

Figure 7 Endothelin-1 production in endothelial cells. Physical stressors or local factors induce transcription of the endothelin-1 gene in the endothelial cell nucleus. Endothelin-1 is produced intracellularly by sequential cleavage of pre-proendothelin

Endothelin-1

Endothelin-1 is a potent and long-lasting vasoconstrictor peptide released from the endothelium in response to a range of stimuli[5]. Endothelin-1 is produced from pre-proendothelin and big endothelin by sequential cleavage (Figure 7). It acts on specific G-protein-coupled endothelin type-A receptors on the underlying smooth muscle, promoting diacylglycerol and inositol trisphosphate production. This induces calcium release from intracellular stores, thereby initiating contraction (Figure 8). By acting on endothelin type-B receptors, which are present on the endothelial cells, endothelin-1 can also induce the release of prostacyclin and nitric oxide.

The long duration of the actions of endothelin-1 suggests that the control of production is at the transcription level of its precursor pre-proendothelin mRNA, but it may be that release is under control of the enzymes involved in cleavage of either pre-proendothelin or big endothelin. Endothelin-1 release is stimulated by angiotenisin II, catecholamines, growth factors, hypoxia and thrombin. As with prostacyclin and nitric oxide, physical forces such as shear stress can also promote release of endothelin-1 from endothelial cells; this release occurs for a prolonged period with a relatively rapid onset. The relatively

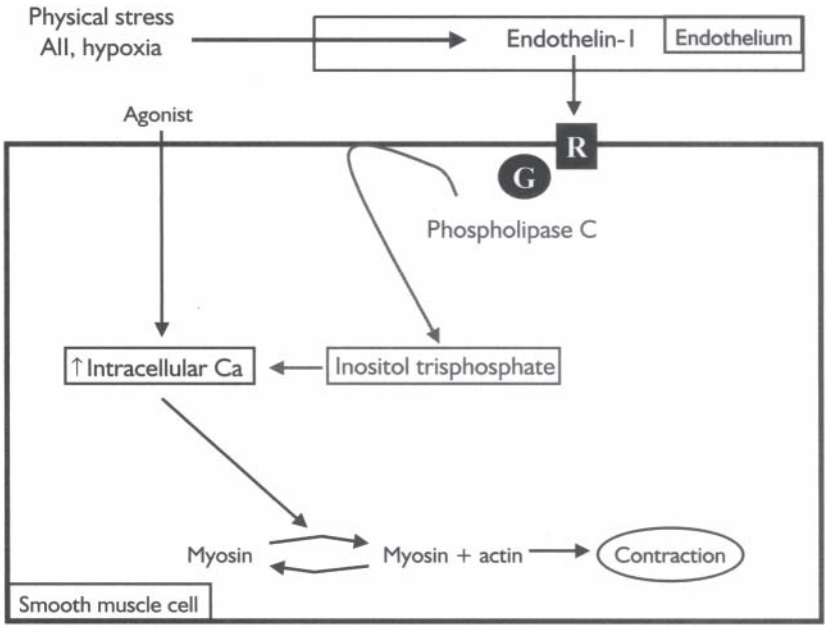

Figure 8 Promotion of vascular smooth muscle contraction by endothelin-1. Endothelin-1 stimulates the production of phospholipase C via the stimulation of receptor (R)-coupled G-proteins (G). Phospholipase C promotes increases in smooth muscle intracellular calcium via the actions of inositol 1,4,5-trisphosphate, stimulating the contractile apparatus. AII, angiotensin II

rapid onset suggests that there is also a pool of endothelin-1 available for release. More work is currently needed to distinguish the controlling mechanisms responsible for endothelin-1 release.

Control of endothelial-dependent constriction and relaxation

We allude above to some of the mechanisms by which the vascular endothelium contributes to the alteration of vessel tone. To summarize:

(1) The release of vasoactive factors such as prostacyclin, nitric oxide, endothelium-derived relaxing factor and endothelin-1 is under the control of neuronal, hormonal and local factors as well as physical factors such as shear stress. The central nervous system promotes the maintenance of basal tone in the vascular system by release of norepinephrine from sympathetic nerves in the adventitia, acting

directly on vascular smooth muscle cells. Neuronal release of norepinephrine or ATP, though, antagonizes this effect by promoting the release of vasodilators from endothelial cells;

(2) Circulating hormones such as angiotensin II, atrial naturetic peptide and vasopressin act via specific receptors on the plasma membrane of endothelial cells to promote constriction or relaxation;

(3) Local factors such as shear stress promote release of vasodilators such as nitric oxide, whereas hypoxia can lead to release of the vasoconstrictor endothelin-1.

It can clearly be seen that there is scope for subtle control of endothelial cell physiology by the interactions between these local and circulating factors. This leads to the release of factors that act on the vascular smooth muscle, balancing vasoconstrictive and vasodilatory influences to provide minute-by-minute control of blood flow via alterations in peripheral resistance.

ADAPTATIONS TO NORMAL PREGNANCY

During normal pregnancy, the main hemodynamic modification is a drop in blood pressure, despite increased cardiac output and increased blood volume (factors that would normally lead to an increase in blood pressure)[6]. The fall in blood pressure, which reaches its nadir in the second trimester of pregnancy before returning to non-pregnant levels by term, can be explained by a reduction in the total peripheral resistance. Data from renal studies of early pregnancy suggest that the fall in peripheral resistance *precedes* increases in cardiac output and volume expansion. Volume expansion is subsequently triggered by the up-regulation of arginine vasopressin and the renin-angiotensin-aldosterone axis in response to increased renal blood flow and glomerular filtration rate (caused by the fall in peripheral resistance).

The reduction in peripheral resistance seen in normal pregnancy could be achieved by a decrease in constrictive factors (a reduced pressor response), an increase in relaxation factors or a combination of both.

Increases in production and secretion of prostacyclin could lead to a reduction in vascular resistance. Data from pregnant rats, though, suggest that indomethacin, an inhibitor of prostaglandin synthesis, does not increase blood pressure. Similarly, assessment of total maternal prostacyclin production by measurement of its metabolites in plasma has failed to provide conclusive evidence for increased prostacyclin production in normal pregnancy. Data from other studies have suggested that localized vascular beds, such as the uteroplacental circulation,

show increased production of prostacyclin in early pregnancy, promoting relaxation, and that production remains high throughout pregnancy. Thus, it may be that prostacyclin is increased in pregnancy, promoting vasodilatation and a reduction in vascular resistance, but that it is acting as a local mediator rather than a circulating hormone.

Data from animal studies have also suggested that the decreased vasoconstriction/increased relaxation associated with pregnancy may be due to increased secretion of nitric oxide[7,8]. Initial data using rats indicated that inhibition of nitric oxide synthesis increased blood pressure in pregnant animals suggesting that nitric oxide is produced under basal conditions. It was noted, though, that the increases in pressure associated with the inhibition of nitric oxide synthesis were not significantly different when compared with non-pregnant control animals, suggestive that nitric oxide production was no greater in the pregnant than the non-pregnant state. Other studies have documented increased plasma and urinary cyclic GMP (a second messenger of the nitric oxide system and a molecule commonly used as an assay for nitric oxide production; see Figure 6) and increased levels of nitrite and nitrate (both oxidation products of nitric oxide) in pregnant rats. These data suggest that nitric oxide production is increased in pregnancy. Similarly, data from guinea-pigs, sheep and dogs have shown that basal nitric oxide production occurs in the fetoplacental and uteroplacental circulations in normal pregnancy. In humans, data from the perfused placental cotyledon and from perfused placental arteries (mounted on pressure myographs) have suggested that nitric oxide contributes to normal vascular tone in the fetoplacental circulation. Additionally, studies have shown increased plasma levels, increased production and increased urinary excretion of cyclic GMP in normal pregnancy, suggestive of increased production of nitric oxide, but other studies have been inconclusive.

Taken together, these data may reflect increased local production but not total production of nitric oxide, leading to vasodilatation of, for example, the uteroplacental and fetoplacental vasculature. In support of this idea is the demonstration of increased expression and activity of the enzymes that produce nitric oxide in human uterine arteries during normal pregnancy.

There is also evidence to suggest that, in normal pregnancy, there is a reduced response to some vasoconstrictors. The renin-angiotensin-aldosterone system is stimulated in early pregnancy, leading to increased circulating levels of angiotensin II and aldosterone. Despite these high levels of angiotensin II, the mother appears to be resistant to the vasoconstrictor effects associated with this peptide hormone. This has been demonstrated using plethysmography to measure forearm blood flow, where normal pregnant women infused with angiotensin II via the

brachial artery showed a reduced pressor response to angiotensin II compared with non-pregnant controls. Similar reduced sensitivities to vasoconstrictors such as arginine vasopressin and epinephrine have been shown, but not for norepinephrine. These data (reduced constriction to angiotensin II, no difference with norepinephrine) have been supported by work directly assessing vascular contractility using omental small arteries obtained at Cesarian section. This reduced pressor response in normal pregnancy has been well documented in animals such as rat, guinea-pig and dog.

The mechanism of the blunted response to angiotensin II in normal pregnancy has yet to be elucidated. Some data suggest that it may be due to a down-regulation at the receptor level, although this has not been supported in work on sheep, and the lack of studies in humans makes this possibility difficult to assess. Other workers have concentrated on post-receptor influences. For example, it has been suggested that up-regulation of prostacyclin and nitric oxide may be sufficient to blunt responses to vasoconstrictors such as angiotensin II through increased smooth muscle cell production of cyclic AMP or cyclic GMP. This non-specific effect on the contractile apparatus would not be sufficient to account for the selective reduction in responsiveness of the vascular smooth muscle to angiotensin II, as, say, compared with norepinephrine.

Much work has focused on the increased production of prostacyclin as the factor that reduces the pressor response. It appears from the data, however, that this may be the case in some but not all vascular beds, although the lack of specific inhibitors of prostacyclin production has made interpretation of these data difficult. Similarly, nitric oxide has received much attention in this regard, but once again animal data have produced evidence both to support and to refute this mechanism of reduced responsiveness to contractile agents.

In summary, current data from both animal and human studies would suggest that prostacyclin and nitric oxide production are increased in normal pregnancy, but that these increases are localized to specific vascular beds such as the uteroplacental circulation and are not a global phenomenon. Coupled with the reduced pressor response to some vasoconstrictors such as angiotensin II and arginine vasopressin, this may be sufficient to account for the reduction in peripheral resistance associated with the normal pregnant state.

ENDOTHELIAL DYSFUNCTION IN PRE-ECLAMPSIA

Pre-eclampsia is defined as new-onset, persistent hypertension after 20 weeks of gestation in association with significant proteinuria (2+ on a dipstick on two separate voided samples or >300 mg/24 h). The

role that defective invasion of the myometrial spiral arteries (Chapter 5) and the placenta (Chapter 6) may have on the pathogenesis of this disease is discussed elsewhere in this book. This section assesses the evidence suggesting that dysfunction of the maternal vascular endothelium is responsible for the wide-ranging maternal symptoms that characterize pre-eclampsia.

The pathophysiological changes associated with pre-eclampsia are indicative of a strongly vasoconstricted maternal circulation, with reduced circulating volume, leading to decreased systemic organ perfusion. The vasoconstriction is thought to be due to increased sensitivity to pressor agents and increased sympathetic nerve activity, producing further increases in vessel tone. There is also activation of the coagulation system and increased permeability of the endothelium (which produces the decreased plasma volume). Since these alterations in normal physiological function are so extensive and intense, it is difficult to form a true analysis of what is cause and what is effect.

Data from a number of sources have suggested that pathological changes are evident in women who develop pre-eclampsia, prior to clinical presentation with the disease, including indicators of endothelial cell activation, platelet activation and increased sensitivity to pressor agents[9]. These data combined with the abnormal glomerular physiology, seen in approximately 80% of women with pre-eclampsia, suggest that dysfunction of the maternal endothelium *early* in pregnancy may lead to the pathophysiological changes that are characteristic of the disease in *late* pregnancy.

Functional alteration of the endothelium in pre-eclampsia has been well documented. Circulating markers of endothelial dysfunction are increased in pre-eclampsia, including von Willebrand factor, thrombomodulin, cellular fibronectin, tissue plasminogen activator and plasminogen activator inhibitor-1, all substances that share the vascular endothelial cell as a common source of production. An increase in endothelin-1 circulating in the plasma of women with pre-eclampsia also suggests increased production of endothelin-1 by activated endothelial cells. Various cell adhesion molecules such as vascular endothelial cell adhesion molecule and growth factors such as vascular endothelial growth factor are also increased in pre-eclampsia. It is well documented that the endothelium plays a role in hemostasis and thrombosis, producing a range of substances that promote (e.g. tissue factor) or inhibit (e.g. thrombomodulin) coagulation. Interestingly, as well as causing vasodilatation, prostacyclin and nitric oxide have also been shown to inhibit platelet aggregation and adhesion. These data support the notion of an inappropriately activated endothelium in pre-eclampsia.

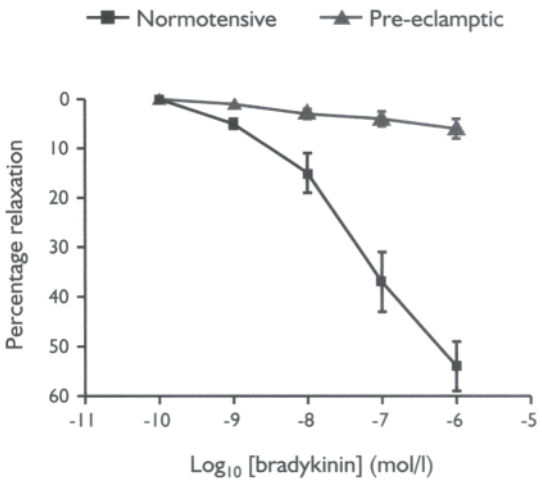

Figure 9 Effect of plasma from pre-eclamptic women on endothelium-dependent relaxation. Percentage relaxation of vessels pre-constricted with arginine vasopressin plotted against $\log_{10}$ bradykinin concentration (mol/l). Incubation of myometrial small arteries taken from normal pregnant women for 1 h with 2% plasma from normal women results in relaxation to increasing doses of bradykinin (squares). Incubation with 2% plasma from women with pre-eclampsia (triangles) leads to an attenuation in this endothelium-dependent relaxation. Adapted from reference 11

Decreased pressor response/alteration in vasodilators

Studies in normal pregnant women suggest that there is a decreased pressor response in early pregnancy, which may be due to decreased sensitivity to pressor agents or an increase in the production of vasodilators such as prostacyclin and nitric oxide (see above). In pre-eclampsia, though, the sensitivity to pressor agents, such as angiotensin II, is increased. It may also be that increased production of endothelin-1 by activated endothelial cells (see above) is involved in this increased pressor response. The first data suggesting altered endothelial responses in blood vessels from women with pre-eclampsia were provided by McCarthy and co-workers, using wire myography[10]. Non-pregnant and normal pregnant subcutaneous arteries showed comparable responses to endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) vasodilators. Vessels from women with pre-eclampsia had a significantly impaired response to acetylcholine. Similar data have since been published from systemic

and myometrial small arteries using endothelium-dependent relaxations to bradykinin (Figure 9)[11].

The role of nitric oxide in this dysfunction in endothelial-dependent relaxation remains controversial. Work with human subcutaneous vessels suggests that in pre-eclampsia the impaired relaxation is due to reduced flow-induced release of nitric oxide. Work on myometrial resistance arteries has shown attenuated responses to bradykinin isolated from pre-eclamptic women, but that the small residual response is entirely mediated via nitric oxide. Data assessing levels of urinary metabolites of nitric oxide in clinical studies of pre-eclampsia are ambiguous, perhaps reflecting the difficulty in drawing conclusions of nitric oxide production in disparate vascular beds using an assessment of overall nitric oxide metabolism (similarly to studies of levels of nitric oxide and prostacyclin in normal pregnancy). Studies using preeclamptic plasma have shown increases in nitric oxide production in cultured endothelial cells and increases in expression of enzymes involved in nitric oxide production.

Studies in normal pregnant subcutaneous and myometrial arteries have suggested that endothelium-dependent responses to vasodilatory agents such as acetylcholine and bradykinin are attenuated but not abolished. These data have been used in addition to those from other studies of non-pregnant vascular beds as evidence for the existence of an endothelium-derived hyperpolarizing factor. It is possible, therefore, that in non-pregnant and normal pregnant women, this hyperpolarizing factor is a significant mediator of endothelium-dependent vasodilatation, but that in pre-eclampsia this factor is diminished, leading to an increase in the pressor response. More studies are required to define the role of a hyperpolarizing/relaxing factor in the control of vessel function in normal and compromised pregnancy.

What prompts endothelial dysfunction?

As noted above, the vascular endothelium appears to be dysfunctional in pre-eclampsia, as indicated by increases in circulating markers of endothelial dysfunction (e.g. von Willebrand factor, cellular fibronectin, etc.)[9]. What is the priming event in pre-eclampsia that causes this shift from a non-thrombogenic to a thrombogenic phenotype with altered endothelium-dependent vascular responses?

Several types of *in vitro* assay have been used to search for a factor or factors that cause endothelial dysfunction, focusing on the use of plasma or serum from women with pre-eclampsia. The presence of a circulating factor(s) stemmed from the observation that central nervous system blockade had a minimal effect on mean arterial blood pressure in pre-eclampsia. This observation suggested that a circulating

hormone or factor must be responsible for the increased vasoconstriction seen in pre-eclampsia. Numerous cytokines and growth factors that are increased in the circulation of women with pre-eclampsia have been suggested as the factor promoting endothelial dysfunction. (The source of such a factor(s) is thought to be the placenta, which releases a factor(s) in response to hypoxic stress experienced in the fetoplacental circulation (see Chapters 5 and 6 for more detail).) For example, using endothelial cells in culture, a range of studies have shown up-regulation of nitric oxide synthesis and increased production of nitric oxide and prostacyclin, and increased secretion of cellular fibronectin, vascular endothelial cell adhesion molecule and platelet-derived growth factor when cells are exposed to plasma from women with pre-eclampsia, compared with plasma from normal pregnant women. These data have demonstrated that serum effects are dependent upon the length of time of incubation, and also that effects are much greater when pre-delivery serum is used, compared with post-delivery. The stimulating factor must therefore have a short half-life in serum. It should be noted that, in these experiments, cultured cells still attach and proliferate as normal in serum/plasma from women with pre-eclampsia, suggesting that a modification in function rather than cell injury and death is responsible for the release of these substances.

A feature of pre-eclampsia is the widespread maternal vasoconstriction and reduction in perfusion of organ systems. Using endothelial cell culture models, it may be surprising, therefore, to find that both nitric oxide and prostacyclin production are increased by exposure to serum or plasma from women with pre-eclampsia. Production of these metabolites depends on the type of endothelial cell under culture and on whether plasma or serum is used in the incubation medium. This observation highlights some of the drawbacks of using the endothelial cell culture model for the study of vascular endothelial dysfunction. By their very nature, these experiments are relatively short-lived (up to 72 h maximum, but very often less than 24 h). Thus, it is difficult to extrapolate data from such a short timescale to describe a disease process that develops over weeks or months. This caveat is highlighted by observations that prostacyclin levels are increased at 24 h of culture, but decreased by 72 h of culture, with plasma from women with pre-eclampsia. Similarly, the source of the endothelial cell culture may also lead to difficulty in determining true endothelial dysfunction. Human umbilical vein endothelial cells are a common cell type used to study endothelial cell dysfunction in pre-eclampsia. These cells are derived from a large conduit vessel, whereas pre-eclampsia is suggested to be a disease of the small-resistance vessels responsible for the production and maintenance of the peripheral resistance. For this reason, some studies have used

microvascular endothelial cells, such as those from bovine tissue or from human skin or decidua. The source of the cell population for study should therefore be carefully ascertained before broad conclusions can be drawn from the data.

Data from another *in vitro* model, using human vessels mounted on wire or pressure myographs, have also suggested that plasma from women with pre-eclampsia can produce alterations in endothelial-dependent vasodilatation. The effects are dependent on the time of incubation and the concentration of plasma in the bath, but alterations in endothelium-derived relaxation have been demonstrated after only 1 h of incubation at 37°C using 2% plasma. Further studies have indicated that this factor(s) responsible for the alterations in endothelial function is heat labile (up to 60°C) and retains its activity after precipitation with ammonium sulfate (70% solution), suggesting a protein/glycoprotein-type molecule. One possible candidate for this circulating factor is vascular endothelial growth factor. Data suggest that vascular endothelial growth factor is increased in women with pre-eclampsia, compared with normal pregnant women, and that it produces alterations in endothelial function similar to those produced by serum/plasma from women with pre-eclampsia using the wire myography technique for studying vessel function. Altered endothelial function can be prevented by the use of a specific antibody to vascular endothelial growth factor in this experimental system (Figure 10).

Another suggested mechanism by which endothelial dysfunction occurs is via increased oxidative stress within the maternal system[12]. This comes from observations of similar pathophysiology of artherosclerosis and pre-eclampsia. Oxidative stress is defined as an imbalance between pro-oxidant and antioxidant forces. Pro-oxidants such as superoxide anions and hydrogen peroxide are continually produced from a number of intracellular sources in all cells. Additionally, interaction of endothelial cells with circulating factors such as low-density lipoproteins or tumor necrosis factor-a can lead to a stimulation of this oxygen free radical production. Oxygen free radicals can interact with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation, an important manifestation of oxidative stress. In pre-eclampsia, placental and serum lipid peroxide metabolites are increased, compared with the levels measured in normal pregnant women. This suggests increased oxidative stress in endothelial cells from women with pre-eclampsia. Vascular function can be affected by oxidative stress through numerous mechanisms, including alterations in nitric oxide and endothelin-1 production. Elevated nitric oxide levels in the presence of increased endothelial cell pro-oxidants may be damaging, since nitric oxide has been shown to react with superoxide anions to produce peroxynitrite, a powerful

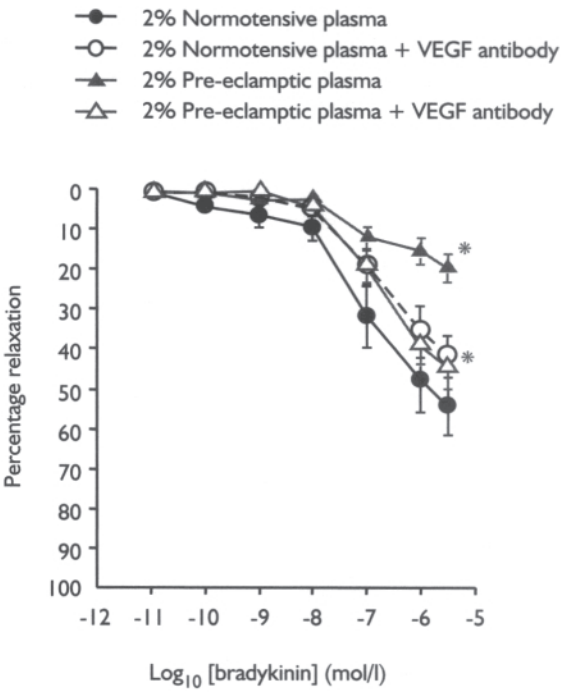

Figure 10 Effect of plasma from pre-eclamptic women on endothelium-dependent relaxation. Percentage relaxation of vessels pre-constricted with arginine vasopressin plotted against log10 bradykinin concentration (mol/l). Incubation of myometrial small arteries taken from normal pregnant women for 1 h with 2% plasma from normal women results in relaxation to increasing doses of bradykinin (solid circles), which is unaffected by incubation with an antibody to vascular endothelial growth factor (VEGF; open circles). Incubation with 2% plasma from women with pre-eclampsia (solid triangles) leads to an attenuation in this endothelium-dependent relaxation, which is reversed in the presence of the anti-VEGF antibody. *p<0.05, significant different. Adapted from reference 13

pro-oxidant. Elevated peroxynitrite levels have been demonstrated in women with pre-eclampsia, suggesting that this pathway may be activated in pre-eclampsia. Altered endothelial function may occur via peroxynitrite acting as a pro-oxidant or by reducing the availability of nitric oxide. Interestingly, it has been suggested that peroxynitrite

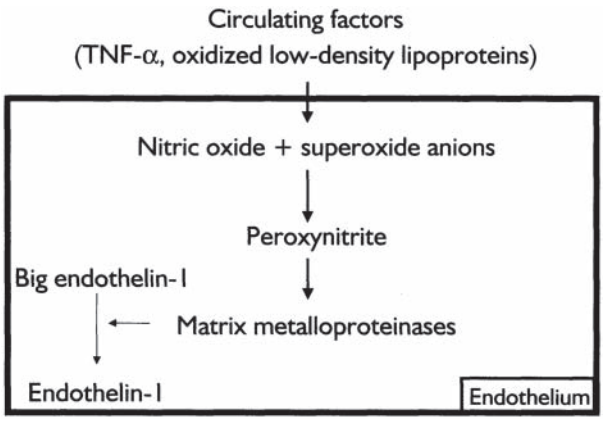

Figure 11 Oxidative stress and pre-eclampsia. Peroxynitrite is formed in endothelial cells from the interaction of nitric oxide with superoxide anions. Altered endothelial function may occur via peroxynitrite acting as a prooxidant, by reducing the availability of nitric oxide or via the actions of matrix metalloproteinases on endothelin-1 production/release. TNF-α, tumor necrosis factor-a

may also have a stimulatory effect via the actions of matrix metalloproteinases on the release of endothelin-1, leading to vasoconstriction (Figure 11).

In summary, multiple factors in the circulation of women with pre-eclampsia could contribute to endothelial cell dysfunction. Specific mediators of vascular dysfunction that may have a predominant role in pre-eclampsia include factors involved in the control of vascular smooth muscle function, factors present in the coagulation cascades and cellular growth factors. There may also be a role for increased circulating levels of pro-oxidants, leading to oxidative stress and endothelial cell activation.

SUMMARY

In summary, small arteries are thought to be responsible for the development of peripheral resistance in the systemic circulation, and therefore have a major role in the control of mean arterial blood

pressure. Arteries are made up of a number of layers of cells that have contrasting anatomical structure and physiological function. The vascular endothelium is thought to play a pivotal role in the control of smooth muscle tone. This is achieved by the release of local factors such as nitric oxide and prostacyclin into the extracellular fluid, and through direct communication with smooth muscle cells via myoendothelial gap junctions. In normal pregnancy, there is a decreased response to pressors that may involve increased production of nitric oxide and prostacyclin in specific vascular beds. In pre-eclampsia, a circulating factor from the placenta leads to maternal endothelial dysfunction, maternal hypertension and proteinuria associated with this important complication of pregnancy.

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8

Screening for pre-eclampsia

F. Reister and J. C. P. Kingdom

INTRODUCTION

About 5% of all pregnant women will suffer some form of pre-eclampsia. As such, this disease continues to have a significant impact on maternal morbidity and mortality, even in countries with well-developed health-care systems. The disease may be associated with intrauterine growth restriction (IUGR) due to placental insufficiency, often in association with uteroplacental vascular disease that can be detected by uterine artery Doppler studies in mid-pregnancy.

Once established, the only known cure for the mother is delivery of the baby. In severe early-onset cases the 'pay-off' is preterm delivery, with consequent risks owing to the need for neonatal intensive care.

The major task of current research is to find ways of selectively identifying those at risk of severe disease, in order to intervene early enough and prevent irreversible maternal vascular injury and placental damage. Increased surveillance for such women may prevent hypertensive emergencies, and unnecessary deterioration of undiagnosed severe disease.

This chapter aims to outline a program to screen women either before pregnancy or in early pregnancy, for early-onset severe pre-eclampsia. This is an important concept, since data currently exist to support the selective use of several prophylaxis agents such as low-dose aspirin, antioxidant vitamins C and E and possibly anticoagulation with heparin to reduce the risk of this disease.

PRE-PREGNANCY SCREENING FOR PRE-ECLAMPSIA

Family and personal history

It is well established that pre-eclampsia has a strong familial tendency[1]. First-degree female relatives of women with a past history of severe pre-eclampsia have a five-fold increased risk of pre-eclampsia. In second-degree relatives the risk of pre-eclampsia is increased two-fold, compared with the general population.

Pre-eclampsia has a baseline risk of 5% in nulliparous women. The risk is reduced to <1% in a second pregnancy with the same partner, following a normal first pregnancy. A pregnancy interval of >10 years appears to restore the baseline risk to that of a first pregnancy[2].

Conception with no or minimal prior exposure to male ejaculate increases the risk of developing pre-eclampsia, compared with women who conceive following repeated ejaculate exposure, for example as a result of prolonged (>1 year) sexual activity protected by hormonal contraception. Pregnancy in a lesbian couple carries increased risks of pre-eclampsia via the same mechanism.

Pregnancies resulting from assisted conception methods are at increased risk of pre-eclampsia, independent of the increased risks resulting from multi-fetal pregnancy.

One of the strongest risk factors for pre-eclampsia is a history of pre-eclampsia in a previous pregnancy. In general, the risk depends on:

(1) The time of onset of the previous pre-eclampsia: the earlier the start of the disease the higher the recurrence risk (in cases of severe pre-eclampsia during the second trimester the recurrence risk may be as high as 65%);

(2) The severity of the previous pre-eclampsia: systolic blood pressure >160 mmHg is associated with a higher recurrence risk;

(3) The time to resolve the previous pre-eclampsia: hypertension and proteinuria lasting >10 days will increase the risk;

(4) The type of pre-eclampsia in the previous pregnancy: HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome and eclampsia have an especially high risk of recurrence.

Thrombophilia and pre-eclampsia

A prior history of deep venous thrombosis (DVT) and/or pulmonary embolism increases the risk of developing pre-eclampsia when pregnant. The risk may be modified owing to heparin treatment, since

heparin may reduce the risk of placental thrombosis-infarction. Nevertheless, these data suggest that underlying prothrombotic disorders may increase the risk of pre-eclampsia even in women with no prior history of venous thromboembolism.

Autoimmune diseases, especially systemic lupus erythematosus (SLE), are associated with venous thromboembolism and an increased risk of pre-eclampsia. The risk of pre-eclampsia may be as high as 50% in the presence of biopsyproven renal disease[3], in the presence of chronic hypertension or if conception occurs while the disease is active. The antiphospholipid antibody syndrome (APS) may cause recurrent miscarriages, and a higher risk of pre-eclampsia than in the general population. Pre-eclampsia with coexistent IUGR due to placental thrombosis is especially common when APS women have a strongly expressed lupus anticoagulant; this is recognized by a prolonged activated partial thromboplastin time (APTT) test (typically >60 s) with normal clotting factors and correction by the *in vitro* addition of normal plasma. Nephritis and vascular disease frequently complicate the rare disease scleroderma, where the risk for pre-eclampsia is around 30%.

Genetic thrombophilias, when expressed as individual maternal (or fetal) heterozygous mutations, may increase the risk of pre-eclampsia 2–3-fold[4]. The extent of the association is controversial because of differences in case-control study design and in populations studied. The extent of the association will be determined by large high-quality population-based studies currently in progress. The subject is discussed in Chapter 4. Homozygous maternal mutation for factor V Leiden, while rare, confers a five-fold increased risk of pre-eclampsia, often with associated IUGR. Compound heterozygotes, or carriers with associated antiphospholipid syndrome, are at increased risk of pre-eclampsia[5].

Polygenic inheritance makes an important contribution to the risk of pre-eclampsia. These ill-defined genetic pathways may confer an increased risk of pre-eclampsia by several mechanisms: an increased rate of oxidative stress, an increased vulnerability to oxidative vascular injury or other mechanisms that lead to increased systemic vascular resistance. The so-called 'syndrome X' is a combination of obesity, diabetes and hypertension, with a central feature being insulin resistance and hyperinsulinemia. As a consequence, complex disturbances in lipid metabolism (hyperlipidemia, hypercholesterinemia and decreased high-density lipoprotein/low-density lipoprotein (HDL/LDL) ratio) may result in premature atherosclerosis and increased vascular oxidative stress. The relationship of this disease to pre-eclampsia is discussed in Chapter 15.

Renal diseases in general increase the risk for pre-eclampsia. Maternal renal biopsy following early-onset pre-eclampsia reveals that up to 20% of women have previously undiagnosed renal glomerular disease, the most common lesion being imunoglobulin A (IgA)

nephropathy[6]. Some of the renal pathways leading to the development of pre-eclampsia are being dissected by transgenic studies in mice. For example, the renal-specific knock-out mouse for podocyte vascular endothelial growth factor (VEGF) develops pre-eclampsia and the typical histological glomerular changes seen in human pre-eclampsia[7].

Paternal/fetal influence

Pre-eclampsia is regarded as a typical disease of the first pregnancy. However, recent epidemiological data have shown that the risk of pre-eclampsia is conferred by *de novo* pregnancy with a new partner, and that the risk of pre-eclampsia can vary by *specific* partner. Some men confer a high risk of pre-eclampsia to women, presumably via genes they express in the developing fetus/placenta. Pregnancy with a new partner is thus an important clinical risk factor for pre-eclampsia.

Pre-pregnancy/early pregnancy risk classification

From a practical standpoint it seems reasonable to classify a woman at her first prenatal visit into one of these three risk groups:

(1) The 'low-risk group': this comprises healthy multiparous women with a history of uneventful term pregnancies, a small (<2 year) interval since the last pregnancy and pregnancy with the same partner.

(2) The 'intermediate-risk group': this comprises all nulliparous women, women with a new partner and any woman with a first-degree family history of pre-eclampsia. This group also includes well-controlled insulin-dependent diabetics or chronic hypertensive patients without signs of vascular damage, as well as moderately obese women and women with heterozygous factor V Leiden mutation. This group has an increased risk of pre-eclampsia, although most cases will be at term, with a lesser risk of perinatal problems (see below).

(3) The 'high-risk group': this comprises women with a history of severe early-onset pre-eclampsia or with significant medical problems suggesting vascular disease or renal disease, long-standing poorly controlled diabetics and significantly obese women. This group is at particular risk of perinatal morbidity and mortality owing to early-onset disease.

SCREENING FOR PRE-ECLAMPSIA IN EARLY PREGNANCY

During the first trimester of pregnancy, development of the placenta and the establishment of normal maternal and fetal placental

circulations are crucial steps for a successful pregnancy outcome. The initial events in placentation center on the invasion of uterine tissues by fetally derived extravillous cytotrophoblast cells (Figure 1). This invasion of maternal tissues begins as early as 12 days after conception. Important maternal cardiovascular adaptations take place in parallel, the overall effect being the promotion of maternal blood flow to the implantation site. First-trimester bleeding is a common complication of pregnancy, and may represent focal abnormalities of early placentation; first-trimester bleeding is associated with a two-fold increased risk for developing pre-eclampsia[8].

In women with a history of pre-eclampsia, or background risk factors (see above), the rapid rise in plasma volume expansion is impaired compared with healthy pregnant women[9]. Pre-pregnancy, the follicular-luteal phase changes in plasma volume are also blunted in women who will develop pre-eclampsia[10]. These data suggest that women with underlying disorders of volume homeostasis are at increased risk of developing pre-eclampsia. The basis for this is currently unknown. The invasive nature of these tests limits their clinical applicability, although more careful attention to weight gain in the first trimester may identify women with poor volume expansion.

SCREENING IN THE SECOND TRIMESTER

Hemodilution

In normal pregnancy, the disproportionate rise in plasma volume above that of red cell mass leads to a decrease in hematocrit and hemoglobin concentration values. This is misleadingly referred to as 'anemia in pregnancy'. Nevertheless, this fall in hemoglobin in the second trimester reduces the risk of pre-eclampsia and associated small infants, compared with women who fail to reduce their hemoglobin concentration[11]. A second-trimester hemoglobin level of >120 g/l is associated with a three-fold increase in the incidence of pre-eclampsia[12].

Blood pressure

In the course of a healthy pregnancy, the blood pressure drops during the second trimester. In patients destined to develop pre-eclampsia, the mean arterial pressure during the second trimester is higher than in women who remain normotensive until delivery[13]. However, the low sensitivity and specificity limit this as a screening test in isolation.

The diagnostic value of changes in blood pressure in mid-pregnancy can be improved by repeated long-term ambulatory blood pressure monitoring. Hermida and colleagues[14] used this approach to

demonstrate, in addition to the absent blood pressure fall during mid-pregnancy, a rise in blood pressure as early as the end of the first trimester in women destined to develop pre-eclampsia. Moreover, ambulatory monitoring has revealed that blood pressure tends to fall when healthy pregnant women are asleep at night, whereas this 'nocturnal hypotension' may be absent in women destined to develop pre-eclampsia[14,15]. Ambulatory blood pressure monitoring (ABPM) offers great promise for community-based screening for pre-eclampsia[16]. ABPM may be used to refine the test characteristics of uterine artery Doppler for the identification of women at greatest risk of early-onset pre-eclampsia[17]. Where ABPM fails to demonstrate a circadian rhythm in blood pressure, the risk of pre-eclampsia is increased 3–5-fold.

Uteroplacental perfusion

Uteroplacental blood flow increases exponentially from about 50 ml/min preconception to over 800 ml/min in the third trimester. Most of this change occurs in the late first and early second trimesters, preparing the uteroplacental unit for the rapidly increasing metabolic demands of the fetus.

Promotion of maternal blood flow to the implantation site is achieved via invasion of the fetally derived extravillous cytotrophoblast cells into the uterine stroma (interstitial invasion), where they surround and invade the muscular walls of the spiral arteries (Figure 1, left panel). The mechanisms by which these cells facilitate dissolution of the vessel medium are currently unknown. These vessels are also denervated during trophoblast invasion, thus losing their ability to constrict. The vessel medium becomes replaced by extracellular matrix produced by the trophoblast cells, and the vessels reach a diameter of eight times that in the non-pregnant state.

These morphological changes are associated with a progressive decrease in impedance to uteroplacental blood flow. These changes can be observed by color and pulsed Doppler ultrasound of the proximal uterine arteries (Figure 2, upper panel). The increased blood flow delivers a greater volume of blood into the intervillous space. Most of these histological and functional changes take place between the 11th and 20th weeks of gestation (Figure 3).

In placental-bed biopsies from women with early-onset pre-eclampsia, the transformation of the spiral arteries from narrow high-impedance vessels to low-impedance wide conduits does not occur normally. Extravillous cytotrophoblast cells seem to invade into the interstitium of the maternal decidua and proximal myometrium, but surround, rather than invade, the spiral arteries. A maternal inflammatory response is present in the vessel wall, represented by a leukocyte infiltration (Figure 1, right panel). Impedance to blood flow remains high, and blood volume

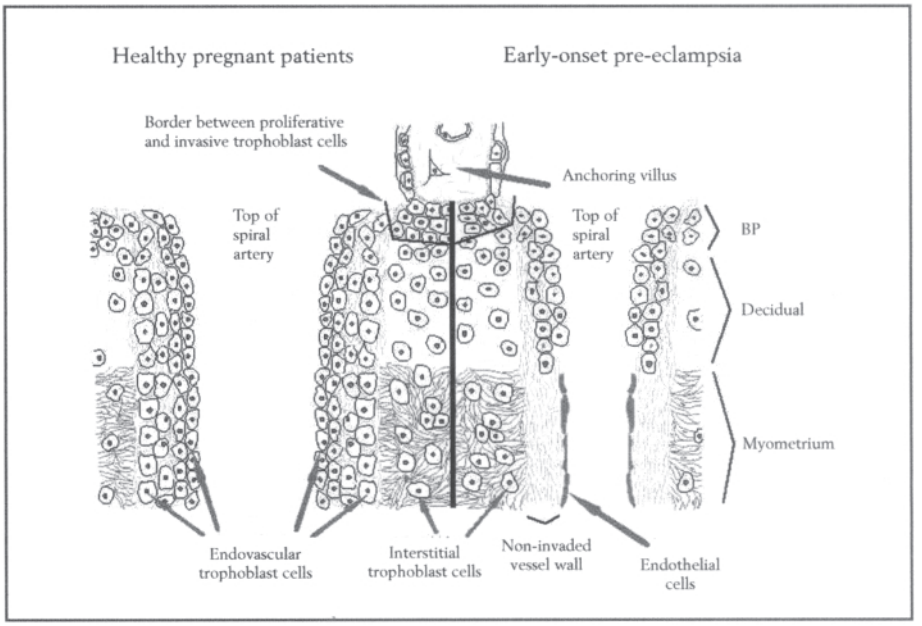

Figure 1 Fetally derived extravillous trophoblast cells are invading the maternal uterine wall (left panel). They are populating the tissue between the maternal vessels as well as the walls of the spiral arteries. This leads to a transformation of these arteries into wide-lumened conduit vessels capable of delivering a high volume of blood into the intervillous space, at a low flow and pressure. In pre-eclampsia, due to impaired trophoblast invasion into the wall of the spiral arteries, this transformation is insufficient and confined to the decidua (right panel)

expansion fails to occur, compared with healthy pregnancies (Figure 2). Doppler ultrasound of the uterine arteries (Figure 3, lower panel) therefore shows persistence of pulsatile, high-impedance flow that is characteristic of first-trimester blood flow, with a persistent early diastolic notch.

During the first half of pregnancy the development of the placenta and the fetus will not be compromised by impaired development of the uteroplacental circulation, because the nutritional demands of the fetus are small in absolute terms. As soon as the maternal blood supply is unable to maintain the fetal and placental demands, the placenta typically increases the vascularization of its villi (to increase fractional extraction of oxygen)[18] and where this mechanism fails to occur, fetal growth is limited and IUGR occurs.

The role of mid-pregnancy uterine artery Doppler as a screening test for pre-eclampsia has been under investigation now for almost 20 years. Early

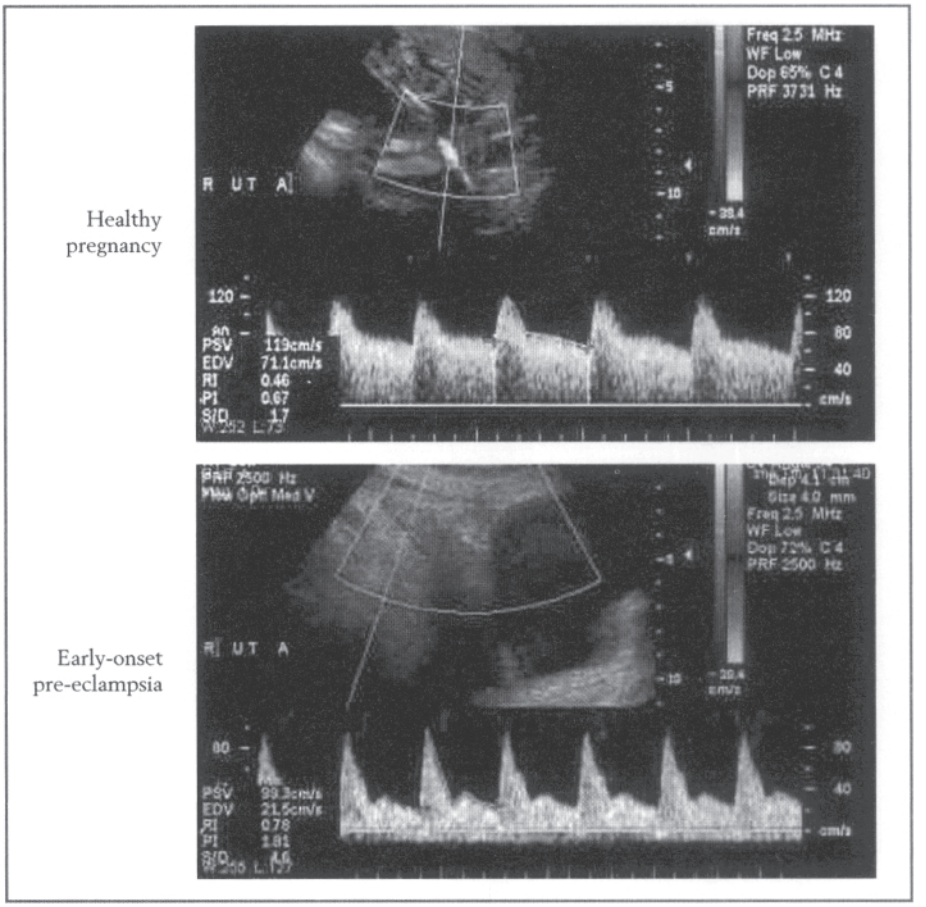

Figure 2 Uterine artery flow velocity waveform in healthy pregnancy is characterized by decreased pulsatility and high diastolic flow (upper panel). Moreover, the early diastolic notch characteristic for great arteries disappears. In pre-eclampsia the pulsatility remains high and there is low diastolic flow velocity (lower panel). The early diastolic notch persists

results were disappointing owing to technical limitations of 'blind' continuous-wave equipment[19], whereas groups that used color and pulsed Doppler[20] found superior results. The sensitivity of uterine artery Doppler for the detection of all pre-eclampsia is low, in the region of 25%. This implies that most women with pre-eclampsia have normal uteroplacental blood flow, or that small reductions in blood flow, or very heterogeneous or unstable blood flow patterns, induce a 'hypoxia-ischemia reperfusion injury' that may induce pre-eclampsia below the detection limits of the Doppler test.

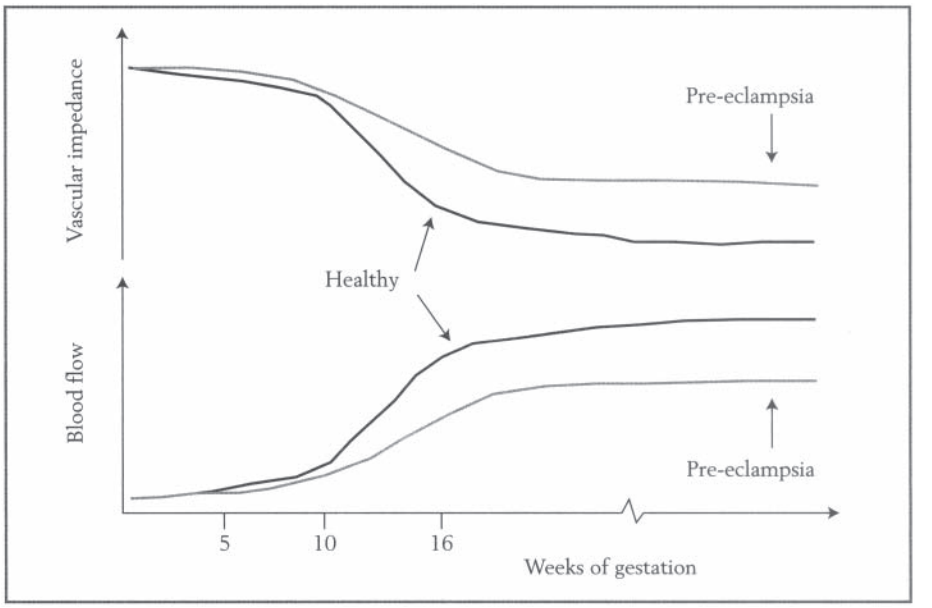

Figure 3 In healthy pregnancies most of the decrease of impedance in the uterine arteries occurs during the late first and early second trimester (upper panel). Blood flow increase parallels these changes. In pre-eclampsia the decrease of impedance is less pronounced (lower panel). As a result, blood flow does not increase as much as in healthy pregnancies

The majority (80%) of women with pre-eclampsia develop relatively mild disease at term ('term pre-eclampsia'), while a minority develop early-onset disease. Studies that restrict the definition of pre-eclampsia to early-onset disease, and/or delivery before 34 weeks, show much improved screening test characteristics, in the range of 75%+ sensitivity[21].

Therefore, uterine artery Doppler should be viewed as a screening test for serious disease that can impact on perinatal morbidity and mortality.

The positive-predictive value (PPV) of uterine artery Doppler for prediction of early-onset disease is in the range of 30%, indicating that perhaps two of three women with clearly abnormal uterine artery Doppler will remain normotensive and probably have normally grown infants. Placentas of pre-eclampsia pregnancies at term are up to 15% heavier than those of normotensive controls owing to adaptive angiogenesis in villi[18]. This mechanism is the likely explanation for the low PPV of uterine artery Doppler for serious disease. The development of early-onset pre-eclampsia is a multilayered pathology that is not based purely upon uteroplacental vascular insufficiency[22].

Practical considerations in uterine artery Doppler

Most centers will integrate uterine artery Doppler assessment of the uteroplacental perfusion at the time of the level-II scan, i.e. at 18–22 weeks of gestation. At this time, over 95% of proximal uterine arteries will be viewed successfully by abdominal imaging.

Color flow mapping is essential, so that the proximal uterine artery is identified at its cross-over point with the external iliac artery (Figure 4). The external iliac artery has a very different high-resistance waveform to that of the normal mid-pregnancy uterine artery, and thus the uterine artery should be sampled at least 1 cm above to avoid vessel interference.

The waveform should be assessed on each side, and the placental location noted.

Mean pulsatility index (PI) values <1.45 are normal, and these waveforms typically do not have early diastolic notches.

Unless the placenta is lateral, the mean PI value for each side should be used for interpretation. In situations where the placenta is completely located on one side of the uterus (lateral location), the waveform on that side (ipsilateral) should be recorded. In these circumstances there is poor transformation of the uterine artery on the contralateral side, and the anticipated high-resistance waveform can be ignored.

A pragmatic interpretation of the above is therefore to report the 'best' uterine artery waveform.

'Late-normalization' of uterine artery Doppler

In most pregnant women the establishment of a sufficient uteroplacental perfusion is fully accomplished by 22 weeks. About 50% of women with abnormal uterine artery Doppler at 20 weeks will show steady improvement to normal by 32 weeks. This subset is associated with a more favorable outcome than in those where severe uteroplacental vascular ischemia persists.

Placental damage

The link between impaired uteroplacental perfusion and the maternal vascular injury that typifies pre-eclampsia is ischemic-thrombotic injury to the placental villi. Infarction of placental villi, or large-scale necrosis of villous trophoblast, may break the integrity of the maternal-fetal barrier, allowing large fetally derived molecules to escape into the maternal circulation, such as a-fetoprotein (AFP). Unexplained elevations in 15–18-week maternal serum AFP are associated with the subsequent development of pre-eclampsia and/or IUGR. Likewise,

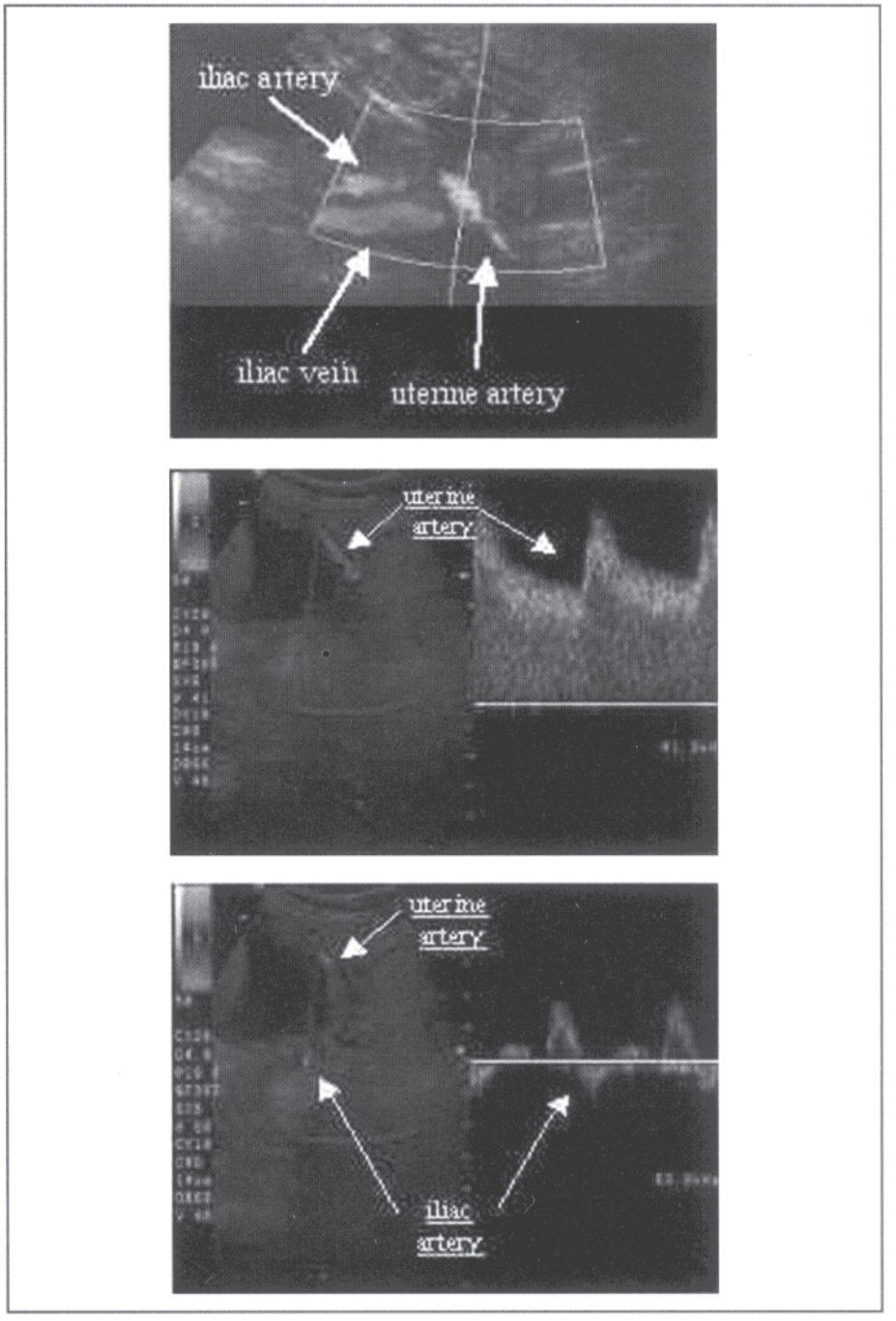

Figure 4 Uterine artery—iliac artery. Topographic relations and waveforms

unexplained elevations in human chorionic gonadotropin (hCG) may be associated with pre-eclampsia[23].

Combined elevations in AFP and hCG confer a 30%+ risk of severe pre-eclampsia and/or perinatal death. Abnormal uterine artery Doppler is present in around 50% of these pregnancies, and, in our recent cohort of 50 cases, had a 100% sensitivity and 75% PPV for death or delivery before 32 weeks (F. Alkazaleh and colleagues, submitted for publication).

Gray-scale imaging of the placenta may reveal small, abnormal 'jelly-like' placentas in a subset of women with abnormal uterine artery Doppler. In addition, some women with an abnormal uterine artery Doppler develop focal placental lesions suggestive of infarcts. In a cohort of 60 consecutive IUGR pregnancies delivering at <32 weeks with absent end-diastolic flow in the umbilical arteries, gray-scale ultrasound had a 70% PPV for the detection of ischemic-thrombotic placental lesions (S.Viero and colleagues, submitted for publication).

Our experience indicates that the indentification of gray-scale placental pathology in women with abnormal uterine artery Doppler increases the risk of developing early-onset pre-eclampsia. In these circumstances, infarcted and necrotic placental villous fragments may enter the maternal circulation and induce systemic vascular injury.

Combination testing for early-onset pre-eclampsia?

The screening test characteristics of uterine artery Doppler for the development of severe early-onset pre-eclampsia may be improved by the integration of other tests, for example blood pressure, maternal serum screening (MSS) for AFP/hCG and the actual gray-scale appearances of the developing placenta. Most studies combining such tests have been either retrospective, or small cohort studies. We are presently screening women at high-risk clinically for developing either early-onset pre-eclampsia or IUGR using the triad of week-16 MSS, uterine artery Doppler and placental morphology at 18–22 weeks. Our preliminary analysis in high-risk women indicates that normal tests have a 95% prediction for survival and delivery at term[24]. The 'triple-test' cohort screening study of 300 high-risk pregnancies will be completed shortly and presented in 2004.

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9

Diagnosis and clinical presentation

R. Hayman and J. Myers

DIAGNOSIS AND ASSESSMENT

As there are no specific diagnostic investigations, the initial diagnosis of pre-eclampsia remains one that is frequently based on clinical observations alone. The subsequent classification of disease severity, while centered on blood pressure level and the presence of proteinuria, may be further characterized by the accompanying signs and results of specific investigations (Table 1).

Symptoms

Most patients with pre-eclampsia are asymptomatic; however, headache, dizziness, tinnitus, drowsiness, general malaise and altered consciousness are more commonly reported by women with severe disease. Frequently, such symptoms herald the onset of eclampsia, and are indicative of poor cerebral perfusion, probably as a consequence of arterial spasm. Likewise, spasmodic changes within the retinal arterioles may lead to symptoms such as blurred vision and diplopia, more severe ischemia and hemorrhage within the occipital cortex, leading to scotoma and blindness. Vague symptoms such as epigastric tenderness, upper abdominal pain and dyspnea may also occur, and although these are not classically the presenting symptoms or signs of pre-eclampsia, inappropriate interpretation of their significance may lead to an inaccurate diagnosis and a delay in initiating the appropriate management.

Table 1 Features of severe pre-eclampsia

Blood pressure	with patient at rest: systolic ≥160± diastolic ≥110 mmHg
Proteinuria	≥5 g in 24-h urine collection (or 4+ on semiquantitative analysis)
Oliguria	24-h urine output <400–500 ml
Pulmonary edema or cyanosis	
Epigastric or right upper quadrant tenderness	caused by stretching of Gilsson's capsule; occasionally pain precedes hepatic rupture
Cerebral or visual disturbances	altered consciousness, headache, scotomata or blurred vision
Impaired liver function	increased transaminases±increased transferases
Thrombocytopenia	<100000 cells/mm^3 *or a rapidly falling count*
HELLP (hemolysis, elevated liver enzymes and low platelets)	*hemolysis: microangiopathic hemolytic anemia, increased bilirubin and lactate dehydrogenase; elevated liver enzymes secondary to parenchymal necrosis; low platelet count <100000 cells/mm^3*

Hypertension

In normal pregnancy, there are substantial cardiovascular changes, with an approximate 40–50% increase in the cardiac output and circulating blood volume. These changes are normally accompanied by a fall in blood pressure due to peripheral vasodilatation. In pre-eclampsia this does not happen and, in fact, tends to be the reverse, i.e. it is associated with a low cardiac output and a high peripheral resistance. However, both high-output and low-output states with measurements of the peripheral resistance in a range from normal to high have been observed among women with pre-eclampsia. More importantly, serial studies have shown a cross-over effect from high output plus low resistance to low output plus high resistance as the disease progresses.

The measurement of blood pressure is pivotal to the diagnosis and management of pre-eclampsia. In the antenatal setting it is a commonly performed procedure, but is often taken incorrectly with equipment that is faulty, which therefore gives erroneous results.

Measurements of the blood pressure should be taken with the patient in a sitting or semi-reclining position. The right arm should be used and the sphygmomanometer placed at the level of the heart. It is now recommended that Korotkoff phase V is used rather than Korotkoff phase IV when measuring diastolic blood pressure, as this corresponds more closely to the intra-arterial pressure, and the KIV/KV difference is smaller in hypertensive pregnancies than in normotensive pregnancies[1]. In a prospective randomized trial, women were managed on the basis of KIV or KV measurements. It was reported that only one fewer case of hypertension would be recorded for every six hypertensive pregnancies using KV, and that there was no difference in the maternal or perinatal outcome parameters measured between the groups[2].

To minimize the standard error between measurements, the patient should either be lying in the left lateral position with a wedge to provide a 30° pelvic tilt or rested supine but reclining at an angle of 45° to avoid aortocaval compression. In either case, the blood pressure cuff should be of appropriate size and placed at the level of the heart. Because of normal blood pressure variation, several readings should be taken to confirm the diagnosis. It has frequently been noted that 'white-coat hypertension' contributes to the overdiagnosis of significant hypertension, and, as a consequence, the measurement should be repeated after a period of rest. Persistent hypertension should only be diagnosed if a high reading is observed on two occasions more than 4 h apart. It is important to realize, however, that persistent hypertension has been observed in 12–22% of pregnancies, depending on the demographic characteristics of the population examined and on the definitions of hypertension employed.

Some clinicians advocate the use of automated sphygmomanometers to overcome these problems, either in the outpatient setting or in the patient's home. While the reliability, sensitivity and specificity of these devices awaits further clarification, preliminary studies have demonstrated that the greatest number of adverse outcomes were in those patients identified as hypertensive by ambulatory monitoring, when compared with those identified by conventional methods. In these series, the improved targeting of those at increased risk allowed the appropriate management to be more specifically directed, with obvious social and economic benefits.

Hypertension in pregnancy can be classified into two main groups: women who are hypertensive when they become pregnant, and those who become hypertensive for the first time in the second half of their

pregnancy. Blood pressure tends to fall in the first and second trimesters; therefore, women with high blood pressure prior to the 20th week of gestation are assumed to have pre-existing hypertension. Although well-controlled essential hypertension is generally a benign condition, the frequency of superimposed pre-eclampsia in such a population may be up to 25%.

Hypertension is a physical sign, and is defined as the upper end of a range of blood pressures and not as a separate/distinct pathological entity. In the research setting, pregnancy-induced hypertension is often rigidly defined as a pressure of greater than 140/90 mmHg in a previously normotensive patient after the 20th week of gestation. Such a level of hypertension corresponds to three standard deviations (SD) above the mean in early and mid-pregnancy to two SDs above the mean between 34 and 37 weeks and to 1.5 SDs above the mean at term, i.e. is significantly raised at all gestations. This classification is also used in relation to perinatal mortality, as a diastolic blood pressure of greater than 90 mmHg corresponds to the points of inflexion of the curve relating diastolic blood pressure to perinatal mortality. Above this point, perinatal mortality is significantly increased.

While the absolute level of blood pressure provides the best guide to fetal and maternal prognosis, diagnostic criteria that include a rise of blood pressure are frequently employed, for example, a rise in systolic and diastolic pressures of 30 and 15 mmHg, respectively. In the majority of patients, such criteria do not increase the precision of diagnosis, and may result in inappropriate treatment. In a series of 1496 women, North and colleagues concluded that normotensive women who showed a rise of ≥30/15 mmHg (below 140/90 mmHg) had uncomplicated pregnancies[3].

A single blood pressure reading of 140/90 mmHg or above is not an uncommon finding in pregnancy, and has been observed in up to 40% of all pregnant women. In this situation, such an observation carries little risk to the mother or fetus. A single blood pressure measurement of 140/90 mmHg may be of greater significance in patients with existing hypertension, especially when on treatment, and in patients with symptoms. In the former case, the booking blood pressure may already exceed this value or may be artificially lowered, and in the latter, when symptoms are present, a diagnosis of pre-eclampsia may be made even if the threshold value fails to be reached.

Consequently, although strict definitions are important for research purposes, they are less important clinically, as all women with elevated blood pressure levels must be carefully monitored for any of the associated symptoms of pre-eclampsia.

Any sudden rise in the blood pressure of a patient may indicate the development of a hypertensive crisis. Such an emergency may

complicate pre-eclampsia and the aim of management must be to avoid the inevitable sequelae of hypertensive encephalopathy, myocardial infarction and cerebrovascular accident (the leading cause of maternal death in pre-eclampsia). Many definitions of the degree of hypertension required to formulate a diagnosis of a crisis exist. Although threshold parameters of a systolic blood pressure greater than 200 mmHg and a diastolic blood pressure of more than 115 mmHg are commonly cited, it should be remembered that cerebrovascular accidents may occur in individuals whose blood pressure has remained consistently below these levels.

The differential diagnoses in patients with a hypertensive crisis include:

(1) Pheochromocytoma (rare tumor of the sympathetic nervous system: 90% adrenal in origin, 25% multiple and 10% malignant);

(2) Renal vein thrombosis;

(3) Cocaine ingestion especially when ingested as the base: 'crack cocaine';

(4) Systemic lupus erythematosus and other connective-tissue diseases.

Hypertensive encephalopathy is a subacute neurological syndrome characterized by headache, seizures, visual aberrations, altered mental status and focal neurological signs in the presence of a raised blood pressure. However, the clinical findings are often non-specific and the diagnosis may be particularly difficult to establish. Treatment must not be unduly delayed, however, as although it is a condition that is usually reversible with rapid intervention, it may be fatal if unrecognized or when treatment is delayed.

Proteinuria

A small amount of protein is normally present in the urine. The average 24-h urinary excretion in healthy non-pregnant subjects is: total protein 18 mg; albumin 10 mg; and β_2-microglobulin 1–2 mg. In pregnancy, protein excretion may be considerably increased, and up to 500 mg of total protein per 24 h is accepted as normal.

In pre-eclampsia, the urinary protein excretion rises above this threshold and significant proteinuria is often defined as 0.5 g per 24 h collection or a concentration of 300 mg per liter in the absence of infection. Such a finding is generally associated with the classic pathological finding of glomeruloendotheliosis, a change in renal architecture that is not permanent and fully resolves in nearly all cases after delivery. The presence of proteinuria in a patient with pregnancy-induced hypertension confirms the diagnosis of pre-

eclampsia and is associated with a concomitant increase in risk for both mother and fetus. The risk is related simply to the presence of proteinuria and is not affected by the absolute value of or the increase in urinary protein excretion. However, the rate of increase in the amount of protein being excreted per unit time may reflect a deterioration in the underlying disease state and careful monitoring may help to plan the subsequent management and timing of delivery.

In common clinical practice proteinuria is initially detected by the use of reagent strips. However, these may give a false-positive result if the urine is alkaline, or contaminated with chlorhexidine-based compounds or excessive physiological vaginal discharge, or in the presence of infection. As a consequence, the incidence of false-positive results in women with normal 24-h urinary protein excretion may be up to 25% in trace reactions, and 6% with one+or more reactions in random urine specimens.

Protein levels within the urine are influenced by several factors and fluctuate in the same patient from hour to hour. For example, the performance of physical exercise and alterations in posture may lead to a five-fold variation in the urinary protein concentration in successive 4-h periods. It is therefore recommended that, in all cases, a 24-h measurement of urinary protein be made to confirm the suspicion obtained from reagent strip testing on random urine samples. Where the clinical progression of the disease is particularly rapid, formal 24-h urinary protein quantification may not be possible. In these cases, quantification should be obtained with a collection recovered over a minimum period of 6 h. The results should then be adjusted for the time over which collection occurred and recalculated for a 24-h period. Additionally, it is imperative that the presence of infection should be excluded as soon as possible, urgent microscopy being performed if necessary.

Edema

In all pregnancies, a decrease in serum protein concentration coupled with an increase in capillary endothelial permeability leads to an increase in tissue edema and a reduction in intravascular volume. Peripheral edema, especially over the tibial tables and other dependent areas of the body, is particularly common, and while it occurs in up to 80% of normal pregnancies, it may be heightened in pregnancies complicated by pre-eclampsia. As it is such a common finding in the normal pregnant state, peripheral edema is no longer considered to be a clinically significant predictive factor in the prognosis of pre-eclampsia, and has actually been excluded from the formal diagnostic requirements of this condition.

'Dry' pre-eclampsia, i.e. hypertensive proteinuria in pregnancy without the presence of edema, is considered by most clinicians to be a worse prognostic indicator, especially in the presence of additional symptoms.

HELLP syndrome

A well-identified constellation of laboratory findings, i.e. hemolysis, elevated liver enzymes and low platelets, has been recognized in pre-eclampsia and the term HELLP syndrome was coined in 1982 by Weinstein. The HELLP syndrome is a serious complication that occurs in 4–12% of patients with severe pre-eclampsia. Among women with severe pre-eclampsia, 6% will present with one abnormality suggestive of HELLP (usually elevated liver enzymes), 12% will develop two abnormalities and 10% will develop the classical triad. HELLP syndrome can manifest itself at any stage during pregnancy, but 30% will occur postpartum, and only 80% of such patients will have had pre-eclampsia diagnosed antenatally.

Hypertension is not always characteristic of this condition in its initial stages, and, as a consequence, it may be confused with other medical conditions such as thrombotic thrombocytopenic purpura hemolytic uremic syndrome and acute fatty liver or pregnancy. Consequently, the presentation of this condition is often with non-specific findings, although disseminated intravascular coagulopathy, placental abruption and fetal demise are not infrequent accompaniments to the maternal morbidity and mortality that can occur.

In patients with HELLP, 80% of patients report right upper quadrant pain, and 50–60% present with excessive weight gain and worsening edema. It is important to note that 20% of patients with HELLP are not hypertensive, and 6% do not have significant proteinuria at the time of diagnosis.

The laboratory criteria for the diagnosis of HELLP most commonly employed are those defined by Sibai[4]. Sibai defined hemolysis as the presence of an abnormal peripheral blood film with the presence of schistocytes; a total bilirubin level of more than 1.2 mg/dl; elevated liver enzymes such as serum aspartate aminotransferase of more than 70 IU/l (>3 SDs above the norm) and a serum lactate dehydrogenase level of more than 600 IU/l; and a low platelet count of less than 100×10^9/l. Based on the severity of the thrombocytopenia HELLP may be further categorized into:

(1) Class 1:< 50×109/l;

(2) Class 2:50×109/l-100×10^9/l;

(3) Class 3:>100×10^9/l.

Although this classification appears to correlate well with the prognosis and speed of resolution, it has not achieved wide acceptance in clinical practice.

INVESTIGATIONS

The British Eclampsia Survey Team (BEST) report stated: 'that screening for proteinuria and hypertension has provided a simple and effective way of reducing the dangers of pre-eclampsia in the past, but new methods need to be sought to reduce the impact of the residual problems not detected by these signs'[5]. Unfortunately, there are still no specific hematological or biochemical parameters that help to formulate the diagnosis of pre-eclampsia, although changes within specific parameters may help to monitor the disease progression.

Hematological

Although a decrease in the absolute platelet count may be an early feature of pre-eclampsia, it has a limited diagnostic value as a consequence of the large variability between individuals in normal pregnancy[6]. Redman and colleagues reported a significant reduction in platelet count that was detectable 7 weeks prior to delivery; however, the population in this study were multiparous with chronic hypertension, in whom the diagnosis of pre-eclampsia was made from an elevation in the plasma urate level[7]. Walker and co-workers subsequently reported that neither platelet volume nor platelet count were useful predictive tests in the general nulliparous population, and should not therefore be used as a screening test[8]. This does not mean that hematological parameters should not be measured, however, as a declining platelet count is indicative of disease progression, and thrombocytopenia is one of the diagnostic requirements for HELLP syndrome.

During the development of pre-eclampsia, platelet activation results in enhanced consumption of the clotting factors. One of the most definitive tests for early coagulation abnormalities in pre-eclampsia is that for demonstrating factor VIII consumption, which depends on measuring factor VIII clotting activity and factor VIII-related antigen.

When the clotting system is activated the circulating levels of both factors increase rapidly as a secondary response, but because factor VIII clotting activity is destroyed by thrombin, its final level is lower than that of the related antigen. The difference between the two is therefore a reflection of factor VIII consumption. However, such changes in the coagulation system are not sensitive enough to form the basis of an accurate screening test, and therefore must remain, at present, as a tool for research.

Biochemical

An elevated plasma uric acid precedes the development of proteinuria and is a simple investigation. However, it is both non-specific and variable in its time course in relation to other features. Uric acid is filtered through the glomeruli, but is primarily excreted through the tubules. The serum uric acid concentrations have been found to correlate inversely with renal blood flow per square meter of body surface area. As a consequence, raised serum urate levels are probably better regarded, not as a diagnostic or specific indicator of pre-eclampsia, but as a sensitive indicator of impaired renal function and renal blood flow. Serum concentrations of uric acid fall in normal pregnancy in line with the increase in its renal excretion. In pre-eclampsia, an impairment of uric acid excretion, when associated with an increase in its production secondary to tissue ischemia and oxidative stress, correlates with a worsening of the outcome for mother and baby.

Renal function is generally maintained in pre-eclampsia until the end stage of the disease. If plasma urea or creatinine is elevated, especially in the presence of a relatively normal plasma uric acid level, underlying renal disease is likely. In a patient with pre-eclampsia, however, a rising plasma creatinine or urea indicates a worsening of the disease.

Fibronectin, a glycoprotein involved in platelet activation, the coagulation cascade and intercellular adhesion, has been shown by Lockwood and Peters[9] to be elevated in the first and second trimesters in patients with pre-eclampsia. As fibronectin is a component of collagen, the elevated plasma levels they detected probably reflect the endothelial damage which is thought to be the primary pathology underlying pre-eclampsia. Estimations of plasma fibronectin may, in the future, provide a predictive test for disease onset.

Liver failure is not a direct consequence of pre-eclampsia, but abnormalities in liver function tests, namely an increase in the enzymes lactic dehydrogenase, aspartate and alanine transaminase, may relate to alterations in liver perfusion or hepatic congestion. Monitoring the liver function is important for detecting disease progression, in particular the complication of HELLP syndrome.

CONCLUSION

Pre-eclampsia remains a disease related to significant maternal and fetal morbidity and mortality. While the vast majority of patients are asymptomatic at diagnosis, the disease may manifest itself with any number of classical and non-classical presentations. The clinician exposed to the antenatal patient must therefore carry a knowledge of

the different guises with which the disease may choose to show itself, so that the atypical may not be mistaken for a less significant illness. Currently, the diagnosis remains one based on clinical symptoms and signs with hematological and biochemical investigations being used to monitor the disease progression.

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10

Maternal complications

J. Gillham and R. Hayman

INTRODUCTION

Following the introduction of organized antenatal care in the UK in the mid-1950s, maternal mortality and morbidity rates from pre-eclampsia/eclampsia have fallen steadily. While advances in intensive-care medicine may be partly responsible for this decline, it is most likely consequence of the pre-eclamptic disease process being identified at an earlier stage than previously. This has enabled the appropriate interventions to be better timed to reduce the incidence of severe disease and its associated maternal complications.

Despite this, the hypertensive diseases of pregnancy remain the second leading cause of maternal death in the UK[1], with 15 attributed deaths occurring between 1997 and 1999. While this 'gross' quantification of maternal mortality remains the standard measure of successful obstetric intervention and management strategies, in the developed world such an outcome is a relatively rare event. While providing 'hard data' it will significantly underestimate the serious maternal morbidity associated with this condition, and thus quantification of maternal morbidity may be a more accurate reflection of our current practice[2]. This should not detract from the seriousness of this syndrome, however, as worldwide, pre-eclampsia continues to account for over 150000 deaths a year.

MATERNAL MORBIDITY AND MORTALITY

Maternal morbidity and mortality may be secondary to the pre-eclamptic disease process. Mortality rates are highest when the disease presents at early gestations (20–28 weeks), with advanced maternal age or after the first live birth. Women of Afro-Caribbean decent are three times as likely to die from pre-eclamptic complications or eclampsia when compared with Caucasian women.

The iatrogenic medical mismanagement of disease complications may be equally to blame. Women who receive no antenatal care are seven times more likely to die from disease complications than those who do[3]. Likewise, although a conservative approach of the management for severe, early-onset pre-eclampsia has been recommended by some, it appears that the women managed in this way suffer from higher maternal morbidity and mortality rates. Consequently, this approach can no longer be supported[4].

Pre-eclampsia is a multisystem disease, and although the well-recognized signs of proteinuria and hypertension are relatively uniform, its clinical manifestations are multiple. The associated causes of maternal morbidity and mortality are equally as widespread, and are considered below.

Renal disease

Acute renal failure is characterized by an abrupt reduction in the maternal glomerular filtration rate that leads to excessive retention of urea and water, as well as other complex electrolyte and acid-base disturbances. It is rare for patients to present with acute renal failure as the primary diagnostic indicator for pre-eclampsia. However, acute renal failure is not common in pregnancy, and when it does occur, pre-eclampsia should be high on the index of suspicion.

During the course of a normal pregnancy, there is an increase in creatinine clearance, with a concomitant decrease in serum creatinine and urea concentrations. If creatinine concentrations are high at an early stage in the disease process, then underlying renal disease should be suspected. However, such a diagnosis does not exclude the possibility of the patient developing superimposed pre-eclampsia at later gestation.

Renal function is generally maintained in pre-eclampsia until the end-stage of the disease. In severe disease, a rise in serum creatinine is often observed, and such a change is often associated with a worsening prognosis.

The causes of acute renal failure in pre-eclampsia can be broadly divided into three categories:

(1) Pre-renal: a consequence of renal hypoperfusion without parenchymal involvement;

(2) Intra-renal: intrinsic renal damage;

(3) Post-renal: secondary to an obstructive uropathy.

Pre-renal and intra-renal failure account for up to 90% of all cases of acute renal failure in pre-eclampsia. Fortunately the majority of patients recover with no long-term renal impairment, as the underlying pathology is commonly acute tubular necrosis. By contrast, in women with pre-eclampsia complicated by disseminated intravascular coagulation or placental abruption, or in patients with known pre-pregnancy renal involvement, bilateral renal cortical necrosis carries a significant risk of maternal and perinatal morbidity and mortality. Acute renal failure is now rare as a complication of pre-eclampsia in more developed countries, and when it does occur most cases are associated with hemorrhage or sepsis.

If there are doubts about renal function, the urine osmolality should be analyzed. If the urine is concentrated, this reflects a working renal system and the reduced output is secondary to reduced perfusion, which will gradually improve. If the urine is not concentrated, this suggests abnormal renal function and underlying renal failure.

The largest review of renal complications occurring in pregnancy included 1433 women with pre-eclampsia and 251 with eclampsia[5]. Only 31 of the women developed acute renal failure, and all of these cases occurred in the postpartum period. Fifty per cent of these women required dialysis and there were two deaths. All renal disease secondary to pre-eclampsia was acute tubular necrosis, and there were no long-term renal problems in patients with this diagnosis.

It is important to note that the kidney is more susceptible to damage by the pre-eclampsia disease process if there is pre-existing chronic hypertension. In the above review, 42% of such women required dialysis and three suffered cortical necrosis[5].

Hepatic disease

The incidence of liver dysfunction complicating pre-eclampsia is uncertain, as it is often diagnosed on finding an elevation of liver enzymes above the normal non-pregnant reference ranges. Mild disease has little or no clinical significance.

Abnormal liver function tests have been reported to occur in 20–30% of pregnancies complicated by pre-eclampsia, although the true incidence based on the appropriate reference ranges is likely to be significantly lower. Genuinely abnormal liver function tests associated with pre-eclampsia may reflect liver dysfunction secondary to vasoconstriction in the hepatic bed. Histopathological examination of the liver in pre-eclampsia reveals periportal fibrin deposition, hemorrhage and hepatocellular necrosis.

At the other end of the clinical spectrum, liver rupture, one of the most severe sequelae of severe pre-eclampsia and HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome is fortunately rare[6]. Liver rupture has a maternal mortality rate of >30%. It occurs most frequently in multiparous women of advanced age; however, the precise cause remains unknown. Postulated etiologies include a cascade of events, commencing with endothelial dysfunction and intravascular fibrin deposition leading to sinusoidal obstruction, intrahepatic venous congestion, subcapsular hepatic hematoma and finally hepatic rupture. Hemorrhage can also occur beneath the liver capsule, and may be so extensive as to cause rupture of the capsule into the peritoneal cavity. This complication is more common in situations where gross disturbances of hematological hemostasis occur such as in the HELLP syndrome.

Following liver rupture, patients may be severely shocked. In the preceding clinical phase, pain in the right hypochondrium and/or epigastric tenderness are the most important clinical features. An abdominal mass extending from below the right costochondral margin should raise the index of clinical suspicion, and the presence of bilateral shoulder-tip pain, and guarding of the abdominal wall with rebound tenderness, should point towards intraabdominal hemorrhage.

Where possible, and with time permitting, imaging techniques such as ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) may enable non-invasive diagnosis.

Cardiopulmonary disease

Although patients with pre-eclampsia are hypovolemic, their tissues are fluid-overloaded and they have increased sensitivity to volume shifts. This is a consequence of low plasma oncotic pressure, raised hydrostatic pressure due to hypertension and increased capillary permeability reflecting endothelial dysfunction. To reduce the chances of complications (pulmonary edema, left ventricular failure and adult respiratory distress syndrome), close monitoring of the fluid balance is essential.

Pulmonary edema is the excessive accumulation of fluid within the interstitial and alveolar spaces, and complicates 2.9% of pregnancies affected by pre-eclampsia. Only 30% of cases develop in the antenatal period, and in the majority of those so affected, chronic hypertension in association with advanced maternal age and/or multiparity has been implicated.

Any factor that results in the following conditions will lead to movement of fluid from within the capillaries and into the extravascular space.

(1) A reduction of the colloid osmotic pressure;

(2) An alteration in the colloid osmotic pressure/pulmonary capillary wedge pressure gradient;

(3) An increase in the capillary permeability;

(4) An increase in the intravascular hydrostatic pressure;

This, in turn, will predispose to the development of pulmonary edema.

Pregnancy is a classic example of a multifactorial insult that is held in careful balance. Rises in the blood volume, cardiac output, heart rate and capillary permeability and a fall in the colloid osmotic pressure are delicately poised, the point of equilibrium being shifted towards increased permeability and tissue edema. In pre-eclampsia these changes are increased. When combined with injudicious iatrogenic fluid replacement therapy it is easy to see why 70–80% of cases of pulmonary edema occur in the postpartum period.

Pulmonary edema is a clinical diagnosis characterized by worsening dyspnea and symptoms and signs of respiratory compromise: orthopnea, tachypnea, hypoxia and auditory crackles on auscultation. Arterial blood gases and an erect chest X-ray may help in the diagnosis, although more complicated modalities (CT, ventilation/quantitation (V/Q) scanning, etc.) may be necessary to exclude other causes of cardiopulmonary compromise (pulmonary embolism, pneumonia, cardiomyopathy). Invasive monitoring of the central venous pressure in these patients can be misleading. The increased interstitial fluid can lead to pulmonary edema occurring in association with a central venous pressure reading in the normal range.

The reduction in intravascular volume causes a raised hematocrit. This also places women with pre-eclampsia at increased risk of thromboembolic disease (the leading cause of maternal mortality in the recent report *Confidential Enquiry into Maternal Deaths*[1]).

Adult respiratory distress syndrome (ARDS) may also be a consequence of pre-eclampsia. This represents an acute respiratory

insufficiency syndrome in patients with a non-physically injured lung. It can have many different etiologies and varied pathogenesis. Pulmonary injury in ARDS results from a systemic injury releasing a variety of proinflammatory cytokines and neutrophil sequestration within the lungs. The endothelial injury results in increased pulmonary capillary permeability leading to interstitial and alveolar edema. Alveolar hemorrhage can then occur, and hyaline membrane formation leading to fibrin deposition within the alveoli. This condition has a substantial maternal mortality rate.

Cerebrovascular disease

The largest single cause of death in the latest maternal mortality report[1] in women with pre-eclampsia was intracranial hemorrhage. This is a consequence of uncontrolled hypertension, and often reflects inadequate or delayed treatment with antihypertensive agents.

The principal postmortem finding of women dying from or with eclampsia is hemorrhage, ranging from petechiae to gross bleeding.

Normally the brain is protected from extremes of blood pressure by autoregulation that ensures constant brain perfusion over a wide range of blood pressures. However, an upper limit for this autoregulation must exist, and thus, if hypertension develops at values greater than this, hypertensive encephalopathy may occur.

Magnetic resonance imaging (MRI) studies have demonstrated hyperintense areas on T_2-weighted images, commonly in the parieto-occipital lobes, in the distribution of posterior cerebral arteries. These lesions are presumed to be a consequence of vasoconstrictive cerebral edema. Their anatomical distribution has been reported to correlate with visual symptoms experienced by the patients, and they are observed to resolve in parallel with clinical improvement.

Hemorrhages associated with pre-eclampsia are usually bilateral and often in the posterior brain areas. It is widely accepted that there is an increased posterior circulation vulnerability to hypertensive vascular disease in pre-eclampsia/eclampsia.

Placental abruption/disseminated intravascular coagulation

Placental abruption is associated with pre-eclampsia, with increased perinatal morbidity and mortality. With accompanying hemorrhage and clotting disorders, there is associated maternal morbidity both directly from the blood loss and indirectly from the effect of hypovolemia on other major organs of the body.

Disseminated intravascular coagulation (DIC) is a disorder in which there is a general increase in both fibrin formation and fibrinolysis, leading to excessive consumption of clotting factors. There is a wide spectrum of manifestations of the process of DIC, from a compensated state with no clinical sequelae but laboratory evidence of increased production and breakdown of coagulation factors, to the condition of massive uncontrollable hemorrhage.

Thrombocytopenia, a common finding in up to 10% of patients with pre-eclampsia, is often the first indication of the development of DIC. It may be an immunologically mediated platelet consumption and increased platelet activation. Several studies have correlated this increased activation with proteinuria and raised serum creatinine, suggesting a link between platelet activation and renal microvascular damage.

Other hematological aberrations that confirm the diagnosis of DIC are an increase in circulating fibrin degradation products (FDPs) (>40 μg/ml) and Ddimer levels, and a decrease in circulating fibrinogen (<300 mg/dl) and protein C levels.

The resulting FDPs interfere with the formation of fibrin clots, myometrial function and possibly cardiac function[7]. DIC can be secondary to pre-eclampsia/HELLP syndrome. (HELLP syndrome is discussed in Chapter 9.) DIC may also be a consequence of excessive antepartum hemorrhage with placental abruption, or occur postnatally with massive postpartum hemorrhage (both antepartum and postpartum hemorrhage have an increased incidence in patients with pre-eclampsia). DIC is always a secondary phenomenon; thus, the mainstay of management is to remove the underlying stimulus.

Eclampsia

Eclampsia (literally 'flashing lights') is the occurrence of convulsions in the absence of other neurological conditions such as epilepsy, subarachnoid hemorrhage and meningitis, in association with the signs and symptoms of pre-eclampsia (Table 1).

However, not all cases present with headaches, flashing lights or epigastric pain, and it is now clear that seizures are only one of several clinical manifestations of severe disease.

The incidence of 4.9/10000 maternities reported in 1994 in the UK[8] was similar to that observed in the USA (4.3/10000 in 1983–86), but higher than that in Sweden (2.7/10000 in 1980). Eclampsia is much more prevalent in the developing countries, with an incidence of 6–100/10000 births[6].

Douglas and Redman commented that the majority of these cases occurred despite a normal frequency of antenatal assessments (70%), and even after admission to hospital (77%)[8]. Furthermore, eclampsia

Table 1 Differential diagnosis of eclampsia

Hypertensive disease	→	hypertensive encephalopathy, pheochromocytoma
Cerebrovascular accident	→	intracerebral or subarachnoid hemorrhage, cerebral arterial or venous thrombosis
Space-occupying lesions	→	brain tumor, abscess
Metabolic disorders	→	hypoglycemia, uremia
Iatrogenic	→	inappropriate fluid management in the severely pre-eclamptic patient
Infectious etiology	→	meningitis, encephalitis
Epilepsy		
Thrombotic thrombocytopenic purpura		

was often (38%) unheralded by hypertension and proteinuria, prompting the comment that, although screening for hypertension and proteinuria may reduce the incidence of eclampsia preceded by pre-eclampsia (evidenced by the reduction in incidence of eclampsia in the UK between 1922 and 1970), atypical cases arising *de novo* would become a proportionally greater problem.

Any combination of neurological signs and symptoms may be noted or reported prior to the onset of the seizure. Headache, one of the defining characteristics of severe pre-eclampsia, is reported in 40% of patients with pre-eclampsia and up to 80% of patients with eclampsia. Some 1–3% of patients with pre-eclampsia report amaurosis (temporary blindness), a suspected consequence of both retinal vascular and occipital lobe injuries. Many others have symptoms that vary from nausea to excitability, apprehension, visual disturbance and alterations in their mental state.

In patients with severe pre-eclampsia, it is interesting to note that the numbers of neurological sequelae, such as eclampsia and stroke, are decreasing. This may reflect improvements in current clinical practice with recourse to earlier intervention and delivery. It may also indicate the influence of changes within environmental factors, since the fall in incidence antedates the use of antihypertensive drugs and magnesium sulfate.

Eclampsia remains a significant cause of maternal mortality (3.4/10000 maternities in the triennium 1991–93). It is often not the

eclamptic seizures that are in themselves dangerous (idiopathic epilepsy has a much lower incidence of fatality), but the severity of the underlying disturbance. In women who die from eclampsia, intracerebral hemorrhage is a frequent postmortem finding. It is interesting to note, that when investigating the clinical course of the disease in those who have died, there is significantly higher blood pressure than in those who have survived. Renal and hepatic function do not differ. It is a sobering thought that it is estimated that eclampsia accounts for over 50000 maternal deaths a year worldwide.

The rate of stillbirths and neonatal deaths has been reported to be up to 34.1/1000 in eclamptic women. If the onset of pre-eclampsia is at a preterm gestation, then eclamptic seizures are more commonly antepartum. They are associated with more maternal complications, fetuses small for gestational age and higher rates of stillbirth and neonatal mortality[8].

The cause of eclamptic seizures remains unknown, although hypertensive encephalopathy, vasospasm, ischemia, hemorrhage and edema have all been proposed in the pathogenesis. Cerebral edema is associated with convulsions, and can be seen on CT and MRI. This disorder has been described as posterior leukoencephalopathy syndrome. It is not a new diagnosis but a radiological description. The cerebral edema may antedate eclampsia, because occipital lobe blindness can occur in the absence of eclampsia and is completely reversible.

Eclamptic seizures are almost always self-limiting and seldom lastlonger than 3–4 min. They are clinically and electroencephalographically indistinguishable from other generalized tonic-clonic seizures. In general, women with typical eclamptic seizures who do not have focal neurological deficits or prolonged coma, do not require cerebral imaging electroencephalographic investigations.

Approximately half of all cases of eclampsia occur before term, with 20% occurring prior to 31 weeks' gestation. Some 75% of the remaining cases develop intrapartum or within 48 h of delivery. Seizures owing to eclampsia always resolve postpartum, often within a few hours. However, late-postpartum eclampsia (>48 h but <4 weeks postpartum) accounts for 25% of postpartum cases.

CONCLUSION

Pre-eclampsia continues to have a massive impact on maternal and perinatal morbidity/mortality. This chapter reviews the major complications affecting the renal, hepatic, cardiopulmonary and cerebrovascular systems, in addition to placental abruption, DIC and

eclampsia. Most maternal deaths and significant morbidity occur after delivery. The vigilance required in the monitoring of these patients must be employed stringently in the postpartum period. Every obstetric unit should have a well-established protocol for the management of a pre-eclamptic patient in the ante- and postnatal periods, and guidelines on how to manage associated complications.

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II

Mild to moderate disease

P. von Dadelszen and L. A. Magee

INTRODUCTION

Pre-eclampsia complicates 3–5% of pregnancies, and is commonly defined as hypertension of ≥140/90 mmHg and proteinuria ≥0.3 g/24 h that appear at ≥20 weeks' gestation, and regress after pregnancy[1-4]. In the USA, pre-eclampsia is estimated to cost the health system in excess of $7.5 billion per annum, $3.00 billion being spent on maternal illness, $4.11 billion on infant illness and $0.41 billion on ongoing pediatric sequelae[5,6]. In addition, 1200 infants die as a consequence of pre-eclampsia per annum. Pre-eclampsia remains one of the two most common causes of maternal death in the developed world[7]. Its definition and etiology are discussed elsewhere (Chapter 1 and Part 2, respectively).

HYPERTENSION DEFINED: THE USE OF AMBULATORY BLOOD PRESSURE MONITORING

Pre-eclampsia is most commonly classified as blood pressure (BP) ≥140/90 mmHg with proteinuria of ≥0.3 g/24 h[1–4]; the Canadian Hypertension Society (CHS) has included criteria only for diastolic BP (dBP)1. For the measurement of dBP, it is clear that Korotkoff V provides more reproducible and reliable measurements than does Korotkoff IV[8], although national guidelines vary on this point[1–4].

Given the importance of BP in the definition of pre-eclampsia, a comment about BP measurement is warranted. As in non-pregnancy

there has been great interest in out-of-office BP measurement, particularly since 'white-coat' hypertension (defined as elevated office BP measurements of ≥140/90 mmHg, with normal ambulatory values) has been documented to be very common in pregnancy. White-coat hypertension has been seen in approximately one-third of pregnant women by 24-h ambulatory monitoring, and in as many as 76–79% by home BP measurements[8].

Some 24-h ambulatory (e.g. Spacelabs 90207, Spacelabs, Redmond, WA, USA) and home (e.g. Omron 705C, Omron Corp, Tokyo, Japan) BP monitoring devices have met published criteria for reliability in pregnancy. However, the deflationary oscillometric devices used in the ambulatory setting have been repeatedly demonstrated to underestimate BP in pre-eclampsia[8]. Recently, an inflationary oscillometric device was validated in pregnant women, but not those with pre-eclampsia, in whom it underestimated both systolic blood pressure (sBP) and dBP[9].

In prospective observational studies, an abnormal 24-h ambulatory BP, versus standard clinic measurements, is better at identifying hypertensive pregnant women at increased risk of adverse pregnancy outcomes: severe hypertension, preterm delivery, Cesarean section, small-for-gestational-age infants and admission to neonatal intensive care[8]. However, there has been no validation of home BP devices against adverse pregnancy outcomes[8]. Regardless of their lack of validation against adverse pregnancy outcomes, the use of home BP monitors four times daily has become the standard of care in many antepartum home-care programs.

PRE-ECLAMPSIA: CURRENT CLASSIFICATION AND EVALUATION

At present, guidelines for the diagnosis and evaluation of pre-eclampsia have been published by the CHS[1], the (US) National High Blood Pressure Education Program[2] and both Australasian[3] and International[4] Societies for the Study of Hypertension in Pregnancy. As stated in the CHS report, much of the evidence is borderline in quality, resulting in mostly grades C and D recommendations (which include expert opinion)[1].

Pre-eclampsia is conventionally defined as the presence of hypertension and proteinuria, and these are most commonly used to distinguish mild from severe disease[1-4]. However, that other maternal and fetal features may be important has been acknowledged in the CHS[1] and Australasian[3] publications (Table 1). The CHS has coined the useful term 'adverse features' that may occur in the setting of gestational (pregnancy-induced) hypertension in the presence or absence of proteinuria. These 'adverse features' include both heavy proteinuria and severe maternal hypertension, as well as other maternal signs (e.g. pulmonary edema), maternal symptoms (e.g. frontal headache),

Table 1 Classification of the hypertensive disorders of pregnancy

Canadian Hypertension Society[1]	*National High Blood Pressure Education Program*[2]	*Australasian Society for the Study of Hypertension in Pregnancy*[3]
Pre-existing hypertension, essential/secondary	chronic hypertension	chronic hypertension, essential/secondary
Gestational hypertension without proteinuria ± adverse features*	transient hypertension	pregnancy-induced hypertension
Gestational hypertension with proteinuria ± adverse features†	pre-eclampsia/eclampsia	pre-eclampsia, mild/severe
Pre-existing hypertension + superimposed gestational hypertension with proteinuria	pre-eclampsia superimposed on chronic hypertension	pre-eclampsia superimposed on chronic hypertension
Unclassifiable antenatally		

*Adverse features[1]: convulsions, diastolic blood pressure > 110 mmHg, thrombocytopenia (< 100×10^{12}/l), oliguria (< 500 ml/day), pulmonary edema, elevated liver enzymes, severe nausea and vomiting, frontal headache, visual disturbances, persistent right upper quadrant pain, chest pain, dyspnea, suspected abruption, HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, intrauterine growth restriction, oligohydramnios, absent or reversed umbilical arterial end-diastolic flow (Doppler); †adverse features[1]: same as above, proteinuria > 3 g/day, hypoalbuminemia (< 18 g/l)

abnormal laboratory tests (e.g. platelets <100×10^{12}/l) and abnormal tests of fetal well-being (e.g. IUGR).

PREDICTORS OF ADVERSE OUTCOME IN PRE-ECLAMPSIA PREGNANCIES

Classification of the hypertensive disorders of pregnancy is aimed at distinguishing between women at differential risk of adverse maternal and/or perinatal outcomes[1–4]. Although hypertension and proteinuria figure prominently among risk factors for these outcomes in pre-eclampsia pregnancies, it must be acknowledged that the maternal and perinatal risks have not been quantified. There are other potential limitations of such a simplified approach to disease severity, which is directly related to management.

First, it is not known whether all of the 'adverse features' proposed by the CHS[1] are predictive of adverse maternal and/or perinatal outcome, and if so, which are and to what degree.

Second, there are other factors that may be predictive. The obvious consideration here is gestational age, which is the most important determinant of perinatal outcome among euploid fetuses and an important determinant of maternal outcome[10]. A greater than 50% chance of intact fetal survival in pre-eclampsia arises only when the delivery gestational age is ≥27+0 weeks or the birth weight is ≥600 g[11]. Also, early-onset pre-eclampsia (<32 weeks), versus that which occurs at term, is associated with a 20-fold higher maternal mortality, and is probably a different disease, with more perturbed neutrophil function and cytokine levels[10,11].

Third, the CHS classification is based on retrospective (ultimate postpartum) diagnoses, and not on the picture at presentation, when plans for management are made. For example, 35% of women who present with gestational hypertension at <34 weeks will develop proteinuria, up to 8 weeks later, and meet the diagnostic criteria for pre-eclampsia. Therefore, in practice, the diagnosis of pre-eclampsia needs to be considered and excluded (by laboratory and fetal investigation) when either of the following arise: non-proteinuric gestational hypertension (present in 20% of women within a week of their first eclamptic seizure[12]) or non-hypertensive gestational proteinuria (present in 10% of women[12]).

In summary, the current classification of pre-eclampsia has not been shown to predict maternal risk. Also, the classification may not include factors predictive of adverse perinatal outcome (e.g. gestational age).

MANAGEMENT OF PRE-ECLAMPSIA PREGNANCIES

Diagnosis and surveillance

In suspected pre-eclampsia, maternal well-being is evaluated by maternal blood and urine tests. The CHS has specified many evaluative tests, which are listed in Table 2. The recommendations are based on inadequate evidence. Most are grade C or D, with the exception of urinary dipstick testing (for proteinuria), which is not recommended for use in making the diagnosis (grade A), and umbilical artery Doppler velocimetry (grade B) and serum uric acid (grade B, but for diagnosis only). While recognizing the limitations of urine dipsticks for the accurate assessment of proteinuria, we believe that dipsticks remain useful in the outpatient setting when identifying women for whom a formal 24-h protein estimation is indicated. Similarly, more recent

reviews of the evidence give stronger support for the use of umbilical artery Doppler in high-risk pregnancies[13].

Fetal well-being is assessed by ultrasound (amniotic fluid volume, estimated fetal weight and umbilical artery Doppler velocimetry) and fetal heart rate (FHR) analysis (Chapter 14). The frequency with which these tests should be performed is not stated.

In practice, practitioners' use of tests for both diagnosis and ongoing surveillance is variable both within and between groups of practitioners (e.g. general practitioner (GP) obstetricians versus obstetricians versus internal medicine)[14,15], reflecting the ongoing controversy that exists between the major documents that advise practice[1]. Based on a postal survey of Canadian clinicians, Table 2 lists the frequency with which clinicians stated that they perform maternal and fetal testing, and Table 3 lists what these clinicians consider to be indications for delivery in the setting of pre-eclampsia (compared with those in the published literature)[16,17]. Fifty per cent of contacted obstetricians, GP obstetricians, midwives, nephrologists and general internists replied[14,15].

We found disparity between CHS recommendations for the evaluation of women with pre-eclampsia, and the stated practice of respondents. For example, 78% of practitioners use urinary dipstick testing and 76% use tests of coagulation[14,15], neither of which is recommended for routine use by the CHS[1]. We found that most clinicians perform evaluative maternal blood tests at least once weekly, with tests of fetal well-being (e.g. ultrasound) performed less than weekly, and dipstick urinalysis performed daily. Also, gestational ages ≥34 weeks are not widely endorsed as an indication for delivery (whereas most other suggested indications are). It would appear from these results that expectant management of pre-eclampsia at ≥34 weeks is not out of keeping with Canadian practice, and that we must assess the predictive ability of standard evaluative maternal and fetal tests to predict adverse outcomes within 1 week, consistent with the current frequency with which most maternal tests are performed. These variations in practice reflect the clinical Gestalt used in determining risk for individual women in the absence of a robust system or model for quantifying that risk.

Predictive models (e.g. acute physiology and chronic health evaluation (APACHE) and multiple organ dysfunction score (MODS)) have been developed for patients with the systemic inflammatory response syndrome (SIRS), which pre-eclampsia resembles to a remarkable degree[18]. Although these models have performed well when modified for defined (usually geriatric) populations, APACHE did not perform well in predicting mortality among women with eclampsia in the intensive-care unit setting[19]. Also, these models predict mortality, which is (thankfully) a rare event in women with pre-eclampsia. Which

Table 2 This table summarizes our recommended maternal and fetal testing, and compares it with both the 1997 Canadian Hypertension Society (CHS) guidelines for the evaluation of women with gestational hypertension[1] in which no specific recommendations were made for women with pre-eclampsia, and the results of our February 2000 survey of Canadian obstetricians about how they evaluate women with suspected pre-eclampsia[14]

Laboratory investigation	*CHS recommendation (and grade) for gestational hypertension*	*Reported use in pre-eclampsia*	*Used at least once/week (by those who use the test)*
Maternal bloodwork			
INR, aPTT, fibrinogen	'of little use in *routine* screening'	75.5%	80.0%
CBC	grade D (but WBC not mentioned)	93.6%	83.9%
Electrolytes	not specified	not asked	
BUN	not specified	not asked	
Creatinine	grade C	82.9%	83.0%
Glucose	not specified	not asked	
Uric acid	grade B	78.2%	82.6%
Albumin	not specified	not asked	
AST and ALT	grade C	92.8%	82.4%
Bilirubin	not specified	not asked	
LDH	grade C	63.6%	81.3%
Maternal urine tests			
Random protein/ creatinine ratio	not specified	not asked	
24-h Urine for protein	grade D (creat CL not specified)	88.6%	83.8%
Maternal bedside			
Urine dipstick proteinuria	not recommended	78.4%	54.6%*
Pulse oximetry	not specified	not asked	
BP q4 and q6 h overnight	not specified		
Intake and output	not specified		

continued

Table 2 *continued*

Laboratory investigation	*CHS recommendation (and grade) for gestational hypertension*	*Reported use in pre-eclampsia*	*Used at least once/week (by those who use the test)*
Fetal evaluation			
NST	grade D	88.4%	64.7%
Ultrasound for EFW	not specified	69.1%	32.2%†
AFI	grade C (as part of BPP)	89.6%	88.1%
Doppler flow velocimetry	grade B (umbilical; middle cerebral not specified)	74.8%	56.0%

*44.8% do this daily; †56.6% do this less frequently than once/week; INR, International Normalized Ratio; aPTT, activated partial thromboplastin time; CBC, complete blood cell count; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alaninine aminotransferase; LDH, lactate dehydrogenase; BP, blood pressure; q, every; NST, non-stress test (cardiotocograph); EFW, estimated fetal weight; AFI, amniotic fluid index; WBC, white blood cell count; creat CL, creatinine clearance; BPP, biophysical profile

maternal and/or fetal features are predictive of adverse maternal outcome in pre-eclampsia pregnancies is highly relevant to management. Although the definitive treatment for pre-eclampsia is always delivery, this is not always the best option for the fetus. At all gestational ages, iatrogenic prematurity is associated with increased perinatal risks. These risks are greatest for the fetus remote from term (i.e. <32–34 weeks). Although the risks are much lower for those fetuses closer to term, the attributable risk of iatrogenic prematurity is still important because most cases of pre-eclampsia present near term[2].

We sought to develop a predictive model, for subsequent prospective validation, through a multicenter, retrospective chart review conducted in both Canada (BC Women's Hospital, Vancouver and Ottawa Hospital, Ottawa) and the UK (John Radcliffe Hospital, Oxford). Included in the study were 556 women who were admitted to hospital with pre-eclampsia, 2000–01, and were not in active labor[20].

The candidate maternal (clinical and laboratory) and fetal (ultrasound) predictors were identified, as was the primary outcome of serious maternal complications, as described. First, a formal literature review determined how pre-eclampsia has been classified, managed and predicted and how complica tions have been

Table 3 Indications for delivery for pre-eclampsia remote from term[15–17]

Maternal indications	Fetal indications
One/more of*:	One/more of:
uncontrolled severe hypertension†	fetal distress by FHR tracing or BPP (≤ 6)
eclampsia	amniotic fluid index ≤ 2
platelet count <100000×10^9/l	US estimated fetal weight ≤ 5th centile
AST or ALT >2 times upper limit of normal with epigastric pain or RUQ tenderness	reversed end-diastolic flow in the umbilical artery by Doppler velocimetry
pulmonary edema	
compromised renal function‡	
persistent severe headache or visual changes	

*Regardless of the magnitude of proteinuria; †blood pressure persistently ≥160 systolic or ≥110 diastolic despite maximum recommended doses of two antihypertensive medications; ‡persistent oliguria (<0.5 ml/kg/h) or rise in serum creatinine of 88 imol/1 over baseline levels; AST, aspartate aminotransferase; ALT, alanine aminotransferase; RUQ, right upper quadrant; FHR, fetal heart rate; BPP, biophysical profile; US, ultrasound

characterized. Special focus was placed on the CHS guidelines[1,21,22]. Reviewed in detail for candidate predictors were existing illness-severity models, including the leading neonatal (score for neonatal acute physiology (SNAP)-II), pediatric (pediatric risk of mortality (PRISM)) and adult (APACHE) models. These rely on physiologically based items from bedside vital signs and laboratory tests. Derangements from physiological norms increase the likelihood of adverse outcome (mortality), and the greater the derangements, the greater the risk. Particular attention was paid to APACHE, which includes variables from seven organ systems; the weighted sum of the derangements across all organ systems is presented as a composite, associated with calculable mortality risk.

To develop a list of candidate predictor variables, we modified the approach taken in APACHE to account for the varied presentation of pre-eclampsia (e.g. hypertension with elevated liver enzymes) and the normal pregnancy changes in clinical and laboratory parameters. We formed a list of candidate maternal and fetal predictors to quantify dysfunction

within each of seven maternal organ systems (included in APACHE), as well as the fetus. The variables chosen fulfil the criteria of Richardson and colleagues[23], in that they are available, measurable, frequently obtained, accurately recorded and reliable. The requirement for measurability led us to exclude maternal symptoms, which are included in the CHS-proposed 'adverse features'[1]. Although BP is associated with significant inter- and intraobserver variability[8], its inclusion was necessary for face validity (as BP defines pre-eclampsia) and generalizability.
The candidate predictors were reviewed, using the iterative Delphic method, by 14 international experts who are leaders in clinical and basic science pre-eclampsia research in North America, Australia and

Table 4 Candidate maternal and fetal predictor variables

Organ system	*Variable(s)*
	gestational age on admission (admission during which delivery occurred)
Maternal	
Cardiovascular	systolic BP diastolic BP
Renal	albuminuria: 24-h urine spot protein/creatinine ratio dipstick proteinuria urine output uric acid creatinine
Hepatic	aspartate aminotransferase (AST) lactate dehydrogenase (LDH) bilirubin albumin
Respiratory	FIO_2/SaO_2
Hematological	platelet count mean platelet volume (MPV) MPV/platelet count ratio fibrinogen
Central nervous	seizures
Fetal	
Growth	estimated fetal weight (centile)
Renal perfusion	amniotic fluid index (centile)
Vascular maladaptation	umbilical artery Doppler (present vs. absent/reversed)

BP, blood pressure; FIO_2, fraction of inspired oxygen; SaO_2, arterial oxygen saturation

the UK. After review of errors of commission and omission, the final list was derived (Table 4). It is recognized that other investigations, such as uterine artery Doppler velocimetry, may have utility in this scenario[24].

Maternal mortality is a rare event, even among women with pre-eclampsia[2]. It is more relevant to predict serious maternal morbidity that would preclude safe pregnancy prolongation. We proposed a list of outcomes based on known serious end-organ complications of pre-eclampsia that we felt would change clinical management, by being considered worthy of avoidance, even in the face of extreme prematurity. As for the predictor variables, this list was reviewed by (the same) international experts, using the Delphic method. Table 5 presents the final list of significant maternal outcomes that were widely endorsed when presented at the International Society for the Study of Hypertension in Pregnancy (ISSHP) meeting, Toronto, June 2002[20]. The time frame considered to be most relevant for prediction of serious maternal complications was 1 week (from admission).

Candidate clinical and laboratory predictor variables were collected at admission to hospital. Data were entered into a customized Windows Access™ (Microsoft Corp., Redmond, USA) database. Unfortunately, most candidate predictor variables (including 24-h urinary protein, on which the diagnosis of proteinuria of pre-eclampsia should be based) were available in <80% of women, making impossible the derivation of

Table 5 Combined adverse maternal outcome (primary outcome)

Organ system	*Outcome(s)*
Maternal (death or one/more of)	
	mortality
Hepatic	failure
	hematoma
	rupture
Central nervous	Glasgow coma scale (GCS) <13
	stroke
	two or more seizures or status eclampticus
	cortical blindness (whether transient or permanent)
Cardiovascular	positive inotrope support
	myocardial infarction
	infusion of any third antihypertensive
Renal	dialysis (whether temporary or permanent)
	renal transplantation
Respiratory	requirement of ≥50% O_2 for >1 h, or intubation
Hematological	transfusion of ≥10 U of blood products (in total)

a development set from retrospective data. We require prospective data collection.

The combined adverse maternal outcome occurred in 58 (10.4%) women, making the maternal outcome sufficiently common to make its prediction feasible. An exploratory analysis of the data indicated that, on the day of admission, lower gestational age, lower fibrinogen and increased mean platelet volume/platelet count ratio were significantly associated with adverse maternal outcome within 1 week of hospital admission, also after logistic regression[20].

In summary, at present, we cannot predict which mothers with pre-eclampsia are at increased risk of maternal complications, and we cannot grade this risk. To improve perinatal outcome safely in the setting of pre-eclampsia, we need to be able to compare quantifiable maternal risk with established quantifiable perinatal risks, and identify which pregnancies can be reasonably prolonged.

Expectant therapy

Randomized controlled trials (RCTs) have shown that, remote from term, prolongation of pregnancy by expectant therapy (delaying delivery until compelled by either maternal or fetal condition) decreases serious perinatal morbidity without increased maternal risk[25]. We systematically reviewed the two RCTs (133 women) that reported the impact on maternal and perinatal outcomes of expectant (versus aggressive) management of pre-eclampsia at <34 weeks' gestation (Table 6)[25]. All women received corticosteroids and antihypertensive therapy (to achieve dBP 90–100 mmHg), and were observed for 24 h. Thereafter, women randomized to aggressive management were delivered. Women randomized to expectant management continued on antihypertensives, and were monitored for maternal and fetal well-being until 34 weeks' gestation or delivery was otherwise considered necessary. Expectant (versus aggressive) management prolonged pregnancy by 2.0 (95% confidence interval 1.4–2.6) weeks[25], resulted in equivalent maternal morbidity (although statistical power was limited to detect a difference between groups) and decreased serious neonatal morbidity despite increasing the risk of small-for-gestational-age (SGA) infants (<10th centile). This suggests that the most important predictor of outcome among fetuses born to women with pre-eclampsia presenting remote from term and being expectantly managed is gestational age, and not fetal growth. Any intervention that might benefit these women should not adversely affect gestational age at delivery.

Table 6 Expectant (vs. aggressive) management of pre-eclampsia remote from term

	Expectant	*Aggressive*	*Number of trials*	*OR (95% CI)*
Maternal outcomes				
HELLP	2/49 (4.1%)	1/46 (2.2%)	1	1.85 (0.19–20.0)
Perinatal outcomes				
Neonatal morbidity*	6/18 (33.3%)	15/20 (75.0%)	1	0.19 (0.05–0.68)
NICU admission†	37/49 (75.5%)	46/46 (100%)	1	0.11 (0.03–0.37)
Respiratory distress syndrome (RDS) or vent > 24 h†	13/66 (19.6%)	30/66 (45.5%)	2	0.30 (0.15–0.63)
Bronchopulmonary dysplasia (BPD)†	2/49 (4.1%)	4/46 (8.7%)	1	0.46 (0.09–2.38)
Necrotizing enterocolitis (NEC)†	0/49 (0%)	5/46 (10.9%)	1	0.12 (0.02–0.69)
Small-for-gestational-age infants	15/49 (30.6%)	5/46 (10.9%)	1	3.23 (1.22–8.33)

*Pooled measure of perinatal death, RDS, NEC and/or pneumonia; †these outcomes are not mutually exclusive; HELLP, hemolysis, elevated liver enzymes, low platelets; NICU, neonatal intensive-care unit; OR, odds ratio; CI, confidence interval

As stated, these RCTs had insufficient power to detect a difference in serious maternal outcomes between groups, and uncertainty about the magnitude of the maternal risk has made some reluctant to use expectant therapy.

Closer to, or at, term (≥34 weeks), expectant management has been advocated[17], and endorsed by Canadian practitioners[15], to achieve smaller neonatal gains and to avoid Cesarean section following failed induction of labor.

Non-drug interventions

Treatment guidelines have recommended the use of non-pharmacological approaches for hypertension in later pregnancy, with or without antihypertensives, particularly for mildly increased BP[1–4]. Six trials (607 women) have tested either outpatient ambulation (versus bed-rest in hospital) or inpatient ambulation (versus inpatient bed-rest) for women with mild gestational hypertension. No RCTs have enrolled women with moderate to severe hypertension. There was no effect of restricted activity (with or without hospital admission) on maternal or perinatal outcomes,

although women managed as outpatients spent an average of 2.3 weeks (95% CI 1.9–2.8) less in hospital[25].

No clinically important benefits were apparent in two small RCTs of psychological support (80 women) or biobehavioral training (45 women).

In summary, the data do not facilitate reliable conclusions to be drawn about the benefits and risks of restricted activity (with or without hospital admission) for mild gestational hypertension. In the absence of definitive data, antihypertensive therapy should not be delayed while recommending non-drug approaches. No data exist to guide the use of rest in either mild or moderate hypertension in later pregnancy[25].

Control of maternal BP: impact on maternal and perinatal outcomes

No published reports address at what level of BP does initiation of antihypertensive therapy, for any type of pregnancy hypertension, optimize perinatal outcome and protect maternal well-being[22]. We have updated our earlier systematic review to include 26 RCTs (four new trials) that examined the impact of antihypertensive therapy (versus placebo or no therapy), for all types of mild-moderate pregnancy hypertension, on maternal and perinatal outcomes (Table 7)[25]. Although

Table 7 Odds ratio (95% confidence interval) for the impact of 'less tight' (vs. 'tight') control of blood pressure on perinatal and maternal outcomes, by type of hypertension

	All types of hypertension	*Pre-existing (n = 7 trials)*	*'Mixed'* (n = 17 trials)*
Perinatal outcomes			
Small-for-gestational-age infants	0.84 (0.65–1.09)	0.78 (0.42–1.45)	0.86 (0.65–1.14)
Respiratory distress syndrome	3.92 (1.96–7.82)	not reported	3.92 (1.96–7.82)
Maternal outcomes			
Severe hypertension	2.53 (2.00–3.19)	3.71 (1.88–7.14)	2.40 (1.88–3.19)
Hospitalization	2.18 (1.54–3.07)	4.36 (1.42–13.37)	2.02 (1.41–2.91)
Proteinuria at delivery	1.32 (1.06–1.63)	1.45 (0.93–2.26)	1.28 (1.00–1.63)
Prematurity	1.03 (0.82–1.29)	0.68 (0.35–1.33)	1.08 (0.85–1.38)

*These trials enrolled mixed populations of women with either pre-existing or gestational hypertension (including pre-eclampsia), without reporting outcome by type of hypertension

there are limitations to these trials (most enrolled mixed populations with pre-existing or gestational hypertension without reporting outcome by type of hypertension, and most were not powered to examine the risk of perinatal complications or SGA infants), they provide the least biased information on treatment effectiveness. Not reviewed here are the 23 trials (three for women with moderate-severe pre-eclampsia expectantly managed) that compared one antihypertensive with another, as the dBP goals were the same for *both* treatment groups[25].

Women randomized to 'tight' control (the antihypertensive arm of trials) received antihypertensives when dBP was ≥90 mmHg, to achieve dBP <90 mmHg. Women randomized to 'less tight' control (placebo/no therapy arm of trials) received antihypertensives only when BP reached 160/100–110 mmHg, to prevent further rises in BP.

'Less tight' (versus 'tight') control decreased the risk of SGA infants (<10th centile), with similar but non-significant effects seen by type of hypertension. In the two pre-eclampsia trials, 'less tight' control did not have an adverse impact on gestational age at delivery (weighted mean difference of +0.39 week (gained) in the expectant (versus aggressive) group, 95% CI -0.18 to 0.95; n=2 trials)), the most important determinant of neonatal morbidity. There was significant between-trial heterogeneity in outcome that could not be explained by type of antihypertensive, with the exception of an atenolol versus placebo trial[26], a statistical outlier[25]. Atenolol is clearly a different drug in this scenario, being associated with reduced fetal growth velocity in RCTs, cohort studies and case-control studies[27].

We have investigated[28], and recently updated[29], this heterogeneity by meta-regression analysis of the difference between groups in the change in mean arterial pressure from enrolment to delivery, compared with parameters of fetal growth. We included data from all treatment trials (excluding reference 26), given that differential control of maternal BP was of interest. There was a significant and linear correlation between magnitude of treatment-induced fall in mean arterial pressure and both SGA infants (for ln (fodds ratio) SGA: slope 0.09±0.03, r^2=0.48, p=0.0006, n=14 trials)[28] and birth weight (slope -7.55±6.67, r^2=0.19, p=0.05, n=27 trials)[29].

On the other hand, our systematic review of antihypertensive versus placebo or no therapy trials[25] revealed that 'less tight' control also increased the risk of respiratory distress syndrome (RDS). This effect was consistent between trials. However, only 6/26 trials reported RDS, and only six additional trials showing no treatment effect would be required to negate the observed effect of 'less tight' control. Second, the incidence of RDS was high in the 'less tight' group (28/438=6.4%) given that most delivered at term. Third, RDS was either not defined,

or defined as endotracheal intubation or ventilation >24 h, and therefore could have included transient tachypnea of the newborn, which is not serious. Finally, we have no adequate biological explanation for why 'less tight' control should increase the rate of RDS, as there was no concomitant change in the rate of prematurity overall or among trials that reported RDS (odds ratio 1.03, 95% CI 0.82–1.29, *n*=13 trials)[25].

'Less tight' control also increased the risk of 'severe' hypertension, hospitalization and proteinuria at delivery[25] for all types of hypertension (where applicable). However, the incidence of prematurity was not decreased, as would have been expected if the decrease in proteinuria at delivery (variably defined) reflected a true decrease in pre-eclampsia. In addition, the relevance of non-sustained 'severe' hypertension as defined (≥160/100–110 mmHg) is uncertain, as it is below the level at which cerebrovascular autoregulation is lost and stroke risk increased.

In summary, expectant (versus aggressive) management of pre-eclampsia remote from term results in better perinatal outcome. Once severe hypertension has been controlled, how best to manage mild-moderate hypertension is unclear, among women with pre-eclampsia who are expectantly managed. 'Less tight' control may be beneficial by decreasing the risk of SGA infants (without compromising gains in gestational age for women with pre-eclampsia). However, 'less tight' control may be harmful by increasing RDS, maternal BP ≥160/100–110 mmHg, antenatal hospitalization and proteinuria at delivery. Nevertheless, sufficient confidence cannot be placed in these results because of reporting bias and uncertainty about the clinical relevance of the outcomes as defined.

Current standard of care

For women with pre-eclampsia who present remote from term, expectant management may be appropriate in the absence of published criteria for delivery[17], which were endorsed by the majority of clinicians who we consulted in a national survey[15]. For women with pre-eclampsia near term, the risks and benefits have not been quantified.

As discussed, there is consensus that severe hypertension should be treated[2,3,7,22]. In addition, there is now no doubt of the utility of $MgSO_4$ for the prevention and treatment of eclampsia in women with mild or severe pre-eclampsia (Chapter 12).

The management of postpartum mild to moderate pre-eclampsia and the follow-up of women following pre-eclampsia pregnancy are discussed in Chapter 15.

CONCLUSIONS

The management of mild to moderate pregnancy hypertension, regardless of type, remains controversial. Much of the uncertainty results from reliance on current definitions of pre-eclampsia and the lack of adequate evidence that can be used to formulate evidence-based treatment guidelines.

First, the definition of 'mild pre-eclampsia' remains unsubstantiated in terms of the ability of that definition to define a level of maternal and/or perinatal risk. At present, there is no accepted definition of 'moderate pre-eclampsia'. A clinical model is required that is predictive of adverse maternal and/or fetal outcomes from the time of first diagnosis[20], which incorporates the influence of gestational age at disease onset[10,20] and which provides a more refined stratification of risk than the current dichotomous definitions[1-4].

Second, the role of ambulatory and/or automated BP monitoring requires validation as its use spreads in both antenatal home-care programs and high-dependency obstetrics units.

Third, the pattern of both the initial diagnostic work-up and ongoing surveillance differs between the various international guidelines[1-4] and between those guidelines and their implementation in practice[14,15].

Fourth, while expectant therapy of pre-eclampsia remote from term clearly reduces perinatal risks, the additional maternal risk associated with this approach has not been quantified. Although there is some support for expectant therapy at or near term by experts[17] and practitioners[15], guidelines generally advise delivery under these circumstances[1-4].

Fifth, the role of non-pharmacological therapy in mild to moderate pre-eclampsia is unclear; it is unsupported by the limited RCT evidence[25] but used by most Canadian practitioners[15].

Finally, 'tight' BP control is recommended by Canadian (therapy for BP >140–150/90–95 mmHg) and Australasian (therapy for BP ≥160/90 mmHg) guidelines[3,22]. 'Less tight' control is advocated in the USA (therapy for dBP ≥100 mmHg)[2]. Leading members of groups that developed the Canadian and American guidelines stated, at a Medical Research Council (Canada)-sponsored workshop, that the evidence is insufficient to assess the relative benefits and harms of antihypertensive treatment for mild-moderate hypertension. Collective uncertainty about the choice of BP goal was also seen among Canadian practitioners whom we surveyed[15]; regardless of the type of hypertension, 49–52% of clinicians stated that they aimed for a dBP of 80–89 mmHg, whereas 45–47% aimed for a dBP of 90–99 mmHg.

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12

Labor ward management of severe pre-eclampsia

J. Tomlinson

DEFINITION OF SEVERE PRE-ECLAMPSIA

Severe disease is not identified by one feature alone but by a combination of factors. Roberts and Villar[1] have proposed the clinical diagnosis of severe disease as patients with a very high blood pressure (>170 mmHg systolic, >110 mmHg diastolic) and heavy proteinuria, or lesser degrees of hypertension (>150 mmHg systolic, >100 mmHg diastolic) with two or more signs of imminent eclampsia such as hyperreflexia, frontal headache, blurred vision or epigastric tenderness. In their consensus statement, the Australasian Society for the Study of Hypertension in Pregnancy[2] do not give criteria for severe disease but they do note indications for delivery, which include inability to control blood pressure, deteriorating liver function, deteriorating renal function, progressive thrombocytopenia, placental abruption, imminent eclampsia or concern about fetal welfare. Thus, there is no standard universally accepted definition of severe disease, and each case will need to be weighed individually. The recently produced North-West Regional Pre-eclampsia guidelines are attached as an Appendix as a practical outworking of these theoretical points.

Measurement of the blood pressure is of critical importance to the diagnosis of pre-eclampsia, and the level of blood pressure has treatment implications; blood pressure should thus be measured as accurately and reliably as possible. Measurement of blood pressure is covered in Chapter 9; however, one important point from the *Confidential Enquiry into Maternal Deaths*[3] is that automated blood pressure devices can seriously underestimate

maternal blood pressure, and thus, at initial assessment, the pressures obtained with an automated cuff should be compared with those obtained using a conventional mercury sphygmomanometer.

On occasion, a patient will arrive on the labor ward, usually after admission from the community, with high blood pressure that settles with relatively little treatment. The cause of this is likely to be 'white-coat hypertension', which is more common in women and in the young[4].

Proteinuria is the other feature that is used to define a patient with pre-eclampsia. There are potential confounding factors such as contamination. If severe disease is suspected, the patient should be catheterized so that the degree of proteinuria can be accurately assessed. The presence of an indwelling catheter will also allow for accurate measurement of the hourly urinary output.

MANAGEMENT

The aims of the labor ward management of such patients can be divided into maternal and fetal. Maternal treatment is to prevent complications occurring while awaiting the resolution that happens following delivery. The fetal aims are to deliver a baby in as healthy a condition as possible, to minimize morbidity and mortality. Sometimes these objectives conflict, and thus acting in the maternal interest takes priority. In the absence of alternative treatment for the cause of the pre-eclampsia, delivery is the ultimate cure, and this will be the normal course of action. This can lead to difficult decisions, however, when pre-eclampsia occurs at early gestations (<24 weeks); the fetal prognosis is poor with very few survivors, and termination of pregnancy may be recommended in this group[5]. Other therapies are aimed at limiting maternal and fetal morbidity.

PREVENTION OF MATERNAL COMPLICATIONS

Hypertension

Use of antihypertensive agents has increased over the past 20 years, at the same time that maternal deaths from cerebral causes related to hypertensive disorders have decreased. However, in the latest *Confidential Enquiry into Maternal Deaths* (1997–99)[3], intracranial hemorrhage was the largest single cause of death; this reflected a failure of effective antihypertensive treatment. There is at present little evidence as to which antihypertensive agent to use, or the threshold

at which treatment should be instigated. The aim of treatment is to prevent end-organ damage.

Cerebral blood flow is under autoregulation, where the controlling mechanisms maintain a constant blood flow over a range of blood pressures. With higher blood pressures the autoregulation system is overcome, the brain is exposed to these high blood pressures and hypertensive encephalopathy is likely to develop. A confounding factor is that patients tolerate hypertension to differing thresholds; those with chronic hypertension are able to manage higher systolic pressures than, for example, teenagers in whom the blood pressure will have started from a lower level. In younger women it has been shown that hypertensive encephalopathy can occur at a diastolic pressure of 120 mmHg[6]. In an individual patient on the labor ward it may be difficult to make such judgements about a threshold for cerebral damage, and thus it would seem reasonable to adopt a trigger level where antihypertensive treatment is started. A commonly used level would be blood pressure persistently above 160/110 mmHg. A systolic pressure persistently higher than 170 mmHg is also thought to be associated with significant risks[5].

The aim of treatment is a diastolic pressure of between 90 and 100 mmHg. Lower levels can result in significantly diminished placental perfusion. A rapid reduction of the blood pressure should be avoided as this may also cause fetal distress. It has not been determined whether blood pressure control will prevent seizures; however, as outlined below, seizure prophylaxis with magnesium sulfate should also be commenced in patients with severe pre-eclampsia.

First-line antihypertensive agents

Hydralazine This has been used widely; there is a large degree of clinical experience with this agent. The mechanism of action of hydralazine is to relax the arteriolar smooth muscle, and thus decrease total peripheral resistance.

There are several unwanted effects with hydralazine. Headaches are a result of vasodilatation. After administration there is believed to be a release of noradrenaline which causes tachycardia, and other symptoms such as hyperreflexia, anxiety and restlessness may also result. All of these effects can give the clinician an impression of imminent eclampsia. Given that hydralazine may cause maternal tachycardia, it may not be wise to give this medication to patients with heart rates greater than 100 beats/min.

Hydralazine can be given both orally and parenterally; when given by the latter route the onset of action is 5–15 min and the half-life is 3 h; however, some patients can experience prolonged effects. This is

explained by the binding of hydralazine to vessel walls, resulting in a prolonged pharmacological response.

Labetalol This can be given orally or intravenously, and has a mechanism of action which is by a combination of a- and (β-sympathetic blockade. The a/(β-blocking ratio is 1:7. The majority of the drug is metabolized in the liver to form an inactive glucuronide conjugate. When given intravenously, the hypotensive effect of labetalol begins within 2–5 min and reaches a peak after 15 min, the action persisting for up to 4 h. Heart rate is maintained or slightly reduced owing to the (β-blocking effect; however, unlike other pure (β-blockers there is no decrease in cardiac output. Peripheral resistance is reduced; however, cerebral, coronary and renal blood flow is maintained. Little placental transfer occurs owing to low lipid solubility.

With bolus doses of 0.5–2 mg/kg there is an almost immediate fall in blood pressure. This is usually followed by an intravenous infusion of 20 mg/h which is increased every 10–20 min until control is achieved, or until a maximum dose of 160 mg/h is reached. Maternal adverse effects include tremulousness, headache and hepatotoxicity; hepatotoxicity is likely to represent an idiosyncratic reaction to the labetalol. Placental perfusion is unaffected by labetalol.

Nifedipine This is a type-2 calcium channel antagonist that inhibits the entry of calcium ions through the slow channel in the cell membranes of cardiac and smooth muscle cells. It is administered orally as 10–20-mg boluses, and repeated every 30 min. Adverse effects include tachycardia, headaches, flushing, dyspnea, chest pain and heartburn. It does not alter uteroplacental blood flow unless there is a precipitous fall in the blood pressure, causing maternal hypotension.

Nifedipine is not absorbed through the buccal mucosa but is rapidly absorbed from the gastrointestinal tract. The mechanism of action is to cause vasodilatation of the arterioles, with onset of action noted after 5–10 min, peak effect at 60 min and duration of action approximately 6 h. It is not available in intravenous form; however, a similar drug, nicardipine, is a water-soluble calcium channel blocker which can be given by this route.

When compared with hydralazine, nifedipine appears to be as efficacious with fewer episodes of hypotension and is easier to administer.

Calcium channel blockers are tocolytics and may affect progress in labor, although the antihypertensive doses are usually much lower than those used for tocolysis. However, nifedipine has the desired effect of improving renal blood flow with an associated increase in urine output.

There are also concerns that nifedipine may potentiate the effects of magnesium sulfate and cause maternal muscle weakness and

hypotension[7]. Animal studies have shown increased hypotensive activity of nifedipine when given in conjunction with magnesium sulfate. This concern is theoretical, and interaction with magnesium sulfate has not been noted in any observational or randomized trials.

Second-line agents

Sodium nitroprusside This is an arterial and venous vasodilator. It is a very potent antihypertensive agent with an onset of action within 1–2 min and a plasma half-life of 3–4 min. It is metabolized by the liver and excreted by the kidneys. Nitroprusside has significant toxicity, with cyanide being released in a dose-dependent fashion. Thus, this treatment should only be considered in patients with normal hepatic and renal function, the dose should be limited to a maximum of 2 μg/kg/min and the duration of treatment limited to the shortest possible time. If higher doses of nitroprusside are used, then thiosulfate should be administered, as this compound is required for the metabolism of nitroprusside. Hydroxocobalamin at an infusion rate of 25 mg/h is also a safe and effective method of preventing and treating cyanide toxicity. Fetal problems have been noted, in the form of fetal cyanide poisoning, transient bradycardia and metabolic acidosis. Nitroprusside may be the drug of choice for hypertensive crises, where end-organ damage is occurring.

Diazoxide This acts by relaxing arteriolar smooth muscle. It has a rapid onset of action when used intravenously, with a peak action at 10 min. The duration of action is 3–18 h. Diazoxide is given as bolus doses of 1–3 mg/kg (up to a maximum of 150 mg). Repeated doses may be used; however, significant side-effects of hyperglycemia and hyperuricemia may occur.

Which is the best antihypertensive agent to use?

In a meta-analysis, Magee and colleagues[7], reviewed 11 trials in which parenteral hydralazine was compared with intravenous labetalol and oral/sublingual nifedipine. In these trials there were 570 participants, and it was shown that use of hydralazine was associated with a greater risk of maternal hypotension, increases in Cesarean section rates, placental abruptions and lower Apgar scores. Neonatal bradycardia was increased with labetalol; however, only one in six of those affected required specific treatment. Another factor in favor of labetalol is that when general anesthesia is required, labetalol diminishes the hypertensive and tachycardic responses that occur during intubation.

Concerns about the theoretical potentiating effects of calcium channel blockers and magnesium sulfate, and the side-effects of tachycardia and cerebral vasodilatation with nifedipine, would again suggest that labetalol is the preferred first-line agent.

Both nitroprusside and diazoxide can cause excessive hypotension, and thus are agents of last resort in patients with refractory hypertension.

Agents under investigation

Calcium channel blockers Isradipine is a second-generation calcium channel blocker. It has the advantage of increasing renal plasma flow. Nimodipine is another calcium channel antagonist that has been shown to decrease cerebral vasospasm; however, there may also be hypoxic effects on the fetus (the fetal middle cerebral artery Doppler pulsatility index has been found to decrease). Nicardipine has the theoretical benefit of being less negatively inotropic than nifedipine, and also has the advantage of intravenous administration.

Serotonin receptor antagonists Ketanserin is a competitive selective serotonin-2 receptor antagonist that decreases systolic and diastolic blood pressure in both non-pregnant and pregnant patients. Stimulation of serotonin-2 receptors causes arterial and venous vasoconstriction, an increase in heart rate and platelet aggregation. It is thus postulated that ketanserin blockade of circulating serotonin in pre-eclampsia results in beneficial effects. Ketanserin has been found to reduce severe hypertension in pregnancy. When compared with hydralazine, it has equivalent efficacy but fewer side-effects. Use in HELLP syndrome (hemolysis elevated liver enzymes and low platelets) has also shown a rise in platelet count, in addition to antihypertensive effects[8].

Seizure prophylaxis and treatment

Eclampsia is defined as a convulsive state occurring for the first time during pregnancy, with hypertension prior to, or after, the fit has taken place[9]. When witnessed, the first priority is to protect the airway and roll the patient over onto her left side. In the British Eclampsia Survey, it was noted that 59% of women experienced one or more prodromal symptoms: headache, visual disturbance or epigastric pain[10]. Fifty per cent of cases of eclampsia occur prior to delivery, and in those who develop eclampsia postnatally it is usually in the first 24 h after delivery (although eclampsia has been reported up to 3 weeks after delivery). Eclamptic convulsions are usually self-limiting, although rare cases can present as intractable fitting. Eclampsia is now a relatively rare event, with an incidence of approximately 1/2000 deliveries in developed countries. This contrasts with a much higher

rate in the developing world where incidences of up to 1/100 have been reported. The aim of prophylaxis and treatment is to prevent the complications of seizures, namely the associated hypoxia with its risk of neuronal death, rhabdomyolysis, metabolic acidosis, neurogenic pulmonary edema and respiratory failure. Aspiration with consequent pneumonia may also occur when the airway is unprotected.

Treatment

Several anticonvulsants have been used over the years, with little evidence to choose between them. Of the most popular agents used, benzodiazepines seem a logical choice because they are used to terminate fits in status epilepticus, despite concerns about the effects of sedation and respiratory depression in both the mother and the fetus. Phenytoin, an anticonvulsant used in epileptics that does not cause sedation, has been used in efforts to prevent eclamptic fits. In North America, magnesium sulfate has been used for many years; however, the use of the agent outside North America has been limited, possibly owing to the absence of a rationale regarding the mode of action.

There have been several randomized controlled trials comparing different anticonvulsant agents. The largest study to date comparing different treatments for eclampsia has been the Collaborative Eclampsia Trial[11], a multicenter international study. In this trial, 1687 women with a clinical diagnosis of eclampsia were randomized to receive magnesium sulfate, phenytoin or diazepam. There were two arms to the trial and comparisons of magnesium with phenytoin and magnesium with diazepam were made. As the medications were in diverse settings, there was an emphasis on safety, and monitoring was not sophisticated. In both arms of the trial, there were significantly fewer recurrent seizures with magnesium sulfate, and also a non-significant trend towards lower maternal mortality. When magnesium sulfate was compared with phenytoin, the maternal morbidity was less, as in the phenytoin group there was a greater incidence of pneumonia, greater requirements for ventilation and more admissions to the intensive-care unit; it was suspected that these complications were due to the phenytoin rather than due to a beneficial effect of magnesium sulfate. It is now clear that, in most cases, magnesium sulfate is the treatment of choice for eclampsia. That said, if a woman fits after having received a 4-g magnesium loading dose and is on a 1 g/h infusion regimen, an alternative agent may be required and the diagnosis should be reviewed.

Prophylaxis

The prediction of which patient is going to experience eclampsia is imprecise. Having demonstrated that magnesium sulfate is the best

agent for the treatment of eclampsia, the optimal prophylactic treatment for eclampsia still remained to be elucidated. A source of debate was whether a patient actually needed a form of prophylaxis other than control of their hypertension, as it has been noted that average blood pressure levels are higher in women who develop eclampsia. In addition, the threshold for treatment was unclear. In the mid 1990s in the USA it was estimated that 5% of pregnant women were receiving magnesium sulfate, whereas in the UK prophylactic anticonvulsants were reserved for the 1% of patients with severe disease. A further large multicenter randomized trial was launched, the Magpie trial[12], to answer these questions. In this study, 10136 patients were randomized, with nearly 5000 women in each arm receiving either magnesium sulfate or placebo. There were significantly fewer women with eclampsia in the treatment group (seven per 1000 women receiving magnesium sulfate had eclampsia as opposed to 19/1000 in the placebo group), alternatively stated as a 58% lower relative risk. This finding was consistent when subgroup analysis of the severity of pre-eclampsia at entry was analyzed. Again, a non-significant trend towards lower maternal mortality was observed with magnesium use. It also reassuringly demonstrated that, at the doses used, there was no greater chance of adverse outcome for babies born to mothers who had been entered into the trial prior to delivery.

Magnesium sulfate pharmacokinetics

The mechanisms of action of magnesium sulfate are not fully understood; however, postulated modes of action include a vasodilator effect (via an increase in the endothelial production of prostacyclin, decreasing cerebral ischemia). Doppler examinations of middle cerebral arteries have shown increased cerebral blood flow in pre-eclamptic women receiving magnesium sulfate. A further mechanism of action is to act as an *N*-methyl-D-aspartate (NDMA) receptor inhibitor. This receptor, when stimulated by agonists, causes neuronal excitability and seizures.

Route of administration The intravenous route allows a more constant plasma concentration to be achieved when compared with the intramuscular route; the latter is also associated with the painful side-effects of large-volume intramuscular injections; several abscesses were reported in the Collaborative Eclampsia Trial.

Protein binding Approximately 40% of plasma magnesium is protein-bound. This protein-bound magnesium is not in glomerular filtrate, as opposed to free magnesium. With higher concentrations of magnesium, the amount that is protein-bound increases slightly.

Pharmacokinetics When administered intravenously, there is a rapid distribution of magnesium followed by a slow elimination. When measured, there is a higher level in the neonates of women who received magnesium prior to delivery, compared with controls; this excess is eliminated in the first 48 h of life.

Maternal elimination of magnesium is solely in urine; renal excretion in patients with normal renal function is directly proportional to the serum magnesium concentration, and once equilibrium has been achieved, excretion is almost equal to infusion. The half-life of magnesium is about 4 h. During magnesium therapy, urinary calcium excretion also increases; this is likely to be because magnesium and calcium compete for common reabsorption sites. Hypocalcemia is thus a potential problem with high doses of magnesium over prolonged periods.

Administration The optimal dose has not been fully elucidated; two popular regimens have been widely employed, an intramuscular regimen proposed by Pritchard and associates[13] and an intravenous regimen recommended by Zuspan[14]. For reasons given above, the intravenous regimen is preferred. After a 4-g intravenous loading dose, intravenous infusions of either 1 or 2 g/h have been suggested. A loading dose of 4 g and an infusion rate of 1 g/h leads to a steady state of magnesium of 1.7 mmol/l; a 2-g/h infusion rate gives a steady state of 2.2 mmol/l. The therapeutic concentrations of magnesium are considered to be between 2 and 4 mmol/l. However, in the large clinical trials (the Collaborative Eclampsia Trial[11] and the Magpie trial[12]), the lower dose was used and was shown to be effective. Thus, 1 g/h is an acceptable regimen to use. At this lower dose, clinical benefits as well as safety have been demonstrated.

Toxicity Reported side-effects of magnesium sulfate include flushing, sensation of warmth, headaches, blurred vision, nystagmus, hypothermia, urinary retention and fecal impaction (at magnesium levels of 3.8–5.0 mmol/l). The patellar reflex is also lost at this level; however, if the magnesium level rises further, then respiratory paralysis occurs at 5–6.5 mmol/l. Cardiac conduction is affected at levels >7.5 mmol/l, and is followed by cardiac arrest at higher levels.

The clinical monitoring of patients receiving magnesium sulfate should include oxygen saturations (using a pulse oximeter) and respiratory rates. Regular assessment of the deep tendon reflexes is recommended; when these are present, toxic levels are unlikely. It is also wise to monitor the urine output, as in the presence of oliguria the chance of toxicity increases (>80 ml in 4 h is suggested as an indicator of adequate renal function). Cardiac conduction should also

be regularly assessed. In the Appendix, the North-West Regional Guidelines give a suggested method of monitoring these patients.

In the presence of renal insufficiency, a loading dose is safe; however, in these circumstances, the maintenance dose may need to be modified or omitted. In the event of toxicity, the antidote is calcium gluconate 1 g, given by slow intravenous injection.

Recurrent convulsions In this situation it is recommended that a further 2–4 g magnesium sulfate is given intravenously over 5 min. Confirmation that a therapeutic level has been reached can be achieved by checking the serum levels of magnesium. If seizures are still not controlled, then a search for alternative diagnoses should be performed.

Thromboprophylaxis

As the plasma volume is reduced and the patient is relatively immobile when being treated for severe pre-eclampsia, conditions are ripe for thromboembolic disease to occur. According to the Royal College of Obstetricians and Gynaecologists guidelines, patients with pre-eclampsia should be treated as at moderate risk[3]. Elasticated compression antiembolic stockings should therefore be worn by all these patients. Use of heparin can be a little more difficult in the antenatal period owing to the risk of spinal or epidural hematoma if regional anesthesia is instigated while the patient has reduced coagulation function. If low-dose unfractionated heparin is used, then an epidural or spinal anesthesia should be delayed for a minimum of 4 h. However, low-molecular-weight heparins are more commonly used; owing to their more predictable bioavailability and prolonged action, a 10–12 h interval is recommended before spinal or epidural anesthesia/analgesia[3]. Similarly, removal of an epidural catheter will need to be timed if heparin has been given, with a minimum of 4 h after unfractionated heparin and 10–12 h for low-molecular-weight heparin.

Following delivery, all patients with severe disease should receive low-molecular-weight heparin until fully mobile.

Fluid management

In the patient with severe disease, careful attention needs to be paid to fluid management. This is because the low plasma oncotic pressure, raised hydrostatic pressure and increased capillary permeability favor water leaving the intravascular space and accumulating in the tissues. These factors, as well as poor left ventricular function, favor the development of pulmonary edema. During the postnatal period the patient is particularly at risk owing to autoinfusion of blood; as the

uterus contracts down, most of the blood contained in the uterine vasculature is expelled. This causes increased pressure in the venous circulation which, in turn, decreases the drainage of lymphatic fluid from the thoracic duct; this includes lymphatic fluid draining from the lungs.

Plasma volume expansion has been one treatment proposed, the rationale being that infused intravenous fluids improve tissue perfusion and increase oxygen delivery (particularly to the placenta, liver, kidneys). This may be particularly appropriate when vasodilators or epidural analgesia are used, to prevent hypotension. There have been small randomized trials of this treatment; with intensive maternal monitoring (including Swan-Ganz catheterization and intra-arterial blood pressure monitoring) there were no serious maternal complications; however, the trials were not large enough to show any beneficial maternal effects, and the monitoring required can be associated with significant morbidity[7,15].

It has also been observed that fluid overload is a significant cause of maternal morbidity. Thus, careful fluid balance by limiting the input is essential in the prevention of pulmonary edema. In the North-West guidelines (see the Appendix), total fluid input is limited to 80 ml/h. There is a theoretical possibility that running the patient too dry will increase the risk of pre-renal impairment. It should be noted, however, that renal function normally recovers completely unless there is another insult such as an abruption. If there is such an insult then more invasive monitoring should be considered, so that fluid requirements and resuscitation are not prescribed blindly.

In the uncomplicated patient in the antenatal period, low urinary output does not trigger specific actions, and it should be noted that, in normal women, urine output decreases in labor. In the postnatal period, fluid restriction is maintained until diuresis occurs, proteinuria clears and edema decreases. For a practical method of dealing with poor urine output, the guidelines in the Appendix can be referred to. If urine output still remains poor, then use of low-dose dopamine (1–5 μg/kg/min) has been shown to increase urine output without increasing the maternal blood pressure[16].

Fetal considerations

The ultimate cure for pre-eclampsia is delivery, and in a patient with severe disease this is often the management option chosen. However, as pre-eclampsia is associated with intrauterine growth restriction (IUGR), an assessment of the fetal condition needs to be made. The plan of management may well be influenced by presentation, presence

of IUGR or other signs of fetal compromise. In the patient with severe disease, assessments may need to occur on the labor ward rather than risk taking an ill patient with all her monitoring and treatment equipment to the ultrasound department. The acquisition of full information regarding the umbilical artery Doppler waveforms, and additional information about the biophysical status of the baby, may not be practical. Assessment of amniotic fluid, however, is easily achieved using low-specification equipment. Further discussion of fetal assessment is discussed in Chapter 14.

Delivery

Patients with severe pre-eclampsia should be stabilized prior to delivery; if delivery is unduly expedited, morbidity may be increased rather than reduced. Thus, delivery should be planned at the best time, in the best place, with the best team and in the best way.

Mode

This will depend upon a number of factors which will include parity, gestation, previous obstetric history, presentation and state of the fetus. In developed countries where there is expected to be a good neonatal outcome, then Cesarean section is usually opted for at gestations of less than 32 weeks. In their retrospective analysis of 145 patients with pre-eclampsia remote from term, Nassar and colleagues[17] showed that, at less than 34 weeks' gestation, only 48% of women who underwent induction of labor achieved a vaginal delivery. In this series, the chances of successful vaginal delivery increased with maturity from six out of 19 patients (32%) at less than or equal to 28 weeks' gestation to 25 out of 40 women (63%) at 32+1 to 34 weeks' gestation. Unsurprisingly, a higher Bishop score was associated with greater degrees of success. A further finding, although not reaching statistical significance, was that, if induction had been attempted, then the rate of classical Cesarean incision was 6.8%, compared with 13.6% in those women with pre-eclampsia who had not undergone induction of labor.

When delivery is expected at gestations of less than 34 weeks, administration of corticosteroids to the mother has been shown to decrease respiratory morbidity in the neonate, risk of intraventricular hemorrhage and neonatal death. The maximal effect is seen after 24 h, and will be of benefit if the fetus is to be delivered within 7 days of the course. When there is severe disease one would normally expect the fetus to be delivered in this time-window. If it is anticipated that delivery will occur before 24 h, steroids should still be given, as there is still a trend towards benefit among those babies whose mothers receive this treatment.

Anesthesia

With respect to analgesia in labor and anesthesia for operative delivery, this is covered in Chapter 13.

Labor

The labor is obviously a high-risk event, and the fetus should have continuous electronic fetal monitoring. Epidural analgesia is recommended, as this decreases the adrenaline output from the adrenal glands, which can exacerbate hypertension and cause incoordinate uterine activity. If the blood pressure is controlled, the mother can be encouraged to push actively in the second stage; however, the second stage should not be prolonged. If the patient has experienced eclampsia, it may be wise to perform elective instrumental vaginal delivery rather than risk raising the intracranial pressure with maternal pushing.

Third stage

Oxytocin 5 IU intravenously is preferred to oxytocin/ergometrine combinations for prophylaxis against postpartum hemorrhage.

TREATMENT OF COMPLICATIONS

HELLP syndrome

This is discussed in Chapter 9. The severity of the disease is reflected by the nadir of the platelet count. The lower is the nadir, the greater is the risk of postpartum hemorrhage. Superimposed disseminated intravascular coagulation (DIC) also increases the risk of other complications. It has been shown that patients will often deteriorate following delivery, before starting to improve. The nadir in platelet count is usually between 24 and 48 h after delivery. Magnesium sulfate may be of benefit, particularly in HELLP syndrome, because, as well as seizure-prophylaxis effects, it may also reduce platelet clumping and thus the platelet nadir may not be as low.

When delivery is expected at gestations of less than 34 weeks, maternal administration of corticosteroids has been shown to decrease respiratory morbidity in the neonate. In a patient with pre-eclampsia this practice should be encouraged, as not only is there benefit to the fetus but corticosteroids may improve the maternal condition. It should, however, be borne in mind that the placental disease process is not

altered by this treatment, and although there may be improvement in the maternal condition, the fetus should be monitored closely.

Once HELLP syndrome is diagnosed it can be associated with rapid deterioration, and delivery will normally be planned. At delivery there is an increased risk of hemorrhage and hematomas, and it is wise to site drains if delivery is by Cesarean section. If a large volume of ascites is encountered, this increases the risk of congestive cardiac failure and adult respiratory distress syndrome, and thus transfer to an intensive-care facility may be appropriate.

Platelet transfusions are given for prophylaxis against hemorrhage. Thus, if a surgical delivery is planned, platelets would be given if the count was <50000/mm^3. If the platelet count falls to <30000/mm^3, a platelet transfusion should be given to prevent spontaneous hemorrhage.

Liver rupture

Clinically, massive intra-abdominal bleeding and associated shock follow bilateral shoulder-tip pain. Aggressive blood transfusion and laparotomy is required. With the liver being edematous, suturing is often not possible, and packing or use of hemostatic gauze may be better. Alternatively, the interventional radiology technique of hepatic artery embolization is another possible method of achieving hemostasis.

Pulmonary edema

As well as demonstrating increasing breathlessness, tachycardia and elevated jugular venous pulse, a falling oxygen saturation will be noted. A chest X-ray (congested lung fields and possible effusions) and arterial blood gases (hypoxia with normal or elevated carbon dioxide) will confirm the diagnosis.

The patient should be treated in a sitting position and oxygen administered. Frusemide (20–40 mg intravenously) should be given to encourage diuresis of intravascular water, thus increasing the colloid oncotic pressure. If renal perfusion is poor, frusemide increases renal cortical perfusion and inhibits sodium transport, thus reducing the renal oxygen requirements.

Disseminated intravascular coagulation

This may occur in association with HELLP syndrome or if there is an abruption. There will be an elevation in fibrin degradation products with a reduction in fibrinogen level and platelet count. Management will be in conjunction with anesthetists and hematologists. Fresh frozen plasma is used to replace the clotting factors. If the fibrinogen is

particularly low, then cryoprecipitate may also be given. The coagulopathy normally reverses after delivery.

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13

Anesthesia considerations of pre-eclamptic patients

K. Mordani and A. Macarthur

INTRODUCTION

The objective of this chapter is to understand the anesthetic difficulties of the pre-eclamptic parturient. The parturient with pre-eclampsia should be delivered in a site with timely access to anesthetic personnel, as they are more likely to require anesthetic intervention and are at increased risk for anesthesia-related morbidity. The following sections outline the general difficulties for anesthetists in dealing with this patient population and the particular anesthetic considerations for labor and operative deliveries. Hopefully, we can provide insight into the anesthetist's perspective and facilitate improved collaborative patient care. The anesthetist providing labor and delivery service expects to evaluate these patients, preferably prior to emergent conditions. In this way, we can provide a safe plan to assist the patient and delivering physician.

GENERAL ANESTHETIC CONSIDERATIONS

Parturients with pre-eclampsia present unique challenges for the anesthetist, and conversely, the anesthetist can provide unique skills in the management of these patients. The systemic nature of this disease can alter the usual actions or responses of anesthetic agents.

The pathological features of pre-eclampsia of concern to anesthetists involve the respiratory, cardiovascular, hematological,

neurological and hepatorenal systems. The changes that can increase anesthesia-related morbidity or mortality include:

(1) Reductions in plasma colloid oncotic pressures are more common in pre-eclampsia than in normal pregnancy, and coupled with increased vascular permeability can result in extensive third spacing of fluids. Increased extravascular fluid within the lungs can predispose the parturient to impaired oxygen exchange or pulmonary edema, and soft-tissue swelling can increase the incidence of airway difficulties at the time of intubation[1,2].

(2) Increased mean arterial pressures arise from increased cardiac output (primarily stroke volume increases), increased systemic vascular resistance or a combination of the two. The end result is an increase in myocardial oxygen demand, which in combination with the pain of labor may exceed the delivery of oxygen to the myocardium. Women with this disease have an increased incidence of high-output cardiovascular disease, including myocardial ischemia and cerebrovascular hemorrhage.

(3) The potential coagulopathies that can accompany the disease may interfere with the ability to provide central axial anesthesia (epidural or spinal anesthesia). Disturbances of platelet counts, platelet function or disseminated intravascular coagulation can increase the risk for excessive bleeding. In the presence of a coagulopathy, anesthetists are concerned about epidural hematoma formation, spinal cord compression and permanent paralysis. There are two periods of risk, the first at the time of epidural or spinal anesthetic insertion and the second during removal of the epidural catheter. Two cases have been reported in the literature of epidural hematomas occurring in pre-eclamptic parturients with epidural anesthesia[3,4].

(4) Significant end-organ dysfunction involving the kidneys and liver can occur in cases of severe pre-eclampsia. The development of oliguria warrants immediate assessment of intravascular volume. The usual etiology of reduced urine output is intravascular hypovolemia, which responds to mild fluid challenges (250–500 ml intravenous isotonic fluid bolus over 1 h). However, renal failure can occur in the absence of hypovolemia, and continued rehydration attempts may precipitate pulmonary edema. Diminished renal clearance can unexpectedly prolong serum levels of anesthetic agents that are dependent upon renal excretion, predominantly muscle relaxants used during general anesthesia. Liver dysfunction is uncommon with pre-eclampsia unless the HELLP syndrome

(hemolysis, elevated liver enzymes and low platelets) develops. In this case, the parturient who develops a subcapsular hematoma or hepatic rupture can present with acute hypovolemic shock.

(5) Massive placental abruption can occur in this disease, leading to diminished fetal reserve or demise, requiring urgent delivery. As well, the normal coagulation cascade can be exaggerated, leading to secondary disseminated intravascular coagulation (DIC).

(6) Central nervous system (CNS) dysfunctions associated with pre-eclampsia include: CNS irritability (headaches, hyper-reflexia, visual disturbances), seizures and intracranial hemorrhage. Any of these disturbances indicate severe maternal disease and necessitate modification of anesthetic management. The parturient may require evaluation of adequacy in protection of her airway from aspiration (appropriate level of consciousness), reduction of paralyzing agents used during general anesthesia owing to interactions with magnesium sulfate therapy or evaluation and documentation of normal intracranial unction/intracranial pressure prior to the administration of central axial anesthesia (spinal or epidural).

Our initial goal as anesthetists, therefore, is to assess the extent of systems involved and optimize the patient as best as possible in the time available before delivery. Immediate goals of management are based upon on our airway-breathing-circulation (ABC) algorithms for resuscitation of critically ill patients. Once the patient's initial assessment and life-saving therapy have been implemented, a more detailed evaluation can be conducted using history, physical examination and laboratory information:

(1) Ensure adequate maternal oxygen exchange: findings indicative of adequate exchange include respiratory rate <25 breaths per min, lack of ausculatory findings of intra-alveolar fluid (inspiratory crepitations) and maternal pulse oximetry findings with room air (saturation >90%). Development of pulmonary edema may be associated with abnormal left ventricular systolic function, altered oncotic pressures or altered capillary permeability.

(2) Assess hemodynamics: non-invasive serial assessments of maternal blood pressure are essential and should be conducted with a monitoring unit dedicated to the parturient. A role exists for invasive monitoring of blood pressure in these patients, although it is not advocated for every patient. We have found a peripheral arterial line useful in a number of circumstances:

(a) Non-invasive blood pressure measurement proves difficult: the accuracy of repeated measurements may be reduced in patients who are obese, swollen or in a non-sinus rhythm (atrial fibrillation);

(b) Severe pre-eclamptic patient: the use of intravenous, potent vasoactive substances can be managed more easily with beat-to-beat monitoring of blood pressure;

(c) Frequent blood sampling required: the parturient with associated disorders of pre-eclampsia (e.g. thrombocytopenia, liver enzyme abnormalities, pulmonary edema) often requires multiple venous samples throughout her care;

(d) Patients for Cesarean delivery under general anesthesia: often the rationale for administering general anesthesia is increased severity of maternal disease. These patients are at increased risk for blood pres sure lability during general anesthesia induction and surgery. The arterial line provides accurate and immediate responses during these times of concern.

Acute hypertensive crisis can be managed medically with the use of short-acting, intravenous agents including: labetalol, nitroglycerine and hydralazine. The maternal organs most prominently affected by severe hypertension include the central nervous system (intracranial hemorrhage) and cardiac system (myocardial ischemia). However, the treatment of hypertension must not reduce placental perfusion or reserve of the fetus. The choice of agent depends upon the predominant pathological characteristic at the time. With tachycardia and hypertension, labetalol provides both heart rate reduction (β_1-antagonism) and reduction in systemic vascular resistance (α_1-antagonism). Nitroglycerine and hydralazine are direct vasodilators, which reduce systemic vascular resistance. However, both agents cause a reflex tachycardia and their effectiveness may be minimal if the patient's abnormal cardiovascular physiology is a normal systemic vascular resistance and a high cardiac output. In this case, a short-acting negative inotropic agent is useful, such as intravenous esmolol or metoprolol (β_1 and β_2 antagonism).

(3) Assess intravascular fluid balance: hourly monitoring of ins and outs are important (urine output, gastrointestinal losses, febrile losses) to pinpoint dysfunction resulting from reduced intravascular volumes associated with pre-eclampsia. If end-organ function is affected, such as with diminished urine output (<0.5ml/kg/h for two consecutive

hours), then modified fluid challenges should be administered (250–500 ml isotonic intravenous solution over 30–60 min). Owing to the heterogeneity of a pre-eclamptic patient's hemodynamic profiles, a failure to respond after 500 ml of fluids suggests that hemodynamic monitoring should be considered. Reports have recommended the use of pulmonary capillary wedge pressure (PCWP) monitoring or transthoracic echocardiography to guide future therapy. Anesthetists recognize two instances where recommendations for PCWP monitoring are useful: the pre-eclamptic parturient who poses a diagnostic challenge because of ongoing oliguria, or in the development of fulminant respiratory failure requiring intubation and ventilation[5]. The ability to determine whether a patient has normal or elevated intracardiac filling pressures can be made with the invasive PCWP monitor, or increasingly with non-invasive transthoracic echocardiographic evaluations. With our ability to determine oxygenation non-invasively, and blood pressure and regional organ function peripherally, the complications of placing a PCWP catheter limit its frequent use.

(4) Assess central nervous irritability and intracranial pathology: altered level of consciousness requires immediate assessment by anesthesia to ensure that the patient is still capable of protecting their airway from aspiration events. The treatment of eclampsia is initially supportive in anticipation of short seizure duration. Initial supportive therapy includes bag and mask ventilation with oxygen, aortocaval decompression by left uterine displacement and monitoring of fetal heart rate if possible. Most women do not vomit during pre-eclamptic associated seizures, and so the goal is simply to maintain maternal-fetal oxygenation and ventilation. Often the patient recovers from the initial cardiorespiratory embarrassment to return to spontaneous ventilation and stable hemodynamics. At this point, if magnesium sulfate therapy has not been initiated, the 2 g loading intravenous dose should be started to reduce the incidence of further seizure activity. Should the seizure duration exceed several minutes, the anesthetist can provide assistance with several short-acting agents that can end the event. These include short-acting barbiturates (thiopental), and short-acting benzodiazepines (midazolam). As well, the anesthetist will then address the issue of intubation to protect the unconscious patient from inadvertent aspiration events. In the presence of already established magnesium therapy, the eclamptic parturient must have further long-acting antiepileptic therapy initiated.

A second consideration in a parturient with CNS pathology is the exclusion of intracranial hypertension or focal abnormalities.

Certainly, pre-eclamptic patients are at increased risk for thrombotic and hemorrhagic intracranial events that may contraindicate specific anesthetic techniques should delivery be required. Therefore, these patients should have, if time permits, an intracranial evaluation by magnetic resonance imaging (MRI), as this examination does not expose the fetus to radiation risk.

(5) Assess laboratory findings for abnormal coagulation: required blood work includes regular assessment of levels that may deteriorate over the course of a trial of labor, i.e. international normalized ratio (INR), activated partial thromboplastin time (PTT), fibrinogen levels, D-dimer levels, platelet count and hemoglobin/hematocrit level. These patients are at increased risk for increased intrapartum blood loss and should have blood work done in preparation for matching blood products.

ANESTHESIA FOR LABOR

The goal of labor pain relief for pre-eclamptic parturients is to provide the most effective analgesia to reduce stimulation of the sympathetic nervous system. Until epidural analgesia was proven safe for use in this population in the 1980s, other pain-relieving modalities were used and studied. The concern with epidurals stemmed from episodes of hypotension that occurred in normal pregnant patients receiving epidural analgesia, an event that would be deleterious in women with reduced uteroplacental reserve. Although these women have exaggerated hypertensive responses to vasopressor medications and pain, they do not have the same lability for hypotensive responses with regional anesthesia as compared with normal pregnant women[6,7]. Concern has existed over the use of intravenous hydration prior to epidural insertions, as pre-eclamptic patients may be predisposed to develop pulmonary edema. However, alternative techniques for labor pain relief have largely failed because they are poorer analgesics for labor than epidurals, the 'gold standard'. For completeness, we address the other techniques that may have to be employed when a contraindication exists for epidural or spinal labor analgesia.

Methods for labor pain relief that do not require particular technical expertise include the use of nitrous oxide with supplemental oxygen and systemic narcotics. The nitrous oxide (N_2O)-oxygen (O_2) mixture contains 50% N_2O gas with 50% O_2 gas. Therefore, the mother receives increased oxygen during the periods of inhalation. If this is timed to coincide with uterine contractions, the mother is receiving increasing oxygen delivery during the period of increased oxygen demand. Most

nitrous oxide delivery systems for maternity units require close fitting of a mask to the mother's face during inhalation. Otherwise, the one-way valve in the system (designed to reduce occupational exposure to nitrous oxide gases) does not open to allow the flow of gases. The pulmonary elimination of nitrous oxide from the maternal and fetal systems is rapid owing to its low insolubility in the blood. Therefore, once the parturient ceases to use the gas, it is quickly exhaled in the breaths following. Nitrous oxide, however, is a poor analgesic, as judged by studies assessing the pain scores of laboring women. It appears to provide a mood-altering quality that allows parturients to tolerate pain. The time span of its effectiveness is quite short, in that parturients will often abandon the technique after an hour. Nitrous oxide would probably be most useful in a multiparous pre-eclamptic parturient with previous rapid labor histories. As well, this modality could be used as an adjunct to other pain-relieving techniques such as a pudendal nerve block or local infiltration of the perineum.

Systemic opioids are the most common form of labor analgesia worldwide and when administered by the best possible delivery system, can be the second most efficacious form of pain relief. Intramuscularly administered narcotics is a poor method of drug delivery for labor pain as relatively slow absorption leads to fluctuating peaks and troughs in serum levels. As well, this method of administration is painful and is contraindicated when the patient is thrombocytopenic (<50000–75000 platelets). Intravenous administration of narcotics is the best method of delivery for the pain of labor. Ideally, the patient-controlled analgesia (PCA) system would be available to these women. This system contains a computerized delivery system to connect to the intravenous line that allows patient demand to access the supplied narcotic. Safety features include barriers to access the narcotic supply and limits to the amount of narcotic administered. Particular narcotics used in this system for obstetrics patients include morphine, fentanyl, meperidine, nalbuphine, remifentanil and alfentanil. The ideal choice of agent is a short-acting narcotic that does not accumulate over time and has a large therapeutic/toxic plasma ratio. Regardless of the drug chosen, particular monitoring requirements must be in place to avoid potential maternal and neonatal complications. Therefore, the minimum monitoring for the mother during administration is hourly respiratory rate and sedation score. Women with respiratory rates below ten breaths per minute between contractions or women who are reusable only with stimulation, require further oxygen saturation monitoring and prophylactic oxygen administration. When magnesium sulfate prophylaxis is concurrently administered, the pre-eclamptic parturient is at greater risk for excessive respiratory depression with the use of systemic narcotics. All narcotics cross the placenta and

therefore will be present in the neonatal bloodstream, depending upon concentration and timing of the narcotic used. At the time of delivery, personnel responsible for neonatal resuscitation should be informed of the use of maternal narcotics and should be familiar with reversing narcotic sedation in the newborn. Monitoring guidelines for nurses, preprinted physician order sheets and pharmacy-provided narcotic mixtures are useful to reduce human errors.

Analgesic techniques that require specific training and whose success depends upon the frequent use include: paracervical block, pudendal nerve block, epidural block (including lumbar and caudal epidural blocks) and spinal block. The paracervical block is the administration of local anesthesia between the 4–5 o'clock and 7–8 o'clock positions of the lateral cervical surface, to block the pain of cervical dilatation. It is not recommended for patients delivering a viable fetus, as local anesthesia uptake can compromise uteroplacental blood flow. This technique is recommended as a first-stage analgesia for mothers with a non-viable fetus, for postpartum dilatation and curettage or repair of cervical lacerations. Pudendal nerve blocks are useful for the pain of vaginal and perineal distension during the second stage; however, the block must be successfully placed bilaterally, and have sufficient time for the local anesthetic effect prior to delivery. It is ideally placed when the fetal head is at or above the ischial spines, to allow proper deposition of the local anesthetic agent. The pudendal block's length of action is time-limited (lidocaine duration of action 60–120 min; bupivicaine 90–200 min). With proper effect, the pudendal block can provide excellent analgesia for operative vaginal deliveries.

Central axial blocks for labor analgesia (epidurals, combined spinal epidurals) in pre-eclamptic women have become the standard of practice over the past 20 years. Provided there are no contraindications to these blocks, such as coagulation abnormalities, overwhelming maternal septicemia or ongoing acute blood loss, continuous lumbar epidural analgesia has many advantages. It is the most effective and reliable method of pain relief of labor with a low incidence of complications. During labor, epidural analgesia decreases maternal oxygen requirements and prevents maternal hyperventilation. Often, in pre-eclampsia, maternal levels of catecholamines are elevated above normal, which can impair uteroplacental perfusion. As well, effective analgesia blunts hypertensive and neuroendocrine responses to labor pain, which may reduce the risk of eclampsia[8]. Another benefit of this technique is that the level of anesthesia can quickly be altered for operative vaginal delivery or for Cesarean delivery. Last, randomized clinical trial evidence has demonstrated that epidural analgesia does not increase the incidence of Cesarean delivery in women with pre-eclampsia[9,10].

Institution of epidural analgesia requires documentation of recent normal blood screening for coagulopathies, because of the concern for epidural hematoma. Epidural anesthesia, and to a lesser extent spinal anesthesia, have been associated with the development of epidural hematomas and permanent neurological deficits in the presence of coagulopathic conditions. This is an extremely rare event, and therefore it is difficult to define the exact risk for each anticoagulant. There are reported cases in the obstetric population, but the relative risk in this population compared with other patients receiving anticoagulation is not known at this time[11]. The periods of greatest risk are at the institution of epidural or spinal anesthesia and on removal of epidural catheters. Future methods for screening may include thromboelastography, the results of which can be immediately processed and available with blood sampling. Parturients who have been taking anticoagulants prior to labor require a window of time between the last dose and administration of central axial anesthesia. The recommendation for pregnant-patient care is based upon current anesthesia guidelines established for all patients[12]. For patients receiving normal heparin (unfractionated heparin), subcutaneous prophylactic doses do not contraindicate the use of epidural or spinal anesthesia. Therapeutic anticoagulation with normal heparin requires cessation of therapy and normalization of laboratory PTT findings prior to insertion of central axial anesthesia (usually within 6 h of stopping heparin infusion). The use of low-molecular-weight heparin (LMWH) prophylaxis or therapy requires the longest waiting period from last dose (12 h following low-dose prophylactic therapy or 24 h from the following high doses: enoxaparin ≥1.5 mg/kg/day, dalteparin ≥200 U/kg/day or tinzaparin ≥175 U/kg/day). The initiation of postpartum LMWH must be carefully timed with the removal of epidural catheters. The use of twice-daily dosing of LMWH has an increased risk of epidural hematoma, and therefore the catheter should be removed at least 2 h prior to the first postpartum dose. Single daily dosing of LMWH allows for the epidural catheter to remain in place for postpartum analgesia continuation while starting the anticoagulation; however, the catheter can only be removed following a 10–12-h window from the last dose.

The optimal time to administer epidural analgesia during a woman's labor must be individualized. The delivering physician with the patient must consider the potential urgency for delivery, the certainty that induction of labor will continue to delivery and not be stopped, and the history of the fetal and placental well-being. There is no evidence to suggest an optimal point in labor to administer epidural analgesia.

OPERATIVE VAGINAL DELIVERY

Epidural or spinal analgesia can prevent the sometimes uncontrollable urge to bear down which is associated with sudden rises in venous pressures in the cardiac and central nervous system. The raised venous pressures may reduce perfusion through these capillary beds, as well as in other susceptible organs (placenta). This method of analgesia also minimizes the likelihood of a precipitous delivery of a preterm or small neonate. If vaginal delivery is imminent, spinal block can safely provide rapid and complete analgesia for vaginal delivery while maintaining motor strength and response. This is achieved by the use of small spinal doses of local anesthesia mixed with narcotics. Left uterine displacement, intravenous access and appropriate blood pressure monitoring is mandatory for any epidural or spinal anesthetic. The combined spinal epidural technique allows prolongation or extension of the initial block if necessary. Often, with pre-eclamptic parturients, epidural analgesia has been instituted earlier in labor, and thus a top-up is required for perineal analgesia prior to operative vaginal delivery. Epidural top-ups require 5–20 min to achieve an effective response, depending upon the volume and specific local anesthetic administered.

ANESTHESIA FOR CESAREAN DELIVERY

There are essentially three methods of anesthesia available for a Cesarean delivery in the pre-eclamptic patient: epidural, spinal and general anesthesia. The most common method provided for this population is epidural anesthesia, as most patients have received an epidural during their trial of labor and have progressed to Cesarean delivery. In this scenario, the epidural catheter in place is topped up with a more concentrated local anesthetic, and requires additional time (15–30 min) to achieve maximal anesthetic effect. The anesthetist can speed up the onset of local anesthetic effect with specific agents such as lidocaine and chlorprocaine, or by alterations of the agent's pH (lidocaine plus CO_2 is faster in onset than plain lidocaine). In the case of an emergent Cesarean delivery, where waiting until full effect is impossible, the anesthetist must choose between two options: providing intravenous sedation until the epidural dose is effective, or administering general anesthesia. Other limitations of the epidural technique include increased unpredictability of the anesthetic when compared with spinal anesthesia or general anesthesia, and requirement for large doses of local anesthesia, which may approach toxic levels.

Prior to the late 1990s, spinal anesthesia for Cesarean delivery was largely avoided in pre-eclamptic patients because of the concern over

precipitous maternal hypotension, compared with epidural anesthesia. However, observational and randomized clinical trials have provided sufficient evidence that the benefits of spinal anesthesia are significant, and that the technique is safe[13,14]. This anesthetic technique can provide almost instantaneous anesthesia with minimal doses of local anesthesia, and is more appropriate in patients with questionable coagulation problems (the potential for epidural bleeding is less with a small, midline-placed spinal needle than with placement of an epidural catheter). Thus, one recent advantage of spinal anesthesia has been to reduce the incidence of general anesthesia. Spinal anesthesia has been reported in pre-eclamptic patients with CNS disease (eclampsia and transient blindness) when the presence of raised intracranial pressure has been ruled out[15,16].

The administration of general anesthesia to a pre-eclamptic parturient is one of the most challenging moments for an obstetric anesthetist. The problems faced include the potential for difficult intubation (accompanying mucosal swelling of the pharynx may interfere with clear visualization of the larynx, or prevent normal passage of the endotracheal tube through swollen vocal cords), the potential for severe hypertension during laryngoscopy and at time of surgical stimulation and the impairment of intervillous blood flow by positive pressure ventilation. Anesthetic goals include adequate assessment of the airway prior to induction of general anesthesia, preparation for blunting hemodynamic perturbations and maximizing oxygen delivery for mother and baby. In the face of a potentially difficult intubation, the anesthetist's first obligation is not to place the mother at further risk. Therefore, a more careful airway assessment (direct laryngoscopy following airway topicalization with local anesthetic sprays) may be required to determine whether general anesthesia can be induced prior to securing the airway or after (awake intubation). These decisions are often required during a period of stress on all care-givers, as this event most commonly occurs with significant fetal bradycardias. Whenever possible, general anesthesia should be avoided in parturients with identified difficult airways.

The consequences of a sudden hypertensive crisis under general anesthesia include intracranial hemorrhage, myocardial ischemia and arrythmias, and pulmonary edema. Identification of neurological complications is not detectable under general anesthesia; however, cardiac and respiratory changes are usually identified with electrocardiography, oxygen saturation and exhaled gas carbon dioxide monitoring. Induction of general anesthesia is often modified with pharmacological agents that will attenuate maternal hypertension. The agents should be intravenously administered for quickest onset of action, should have a short duration of action in case of sudden hypotension

and should not negatively affect the fetus if they cross the placenta. Suitable agents include nitroglycerine, trimethaphan, labetalol and narcotics such as fentanyl, sufentanil and remifentanil. In the case of narcotics, the fetus may need respiratory assistance at birth and reversal of prolonged opiate effect with naloxone. The anesthetist, as with all Cesarean deliveries, will be unable to provide primary neonatal resuscitation measures while responsible for the mother.

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14

Antepartum assessment and monitoring of the fetus

M. J . Simchen and J. C. P. Kingdom

INTRODUCTION

Fetal growth and well-being during the third trimester are dependent upon sustained placental development. This implies that the fetal-placental blood vessels continue to grow to make gas-exchanging terminal villi for oxygen transfer, and that the surface of these villi remain covered with healthy trophoblast to transport nutrients and oxygen actively to the rapidly growing fetus[1]. The syndrome of pre-eclampsia implies abnormal endocrine signalling between the placenta and maternal host and is initiated by a combination of abnormal placental development, pre-existing maternal ill-health and pathological processes damaging the placental villi. The majority (80%) of previously healthy women who develop pre-eclampsia do so at term, and have normal, or even heavier, babies compared with women whose blood pressure remains normal[2]. In this 'late-gestation' form of the disease, the placenta has adapted to a limited degree of uteroplacental ischemia, and has not suffered a significant degree of thrombotic damage. Indeed, such placentas are around 15% heavier than normal at delivery[2]. By contrast, a minority (20%) of women with pre-eclampsia deliver before 34 weeks of gestation from combinations of severe maternal disease, abruption or fetal compromise from intrauterine growth restriction (IUGR), placing them at risk of neurological injury[3]. Placentas from these pregnancies have limited degrees of adaptation to uteroplacental ischemia, have maldeveloped villi and have patchy areas of placental thrombosis and infarction[4].

The aim of this chapter is to describe the use of ultrasound methods for screening, diagnosis and management of pregnancies complicated by pre-eclampsia and coexistent IUGR, as practiced in our centers.

SCREENING FOR EARLY-ONSET PRE-ECLAMPSIA AND INTRAUTERINE GROWTH RESTRICTION

Uterine artery Doppler

Uterine blood flow increases from 50 ml/min in the non-pregnant state to over 700 ml/min in the third trimester of a normal pregnancy. Extravillous cytotrophoblast invasion transforms the spiral arterial segments in the inner myometrium from narrow innervated vessels to widely dilated sinusoids, and maintains their patency by producing locally acting vasodilator peptides. As a result, the Doppler waveform of the artery is radically transformed from one with low diastolic flow and an early diastolic notch to one with high diastolic flow (Figure 1). This transformation is largely completed by the time that the 18–20-week detailed level 2 ultrasound anomaly screening examination is performed. Mean pulsatility index (PI) values from the left and right proximal uterine arteries (located by color Doppler at the cross-over point) at this stage of pregnancy are typically <1.2, with only 5% of pregnancies with mean PI values above 1.45[5]. Some investigators perform a combined cervix and uterine artery screening ultrasound at 23 weeks, when only 2–3% of unselected pregnancies will have bilateral abnormal waveforms[6]. Embolization studies in the chronically catheterized sheep fetus[7,8] show that bilateral, deep uterine artery notches equate to a 50% reduction in uterine artery blood flow. Consequently, the detection of bilateral abnormal uterine artery waveforms at 18–20 weeks (mean PI >1.45 with bilateral notches) is reported as implying significant uteroplacental vascular ischemia.

Uterine artery Doppler is not considered to be a valid screening tool for the detection of pre-eclampsia and/or IUGR in low-risk unselected pregnancies[9,10]. However, if screening is aimed at detecting the subset with the most perinatal morbidity and mortality, i.e. severe early-onset pre-eclampsia and IUGR (presenting before 32–34 weeks), and the screening test is applied to clinically high-risk groups, the test characteristics are substantially better[6]. A mean PI value of 1.45 at 23 weeks had 80% sensitivity for the detection of pre-eclampsia that required delivery prior to 34 weeks[5]. Reporting using bilateral notches as opposed to PI values gave similar results. In circumstances where the placenta is exclusively on one side of the uterus, only the ipsilateral

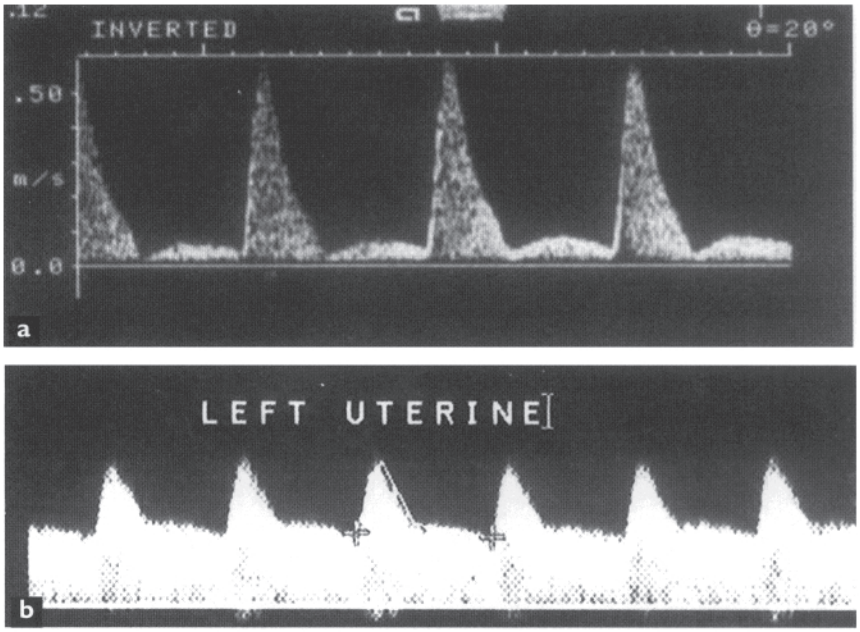

Figure 1 Uterine artery Doppler waveforms, (a) Normal uterine artery waveform in the first trimester. (b) Normal uterine artery waveform at 25 weeks' gestation

waveform should be reported, since the contralateral waveform will be falsely abnormal owing to minimal trophoblast invasion.

Maternal serum screening

The 15–18-week maternal serum screen (MSS) test calculates an individualized risk for trisomy 21 and neural tube defect (NTD) based upon age and serum levels of α-fetoprotein (AFP), estriol and human chorionic gonadotropin (hCG), expressed as multiples of the median (MoM) value for gestation. Elevated levels of hCG in the absence of trisomy 21, or of AFP in the absence of any structural abnormality, may be associated with subclinical placental insufficiency, the association being stronger with the magnitude of rise[11]. Elevations in hCG are thought to be due to persistent uteroplacental ischemia[12]. Elevations in AFP are thought to occur as a result of a breach of the fetomaternal interface of the placenta, for example owing to villous infarcts, since the fetus synthesizes AFP almost exclusively[13]. Combined

elevations in both analytes represent a serious risk, with perinatal mortality and preterm birth occurring in over 25% of cases[14].

Integration of uterine artery Doppler and maternal serum screening

Combined testing may increase the screening test characteristics for early-onset pre-eclampsia/IUGR. The evidence for this is as follows:

(1) Uterine artery Doppler studies identify more than 70% of women with abnormal MSS who are destined to deliver before 34 weeks owing to placental disease[12].

(2) Half of women who develop early-onset IUGR with absent end-diastolic flow in the umbilical arteries have prior abnormal MSS results (S.Viero and colleagues, submitted for publication). Over 85% of women with combined elevations in AFP and hCG have abnormal uterine artery Doppler studies and dysmorphic placentas, and perinatal mortality is over 30%[14].

(3) Placental shape and texture may be abnormal in women destined to develop early-onset IUGR, many of whom had prior abnormal MSS results. An example of a dysmorphic placenta is shown in Figure 2.

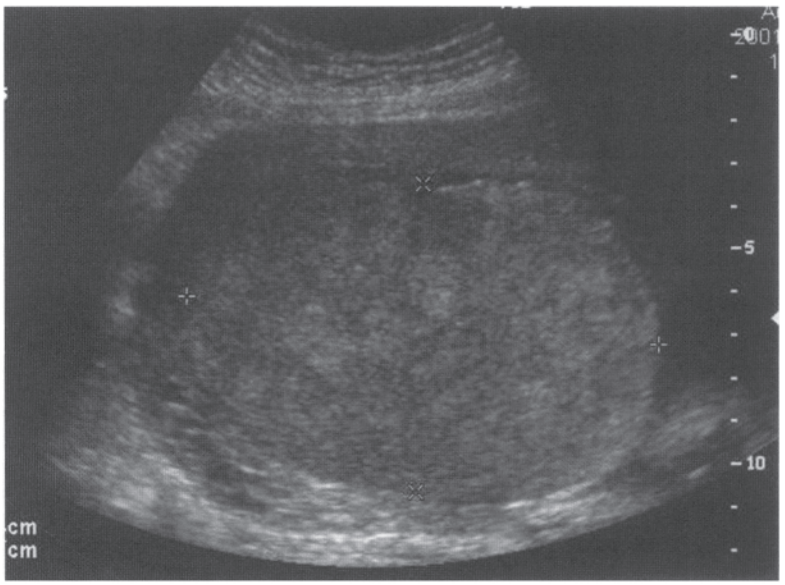

Figure 2 An example of a dysmorphic placenta. The placenta appears small, thick and non-homogeneous

Guidelines for fetal screening in the high-risk population

Uterine artery Doppler screening can be performed as part of the 18–20-week anatomical examination. Mean uterine artery PI values >1.45 should be considered as indicating uteroplacental vascular insufficiency. The significance of abnormal uterine artery Doppler is increased if the prior 15–18-week MSS results were abnormal. In such instances, review of placental shape and villous texture is worthwhile, and a thrombophilia screen should be considered in those with evidence of thrombotic placental damage (S.Viero and colleagues, submitted for publication). Women with complex medical or past obstetric histories may benefit from consultation with a perinatologist, or interested radiologist, in a high-risk tertiary-care center for risk assessment, specialist testing and counselling. An initial ultrasound evaluation can guide planning of the subsequent need for intensive fetal monitoring of growth and well-being, the need for elective antenatal steroids for fetal lung maturation and transfer to anticipate delivery of the infant into a neonatal intensive-care unit.

Fetal response to uteroplacental vascular insufficiency

In mid-pregnancy, placental reserve is substantial and exceeds the demands of the rapidly growing fetus, even when significant uteroplacental vascular insufficiency has been demonstrated at 18–22 weeks. Occasionally IUGR is evident at this stage, usually with complex maternal medical problems, abnormal MSS results and a damaged small placenta. The more typical outcome is for normal fetal growth until 26–30 weeks, the explanation being that uteroplacental vascular insufficiency, producing intraplacental hypoxia, is a stimulus for enhanced development of placental villi to preserve maternal-fetal exchange[15]. The default pathway is for this placental adaptation to increase placental weight by as much as 15%[2]. IUGR occurs when this adaptive mechanism is compromised[16]. The possible mechanisms include chronic maternal disease, placental infarcts or an inability of the placental villi to increase angiogenesis in the face of tissue hypoxia[17].

IDENTIFICATION AND MONITORING OF THE 'AT-RISK' FETUS

Uterine artery Doppler and placental morphology are diagnostic tests at either 18–20 weeks or the first subsequent referral examination, but do not contribute further to management once the diagnosis has been established. At 24–28 weeks of gestation, baseline fetal growth, symmetry (head circumference/abdominal circumference ratio), umbilical artery Doppler, amniotic fluid index and placental texture are used to screen

for pregnancies that have adapted poorly to uteroplacental vascular ischemia. Such fetuses require weekly surveillance, as do their mothers, since the incidence of early-onset pre-eclampsia is at least 40%[18].

Methods of fetal health surveilance

Bio.physical profile score

The ultrasound component of the fetal biophysical profile score (BPS) is made up of four parameters each scoring either 2 or 0. These are fetal tone (limb movements), sustained fetal breathing movements, fetal gross body (trunk) movements and the amniotic fluid level (one cord-free pocket >2 cm depth)[19]. A normal score of 8/8 indicates a healthy fetus. Fetal movements occur in cycles. These cycles increase in length as pregnancy progresses, and up to 75 min of no fetal movements has been observed in normal pregnancies. Hypoxemia has been shown to be associated with both reduced fetal body[20] and breathing movements in the lamb model[21] and in the human fetus. In addition to being part of the biophysical profile assessment, fetal movements can also be monitored by the pregnant woman herself as a subjective method of assessing fetal well-being.

Routine biophysical profile examinations have not gained worldwide popularity, largely because of the time required (up to 30 min) to complete an examination, e.g. to observe sustained fetal breathing in a 28-week fetus. The concept of a modified biophysical profile has been suggested[22], incorporating fetal weight estimation, amniotic fluid and umbilical artery Doppler as a first-line screen, confining a full biophysical profile and/or fetal Doppler studies to those pregnancies with abnormal modified screening examinations. This is an attractive concept, and is the preferred approach in most UK centers.

Cardiotocography (non-stress test)

Cardiotocography (CTG) or non-stress test (NST) monitoring of the fetal heart rate has been utilized in obstetric units since the 1970s. Nevertheless, when used for obstetric decision-making in the absence of ultrasound, the test may be associated with increased perinatal mortality[23]. Cardiotocography is probably not necessary if the mother reports normal fetal activity, and the ultrasound shows normal fetal growth and a normal biophysical profile.

As gestation advances, fetal accelerations become larger and more frequent, and are used to interpret a normal tracing. Accelerations are smaller and less frequent in preterm fetuses, especially before 32 weeks. In these circumstances, a normal tracing is defined by the baseline heart rate (120–160 beats/min), short-term heart rate variation and the lack of decelerations.

The preterm fetus of a mother with pre-eclampsia may be exposed to a number of drugs that can affect the CTG-NST, such as magnesium sulfate[24] or steroids[25]. Despite these limitations, the CTG-NST is a commonly used daily test of fetal well-being, because it can be performed by physician assistants and reviewed as hardcopy. A recent development has been the introduction of computerized CTG-NST recordings, whereby all components, including an objective assessment of heart rate variability (short-term fetal heart rate (FHR) variation), can be objectively reported. Used in conjunction with intermittent ultrasound examinations, the computerized CTG-NST offers a robust method of daily fetal health assessment in preterm preeclamptic and IUGR fetuses, since short-term FHR variation correlates closely with fetal oxygenation[26]. It provides information on a 'window of opportunity' for delivery prior to the development of spontaneous decelerations, defined by low short-term FHR variation[27].

Umbilical artery Doppler

Throughout the first trimester, the umbilical artery waveform is characterized by absent end-diastolic flow. Progressive growth of the placental villous tree, together with an increase in fetal cardiac output, increases both systolic and diastolic velocity in the umbilical artery[1]. Pulsatility index values therefore decrease progressively as pregnancy advances (Figure 3). Diastolic velocities are typically present in normal pregnancies by 14–16 weeks of gestation, such that absent end-diastolic velocity in the umbilical artery is an abnormal finding by the time of the 18–20-week ultrasound examination. Typically, the PI value ranges from 2.0 in the second trimester to 1.0 as term approaches. Declining PI values indicate increasing fetal cardiac output, and a progressive fall in fetoplacental vascular impedance as the placental cotyledons develop[28].

Early-onset IUGR pregnancies exhibit an abnormal PI value in the umbilical arteries, the more extreme examples represented by absent or reversed end-diastolic flow velocity (Figure 4). The pathology is multilayered and includes: a small dysmorphic placenta; short, eccentric cord, narrowed hypertrophied stem arteries; maldeveloped gas-exchanging villi; oxidative stress in the placental vascular endothelium; and thrombosis both in and around individual villi[1]. Increasing umbilical artery PI is correlated with increasing degrees of fetal hypoxemia and acidemia[29,30], resulting in an increase in perinatal mortality to around 30%[18].

A systematic review of available randomized trials demonstrates no benefit from screening low-risk pregnancies for IUGR with umbilical artery Doppler[10]. However, umbilical artery as well as middle cerebral artery Doppler studies are helpful in the recognition of a subset of preterm small-for-gestational-age fetuses at most risk of serious perinatal morbidity and death[31], and can therefore be used logically

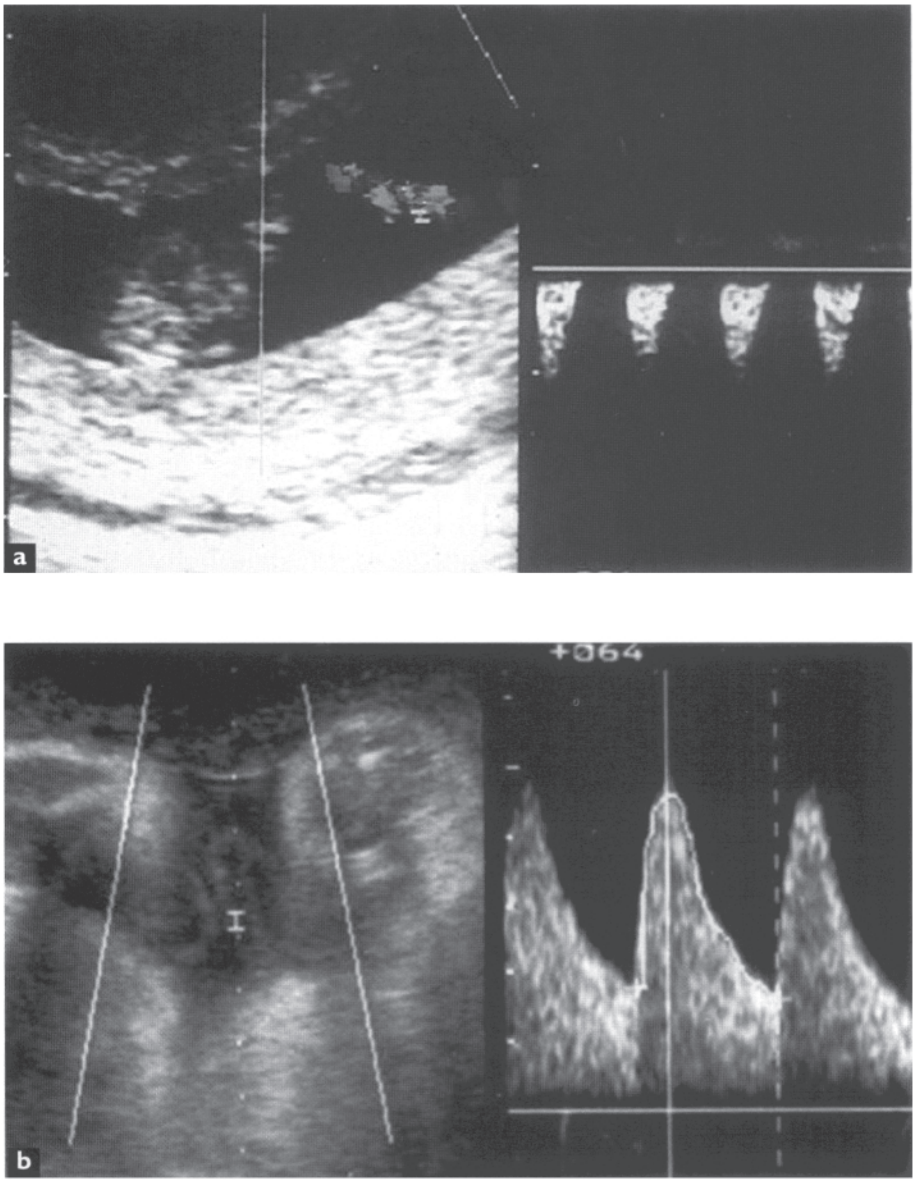

Figure 3 Umbilical artery Doppler waveforms. (a) Typical umbilical artery waveform at 8 weeks' gestation. (b) Typical umbilical artery waveform at 24 weeks' gestation. As gestation progresses, both systolic and diastolic blood flow velocities increase, and as a result the pulsatility index decreases

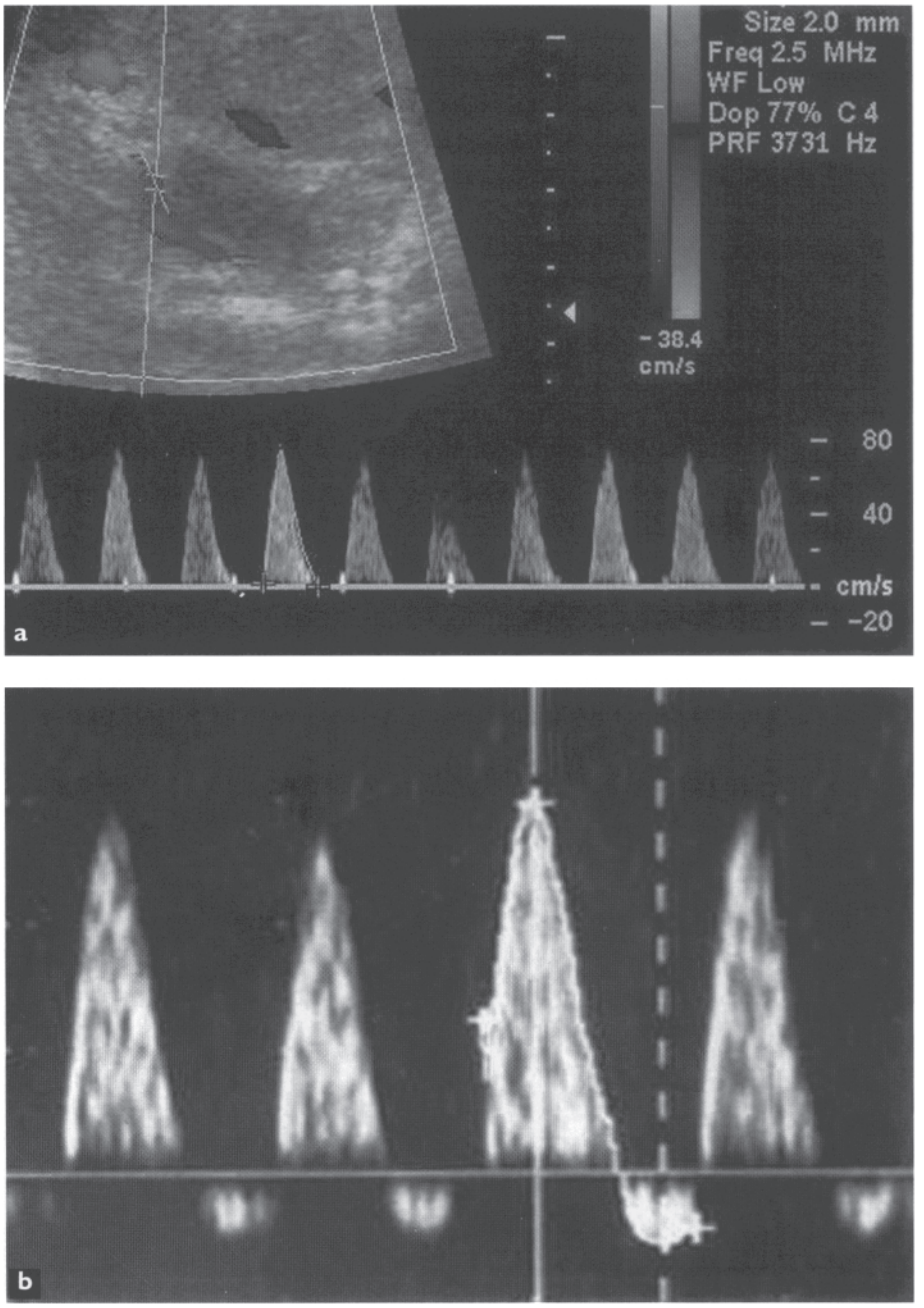

Figure 4 (a) An example of absent end-diastolic blood flow velocity in the umbilical artery. (b) An example of reversed end-diastolic blood flow velocity in the umbilical artery

as part of the ultrasound examination in pregnancies complicated by pre-eclampsia before 32 weeks.

A systematic meta-analysis of 11 randomized controlled trials of umbilical artery Doppler in the assessment of clinically high-risk pregnancies before 34 weeks, involving mainly pre-eclampsia and IUGR, resulted in a trend towards reduction in perinatal death by 30% (odds ratio 0.71, 95% confidence interval 0.50–1.01)[32].

Umbilical artery Doppler can be reported either quantitatively using PI values, or in a categorical manner. Categorical reporting is popular in Scandinavia[33].

Middle cerebral artery Doppler

The circle of Willis is easily demonstrated by color Doppler ultrasound of the base of the skull in the transverse fetal head position (Figure 5). The typical middle cerebral artery (MCA) waveform at 28–32 weeks' gestation is characterized by high systolic velocities and minimal diastolic velocities, resulting in high PI values. A low diastolic velocity indicates normal autoregulatory mechanisms limiting brain blood flow. In chronic fetal hypoxia, vascular tone is reduced in the MCA resulting in increased diastolic velocity and reduced PI values (Figure 6). This is

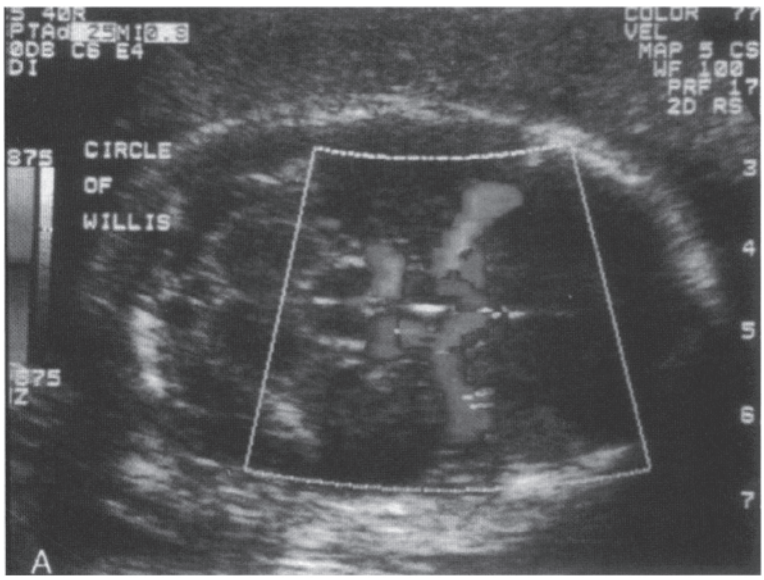

Figure 5 Color Doppler imaging of the circle of Willis in a transverse view across the base of the fetal skull

known as 'cerebral blood flow redistribution' to the fetal brain, and is found in more severe forms of IUGR. In a study of nearly 300 IUGR fetuses, Fong and colleagues demonstrated decreased MCA PI to be a more sensitive indicator than umbilical artery Doppler in identifying preterm fetuses at risk of major adverse perinatal outcomes, with a sensitivity of 92% versus 58%[31].

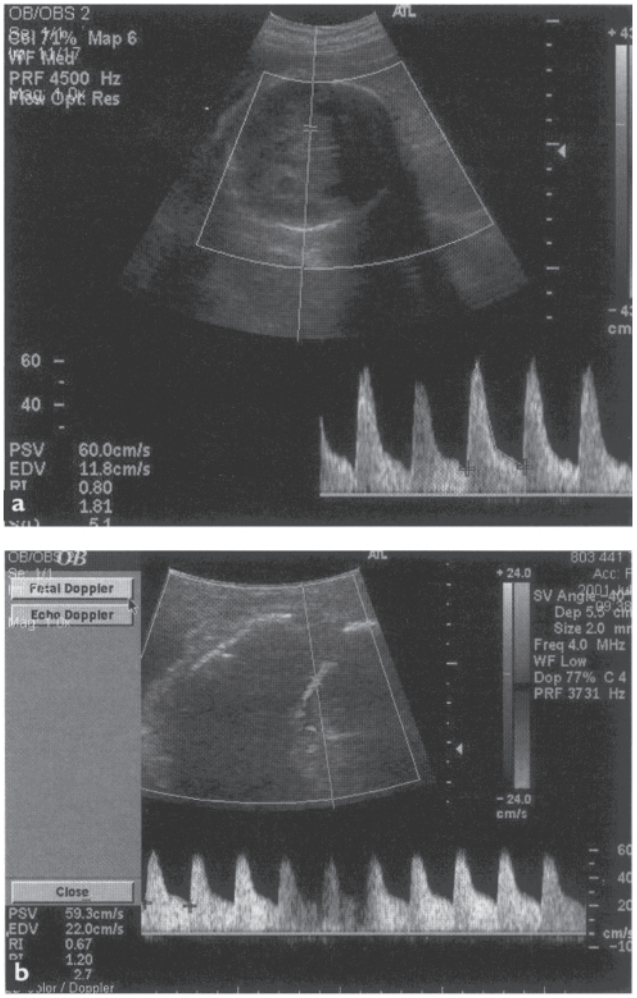

Figure 6 (a) Normal middle cerebral artery Doppler waveform. Normal pulsatility index (PI) typically above 1.5. (b) Middle cerebral artery Doppler waveform in a severely growth-restricted fetus demonstrating increased diastolic flow velocity with a resultant low PI, indicating significant cerebral redistribution

Venous Doppler

Venous blood flows back to the right atrium of the fetal heart along three vessels, namely the superior vena cava (SVC), the inferior vena cava (IVC) and the ductus venosus (DV). Approximately 50% of the blood returning to the heart via the intrahepatic umbilical vein (IHV) enters the narrow muscularized DV, directing oxygenated blood at high velocity via the foramen ovale into the left atrium, and on up the aortic arch to the fetal brain. The remainder of IHV flow enters the hepatic circulation returning via the IVC at relatively low velocities. The normal DV waveform has two interruptions caused by atrial contraction (the A wave) and closure of the tricuspid valve (E wave; Figure 7). Under normal circumstances these interruptions are small, and are not transmitted in a retrograde manner into the IHV. As a result, venous flow in the free umbilical vein and IHV is smooth and

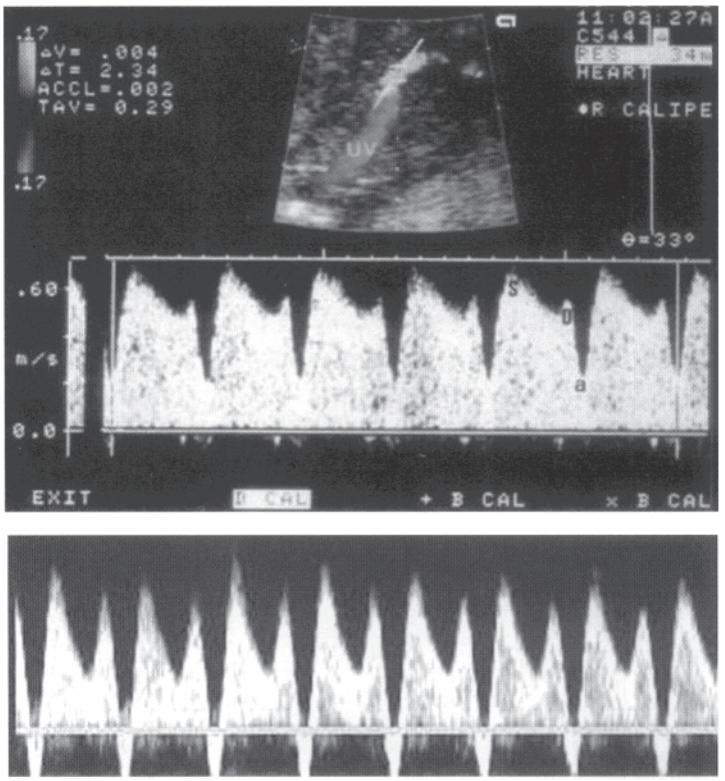

Figure 7 (a) Normal Doppler waveform in the ductus venosus. The A wave depicted by 'a' in the figure denotes the interruption to flow caused by atrial contraction. (b) Reversed flow velocity in the ductus venosus during atrial contraction indicates significant right-sided cardiac decompensation

uninterrupted. Flow velocity in the DV and IVC is similar to that at the commencement of the circulation, namely the pulmonary and aortic valves. As the hypoxic fetus becomes gradually acidotic and cardiac output falls, progressive changes are apparent in the venous circulation. Right atrial contractions cause deeper indentations in the DV waveform, resulting in absent or even reversed 'A waves' (Figure 7b). These are transmitted distally into the IHV, and eventually out into the umbilical vein of the cord, where they are detected as synchronous umbilical vein pulsations (Figure 8). These venous waveform changes relate closely to fetal metabolic acidemia[29,34], right-sided cardiac dysfunction[35] and increased central venous pressure[36]. These abnormal DV waveforms are ominous signs of fetal decompensation, and precede the development of a decelerative NST in preterm fetuses by 1–3 days.

TEMPORAL SEQUENCE OF FETAL HEALTH DETERIORATION

Several investigators have recently outlined the temporal sequence of deterioration of the IUGR fetus as placental disease progresses. Baschat and colleagues[37] described the sequence of deterioration of Doppler and biophysical profile parameters in a group of 236 IUGR fetuses. Sequential deterioration of first arterial, and then venous, flows

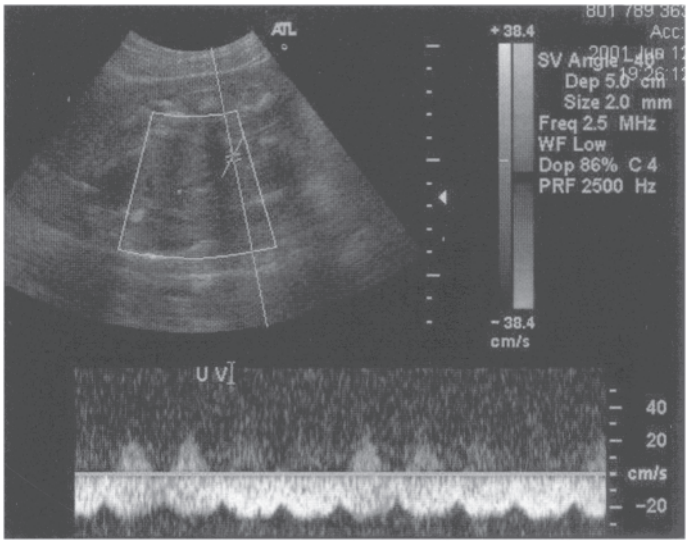

Figure 8 Doppler waveform of the umbilical vein demonstrating synchronous umbilical venous pulsations

generally preceded biophysical profile score deterioration. Ferrazzi and associates[38] divided Doppler changes into early and late changes. Early changes occurred in arterial vessels (umbilical and middle cerebral arteries) in half the patients, approximately 2 weeks prior to delivery, while late changes, including reversed end-diastolic flow and abnormal changes in the ductus venosus, aortic and pulmonary outflow tracts (where 50% of patients were affected 4–5 days prior to delivery) were significantly associated with perinatal death. Therefore, adding serial Doppler evaluation of the umbilical artery, middle cerebral artery and ductus venosus to IUGR surveillance can enhance the performance of the biophysical score in the detection of fetal compromise, consequently optimizing the timing of intervention. Hecher and colleagues[39] investigated 110 IUGR fetuses and compared short-term variation of NST heart rate, arterial and venous Doppler parameters and amniotic fluid index for optimal timing of delivery. Amniotic fluid index and umbilical artery PI were the first variables to become abnormal, followed by the middle cerebral artery, aorta, short-term variation, ductus venosus and inferior vena cava. These investigators concluded that ductus venosus PI and short-term variation of fetal heart rate are important indicators for the optimal timing of delivery before 32 weeks of gestation, and that delivery should be considered if one of these parameters becomes persistently abnormal. In trying to summarize these studies and other similar ones, it can be concluded that worsening placental disease in the IUGR fetus can be observed as a sequential, step-wise progression. Our experience of 37 consecutive severe IUGR fetuses at <32 weeks with absent end-diastolic flow in the umbilical arteries confirms the strength of fetal Doppler studies, and that serious circulatory changes can exist even in the presence of normal amniotic fluid and a normal biophysical profile[40]. We have therefore begun to use a Doppler-based staging system in compromised fetuses, as outlined in Table 1.

Table 1 Toronto staging system for fetal health deterioration in early-onset intrauterine growth restriction with absent end-diastolic flow in the umbilical arteries

Stage	*BPS*	*MCADoppler*	*Ductus venosus Doppler*	*Non-stress test*
1	8/8	normal	normal	normal
2	8/8	redistribution	normal	normal
3	8/8	redistribution	reduced A wave	normal
4	2–4/8	'normalization': loss reversed	of redistribution A wave	absent/ decelerative

BPS, biophysical profile score; MCA, middle cerebral artery

Late-gestation IUGR

In late gestation, beyond 34–36 weeks, it is uncommon to find absent or reversed end-diastolic flow velocity in the umbilical arteries caused by uteroplacental vascular insufficiency. Therefore, at term, evidence of fetal hemodynamic redistribution may exist in the presence of a normal umbilical artery PI[41]. This cerebral redistribution is none the less important clinically in marking those fetuses at risk for adverse perinatal events[42], and may therefore be a useful tool for the management of the near-term IUGR fetus.

PREGNANCY MANAGEMENT: FETAL PERSPECTIVE

Intrauterine growth restriction (IUGR) is one of the main fetal consequences of pre-eclampsia. Severe early-onset IUGR is defined as the detection of restricted fetal growth that results in death of the fetus or delivery for fetal indications before 32 weeks of gestation[43], and is associated with significantly increased perinatal morbidity and mortality[3,44]. It is important to keep in mind that IUGR resulting from uteroplacental insufficiency has an important differential diagnosis, necessitating re-evaluation of fetal anatomy, and consideration of prior screening tests for aneuploidy[45]. For the purpose of this chapter, we consider IUGR as resulting from progressive uteroplacental insufficiency, based on the pathological changes in placental structure which are characteristic of pre-eclampsia.

Frequency of fetal monitoring

The optimal assessment protocol required for safe monitoring of the fetus in pre-eclampsia with uteroplacental insufficiency is debated, but depends upon the stage of fetal health and the maternal condition. The recently reported GRIT trial supports the overall strategy of intensive fetal monitoring for early-onset IUGR as a valid alternative to immediate delivery[46]. The routine use of umbilical artery Doppler in small-for-gestational-age fetuses before 32 weeks to identify and report those with absent/reversed end-diastolic flow in the umbilical arteries will, owing to subsequent measures, reduce perinatal mortality by around 30%[47]. Generally speaking, any method that safely prolongs gestation is likely to be beneficial[46]. The initial screening procedure, which encompasses level 2 ultrasonography with uterine artery Doppler and placental morphology studies (MSS and placental ultrasound appearance), may identify those fetuses at increased risk that will benefit from further evaluation and a more intensive monitoring protocol. If placentation studies are normal, a 4-weekly repeated

evaluation may be sufficient to detect those patients in whom placental insufficiency will develop, in the absence of maternal ill-health. IUGR fetuses with normal Doppler findings may be monitored weekly, while those IUGR fetuses with evidence of significant cerebral blood flow redistribution may be monitored twice-weekly. Fetuses with evidence of venous Doppler changes should be monitored more intensely as inpatients in a perinatal center capable of dealing with very preterm, compromised neonates. An alternative or adjunct to intensive fetal Doppler surveillance is frequent biophysical profile evaluations and NST studies, with consideration for delivery given for a persistent BPS of less than 6/10 or loss of short-term variability with or without late decelerations.

Antenatal glucocorticoid administration

The detection of severe IUGR with absent end-diastolic flow before 32 weeks should prompt the consideration of steroids to improve fetal lung maturity. Interestingly steroids have been shown to improve umbilical artery Doppler waveforms transiently in this situation, for 4–7 days[48]. IUGR fetuses are at risk of lactic acidosis[49], especially if they are compromised (Toronto stages 3 and 4), and steroids have been shown to induce lactic acidosis in sheep fetuses[50]. We therefore prospectively monitored fetuses with absent or reversed end-diastolic flow by daily umbilical and fetal Doppler studies through steroid administration. Approximately two-thirds developed a positive end-diastolic flow response and remained well, while one-third deteriorated acutely[51]. These data suggest that IUGR fetuses with absent or reversed end-diastolic flow receiving steroids before 32 weeks should undergo short-term intensive daily fetal Doppler studies either to detect a favorable positive end-diastolic response, or to recognize the need for delivery (Toronto stage 3 or 4). The placental steroid barrier may already be broken in severe IUGR, exposing the fetus to maternal, rather than exogenous, steroids[52]. Future research may therefore suggest that stage-3 fetuses benefit more from delivery than from steroids and intensive monitoring.

Timing and mode of delivery

Early consideration of other causes of early-onset IUGR is important in terms of the management plan. Information regarding associated anomalies or an abnormal karyotype can affect urgent management decisions such as mode of delivery as a result of an acute deterioration in either fetal condition, or maternal condition (i.e. severe pre-eclampsia or HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome).

The threshold for delivery depends on gestational age, and is lowered the more advanced is the fetal age. There is little justification for

prolongation of pregnancy after 32–34 weeks' gestation in a woman with severe pre-eclampsia, regardless of the degree of fetal compromise. Expectant management in severe pre-eclampsia for the fetal benefit of increased maturity is generally discussed prior to 28 weeks[53,54], and is difficult to justify after 32 weeks[55].

Most uncompromised fetuses can withstand a vaginal trial of labor, which would be the preferred method of delivery in the relatively stable pre-eclamptic patient. This is also true for most growth-restricted fetuses, if induction of labor is done gradually and continuous fetal monitoring is employed to detect signs of fetal compromise in a timely fashion. Nevertheless, in the preterm preeclamptic population (below 34 weeks), the rate of successful vaginal delivery after induction of labor is only 50%, with an inverse relationship to gestational age at induction[56]. A severely growth-restricted fetus with Doppler evidence of cerebral redistribution and oligohydramnios is not likely to be able to withstand a trial of labor. In these cases, a preliminary oxytocin challenge test (OCT) may be of help in deciding whether there is enough placental reserve to allow the fetus to withstand uterine contractions in labor. If the OCT is positive or equivocal, Cesarean delivery may be the wiser choice.

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15

Long-term implications of pre-eclampsia

J. E. Ramsay, N. Sattar and I. A. Greer

INTRODUCTION

Epidemiological studies have recently demonstrated a relationship between a pregnancy complicated by pre-eclampsia and an increased risk of maternal coronary heart disease in later life. In summary, the reported increased relative risk of death from ischemic heart disease in association with a history of pre-eclampsia/eclampsia is around two-fold (1.7–2.6) (reviewed in reference 1). This increase in relative risk, however, must be put into context by considering the absolute risk. From the Framingham data, in women aged 35–75 years, the absolute risk of a woman dying from causes related to coronary heart disease can be estimated. With a body mass index (BMI) less than 25 kg/m^2 this risk is 4/10000 woman-years[2]. Therefore, the two-fold relative risk translates to an absolute risk of around an additional four deaths in 10000 woman-years. Obviously this figure would be greater with increasing accumulation of risk factors such as age, smoking and BMI. However, there is purpose to the demonstration of such statistics. First, these observations stimulate further research exploring possible mechanisms linking these two conditions, which, hopefully, will provide insight into potential preventive or interventional therapies. Second, pregnancy is a time when young, apparently healthy women may have their only contact with health professionals, and so provides an opportunity for health education and awareness. A link between pregnancy complications and risk of vascular disease will therefore allow us to consider pregnancy as an opportunity for the identification of vascular risk and subsequent intervention by life-style modification. Obviously, in

areas of the world where vascular disease has a high prevalence, such as the west of Scotland, this opportunity for life-style advice would be potentially very useful.

WHAT LINKS PRE-ECLAMPSIA AND CARDIOVASCULAR DISEASE?

At present, few data exist to explain the underlying mechanisms linking pre-eclampsia and cardiovascular disease. However, the pathophysiologies of both conditions have been independently and comprehensively investigated, and it appears that pre-eclampsia shares many common pathological features with arteriosclerosis. Some of these features may best be described under the banner of the metabolic syndrome, a spectrum of abnormalities known to play a key role in the pathophysiology of cardiovascular disease and, in particular, coronary heart disease. Such features include impaired insulin sensitivity, dyslipidemia and coagulation disturbance (Figure 1).

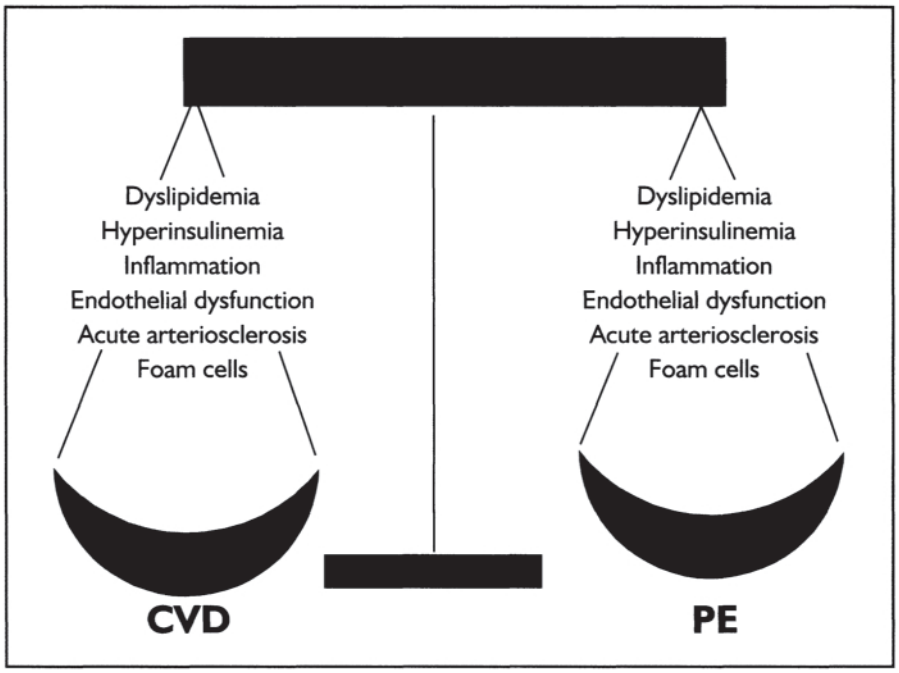

Figure 1 Shared pathophysiology between cardiovascular disease (CVD) and pre-eclampsia (PE)

Uncomplicated pregnancy

The normal physiological response of pregnancy represents a transient excursion into a metabolic syndrome where several components are acquired: a relative degree of insulin resistance, significant hyperlipidemia with elevations of plasma cholesterol and triglyceride concentrations by 25–50% and 200–400%, respectively and an increase in coagulation factors[3]. In the latter stages of the second trimester of pregnancy an increased concentration of free fatty acids (FFAs) is observed. This response occurs as a result of stimulation of hormonesensitive lipases (HSLs) by human placental lactogen (hPL) and/or the development of a relative resistance to insulin with a loss of the usual suppressive effect of insulin upon FFA release from adipose tissue (reviewed in reference 4). Therefore, an increased delivery of FFAs to the liver results in increased synthesis of triglycerides and subsequent hepatic assembly of very-low-density lipoprotein (VLDL) cholesterol particles[3]. Pregnancy also results in a significant reduction in lipoprotein lipase activity, with a consequent reduction in maternal triglyceride catabolism. Driven by elevated estrogen concentrations which stimulate increased apolipoprotein A1 (apo A1) production, the principal protein of high-density lipoprotein (HDL) cholesterol particles, HDL also increases in pregnancy, achieving a peak at around 24 weeks' gestation. This may act as a buffer for the detrimental vascular effects of hypertriglyceridemia. Low-density lipoprotein (LDL) cholesterol achieves a less significant elevation, although as triglyceride concentrations rise, a threshold is reached after which proportionally more production of the small dense LDL subfraction takes place. In the oxidized form, this molecule is believed to be highly atherogenic, promoting foam-cell production and initiating endothelial dysfunction[3] (Figure 2).

Therefore, as discussed above, excess peripheral lipolysis of fat stores provides increased non-esterified fatty acid (NEFA) concentrations. NEFAs contribute to the dyslipidemia of pregnancy, but in addition activate and damage endothelial cells, impair peripheral glucose uptake and enhance glucose production, as well as possibly inhibiting portal insulin extraction, resulting in increased insulin concentrations and insulin resistance. A progressive increase in nutrient-stimulated insulin responses consistent with insulin resistance has been described in normal healthy pregnancy. Hyperinsulinemic, euglycemic clamp techniques have demonstrated insulin efficacy to be as much as 50–70% less in late pregnancy, compared with non-pregnant women. To meet the increasing demands of the developing fetus and placenta, basal endogenous hepatic glucose production is thought to increase by 16–30%, with an increased contribution of carbohydrate to oxidative metabolism in late pregnancy (reviewed in reference 5).

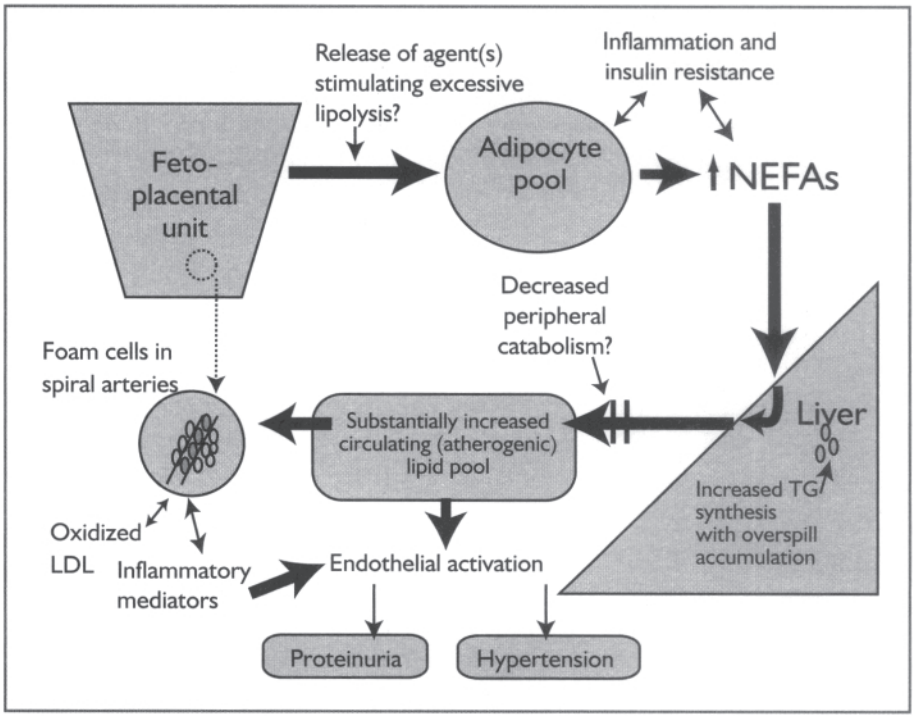

Figure 2 The potential role of a disturbance in lipid metabolism in the pathogenesis of pre-eclampsia. LDL, low-density lipoprotein; NEFA, non-esterified fatty acid; TG, triglyceride

Normal pregnancy also involves up-regulation of the inflammatory cascade. In the non-pregnant, inflammatory markers such as C-reactive protein, interleukin-6 and white-cell count have all been found independently to predict future risk of cardiovascular events and diabetes (reviewed in reference 6). Therefore, it is not surprising that many studies have demonstrated a small but significant association between increasing parity and cardiovascular risk.

Yet, despite this particularly hostile metabolic and inflammatory milieu, pregnancy is a time when peripheral vascular resistance falls, despite a 40% increase in cardiac output and a 50% increase in circulating blood volume. This is believed to be a result of up-regulation of endothelial-derived nitric oxide production, and it may be that factors such as this enhancement of endothelial-dependent vasodilatation, increased concentrations of HDL cholesterol and up-regulation of endogenous antioxidant enzymes act as physiological buffers to the metabolic disruption as described above (Figure 3).

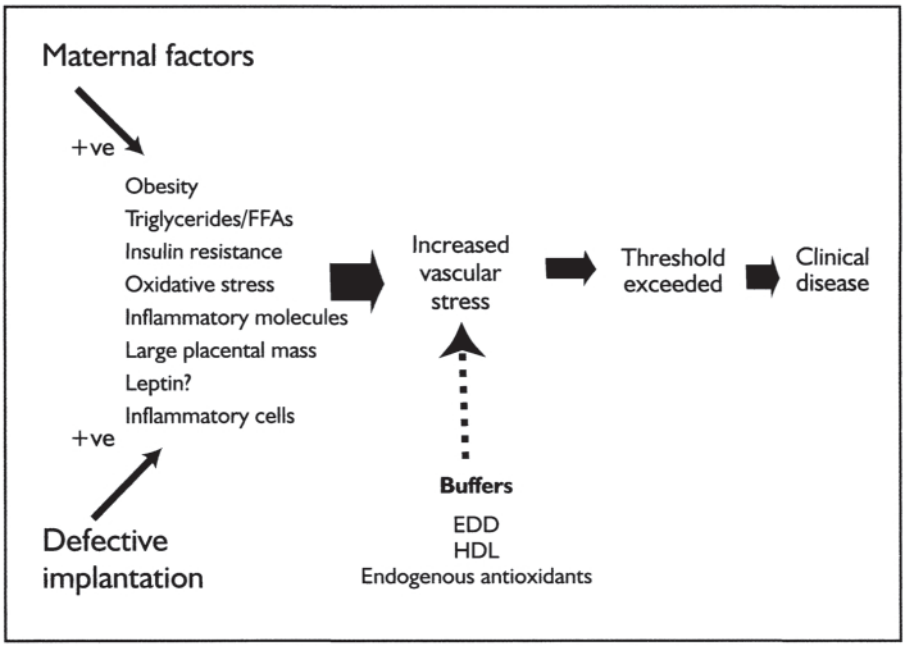

Figure 3 Threshold theory of pathophysiology of pre-eclampsia. FFA, free fatty acid; EDD, endothelial-dependent vasodilatation; HDL, high-density lipoprotein

Pre-eclampsia

Therefore, like coronary heart disease, pre-eclampsia is unlikely to be associated with a single causative factor. Instead, perhaps the mechanism can be thought of as a threshold of risk. Both maternal factors, such as adiposity, insulin resistance and an inflammatory phenotype, and fetal factors, such as an inadequately implanted placenta resulting in hypoxia and liberation of 'toxic' inflammatory molecules, produce an increased vascular stress until a threshold is exceeded. Therefore, these detrimental effects will outweigh the beneficial effects of increased endothelial-dependent vasodilatation and HDL cholesterol concentrations, as discussed above, and clinical disease ensues. In preeclamptic pregnancies, the physiological hypertriglyceridemia observed in healthy pregnancies is exaggerated. Fasting triglyceride concentrations are doubled, compared with normal pregnancy, and a three-fold increase in VLDL and LDL-III concentrations is also observed[4]. Significantly, this hypertriglyceridemia is observed well in advance of the clinical manifestations of the disease.

As described above, these changes could contribute to endothelial damage and insulin resistance. It is also notable that the specific vascular lesion of pre-eclampsia 'acute atherosis with lipid-laden foam cells', as observed in the intima of the spiral arterioles of the placental bed, is similar to that seen in arteriosclerosis in the non-pregnant[4] (Figure 2).

Pre-eclampsia is also believed to be a disease of inflammation and there is now substantial published evidence in this field (reviewed in reference 7). Granulocytes and monocytes are activated with increased release of proinflammatory cytokines such as tumor necrosis factor-a (TNF-α) and interleukin-6 (IL-6). An increase in serum concentrations of the cell adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), has been demonstrated in women with pre-eclampsia, also suggesting a state of inflammation and endothelial activation resulting in activation of the coagulation cascade and production of fibrinogen, known outwith pregnancy to be associated with an elevated cardiovascular disease risk[6]. Adhesion molecules are expressed on the surface of vascular endothelial cells in response to stimulus by inflammatory cytokines, and are believed to encourage adhesion of circulating leukocytes and their subsequent transendothelial migration. This process is believed to be critical in the progress of early atherogenesis, and cross-sectional data suggest that soluble forms of these proteins are elevated in patients with atherosclerosis. ICAM-1 in particular has recently been established to be independently predictive for coronary heart disease, and, from the Atherosclerosis Risk In Communities (ARIC) study, those with baseline concentrations of ICAM-1 in the highest quartile, when followed up for 5 years, were found to have a five-fold increased risk of incident coronary heart disease (relative risk (RR) 5.5, 95% confidence interval (CI) 2.5–12.2)[8]. These data are proposed to support the hypothesis that endothelial activation and damage, or 'inflammation' occur early in the atherosclerotic process.

Therefore, such metabolic and inflammatory responses could be considered as a 'stress' test, in which the maternal vasculature is challenged to cope with the cardiovascular adaptations to pregnancy. In the majority of individuals such changes are absorbed by physiological buffers, as discussed above. However, in those women who develop complications of pregnancy such as pre-eclampsia, a phenotype may exist whereby the inflammatory and metabolic response to pregnancy is exaggerated, and buffering mechanisms are inadequate. Such a phenotype may provide a key to the link between pre-eclampsia and coronary heart disease, and one could hypothesize that this phenotype may be either genetically inherited or acquired through intrauterine fetal programming, or through later acquired risk factors such as central obesity. Therefore, in such an individual, the threshold for clinical cardiovascular disease is

breached during pregnancy and again in later life, as with increasing age other acquired cardiovascular risk factors are encountered. In this way, adverse pregnancy outcome may reveal women at increased risk of metabolic and vascular diseases in later life (Figure 4).

THE 'PRE' PRE-ECLAMPTIC PHENOTYPE

Unfortunately, to date, there are limited prospective data available prior to conception to examine the metabolic phenotype of women predisposed to pre-eclampsia. The alternative is to examine women after pregnancy, although this approach cannot establish whether abnormalities observed arose consequent to pregnancy or predated it. Early work suggested that women with pregnancies complicated by pregnancy-induced hypertension and eclampsia did not develop later chronic hypertension, but others have since found an increase in risk of hypertension, especially where the hypertensive problem arises before 30 weeks' gestation (reviewed in reference 1). There does appear to be agreement, however, that mothers who experience uncomplicated

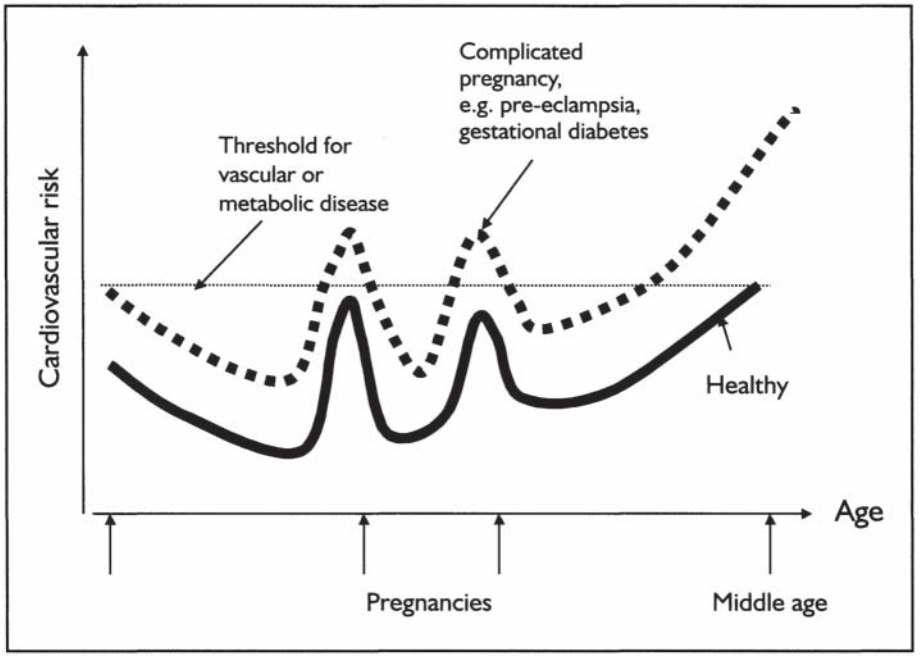

Figure 4 Risk factors for vascular disease identifiable during excursions into the metabolic syndrome of pregnancy. Adapted from reference 1

Table 1 Association of adverse pregnancy outcomes with coronary heart disease (CHD) risk factor status and vascular risk. Adapted from reference 1

Pregnancy outcome	*Incidence in pregnancy*	*Risk factors shown to be perturbed after pregnancy*	*CHD mortality or morbidity (hazard ratios (95% CI))*
Pre-eclampsia	2–4%	lipids	1.9 (1.0–3.5) vs. PIH alone
		clotting	1.7 (1.3–2.2) vs. no pre-eclampsia
		fasting insulin	2.0 (1.5–2.5) vs no pre-eclampsia
		large vessel function	

PIH, pregnancy-induced hypertension; CI, confidence interval

pregnancies have a lower incidence of subsequent hypertension compared with the general female population of similar age and race. Recent studies demonstrate that women with a history of pre-eclampsia have persistently higher circulating levels of fasting insulin, testosterone and lipid and coagulation factors, relative to BMI-matched controls, up to 17 years following the pregnancy (Table 1). They also appear to exhibit a specific defect in endothelial-dependent vascular function, relative to women with a history of a healthy pregnancy, independent of maternal obesity, blood pressure and metabolic disturbances associated with insulin resistance or dyslipidemia (reviewed in reference 1). We have also demonstrated from our own work in this field that women with a past history of pre-eclampsia have increased plasma concentrations of the inflammatory markers, VCAM-1 and especially ICAM-1, and impaired endothelial-dependent vasodilatation, 15 years or more after the index pregnancy. This pattern of metabolic and vascular changes in women with a history of pre-eclampsia is almost identical to the abnormalities seen in this condition at diagnosis, namely exaggerated lipid and insulin levels, disturbed hemostatic and endothelial parameters and endothelial dysfunction.

Epidemiological studies have borne out the inferred increase in coronary heart disease risk as suggested by the above changes in vascular risk markers (Table 1). A study of 374 Icelandic women with a past history of hypertensive complication of pregnancy revealed that

death rate from coronary heart disease was significantly higher (RR 1.47, 95% CI 1.05–2.02) than expected from the analysis of population data from public health and census reports during corresponding periods. Moreover, the relative risk of dying from coronary heart disease was significantly higher among women who had suffered eclampsia (RR 2.61, 95% CI 1.11–6.12) or pre-eclampsia (RR 1.90, 95% CI 1.02–3.52), compared with those with hypertension alone (reviewed in reference 1). A prospective cohort study using the Royal College of General Practitioners oral contraceptive study data also reported that a history of pre-eclampsia increased the risk of cardiovascular conditions in later life. For all ischemic heart disease the relative risk was 1.7 (1.3–2.2). Furthermore, the authors found that the increased risk could not be explained by underlying chronic hypertension (reviewed in reference 1). A retrospective cohort study from Scotland using hospital discharge data has also recently reported an association between pre-eclampsia and subsequent ischemic heart disease in the mother (hazard ratio 2.0, 95% CI 1.5–2.5) (reviewed in reference 1), and suggested that risks are cumulative in association with multiple poor outcome in pregnancy (e.g. pre-eclampsia and preterm birth: hazard ratio 6.4, 95% CI 1.9–21.3). This may reflect a relationship between the severity of the disease and lifetime effect. Prospective evaluation of women in pregnancy with long-term follow-up is now required to elaborate the mechanisms underlying this association. It is also important to determine whether this finding represents a new source of risk identification that might not have been evident otherwise, or whether these women would have been identified as being 'at risk' from the use of established risk factors such as hypertension and obesity. Only then will we know whether this finding represents an opportunity for primary prevention.

NEW OPPORTUNITIES FOR SCREENING

A major problem in the prevention of vascular disease has been the difficulty in identifying individuals at risk at an early enough stage to benefit from intervention such as life-style modification. For example, by the time type-2 diabetes is diagnosed, more than 30–50% of patients will have evidence of vascular disease. Clearly, therefore, women with a history of gestational diabetes are candidates for screening for diabetes. However, as described above, pre-eclampsia is associated with a more subtle, subclinical metabolic disturbance. Hyperinsulinemia can be demonstrated in the absence of frank glucose dysregulation in women with a past history of pre-eclampsia, up to 17 years postpartum.

Obesity and insulin resistance

Obesity is the modern health epidemic of Western society, with 10% of children and 20% of adults in the UK now classified as clinically obese (BMI >30 kg/m^2). Worldwide it is estimated that more than 300 million people are clinically obese, and it is now well established that obesity and particularly a central body fat distribution correlate strongly with deranged metabolic function and are associated with an elevated incidence of cardiovascular disease (CVD). Many of these risk factors are modifiable, and compelling evidence indicates that a major proportion of the current CVD burden is either totally or partially preventable. Data from the Nurses' Health Study[9] suggest that women who maintain a desirable body weight, consume a healthy diet, exercise regularly, avoid smoking and drink alcohol in moderation reduce their risk of CVD by 84%. Yet, only 3% of the women studied followed these recommendations, which points to the need for more effective public-health messages and the implementation of standardized preventive strategies. Additionally, it is important that primary preventive efforts be targeted at younger people, so that the effects of decades of unattended risk factors can be averted.

In the non-pregnant individual, an inverse relationship has been demonstrated between blood pressure and insulin sensitivity, as well as a strong positive relationship between microvascular function and insulin sensitivity. Abdominal fat is therefore proposed as a source of FFA and cytokine production, both of which factors would promote vascular inflammation and endothelial dysfunction, potentially resulting in insulin resistance and hypertension as described above. One may hypothesize, therefore, that either a reduction in BMI or a remodelling of body fat distribution may improve both the inflammatory profile and insulin sensitivity, and may provide some hope of altering this predisposed risk of cardiovascular disease. Interventional studies have demonstrated in a group of obese premenopausal women that weight loss over 1 year was associated with a reduction in inflammatory cytokine concentrations, a reduction in adhesion molecule concentrations including ICAM-1 and an improvement in endothelial-dependent vascular function[10]. These latter observations are important, since, on the basis of conventional risk factor charts for CHD risk based on Framingham data, only a very small minority of women with a history of pre-eclampsia in our local follow-up of 15 years would be considered for primary prevention[11]. Indeed, the average total cholesterol/HDL cholesterol ratio in our group was less than 4, and only two of the 40 women with a history of pre-eclampsia were on lipid-lowering therapy. Another recent interventional study randomized over 3000 individuals with elevated fasting and post-load plasma

glucose concentrations to either life-style change or drug therapy, with the aim of reducing progression from high risk to clinical disease. The findings demonstrated that a program of life-style modification including exercise and weight loss was more effective than metformin in reducing the progression to type 2 diabetes[12]. Therefore, breaking the cycle of weight gain, insulin resistance and hyperlipidemia should be addressed in the young adult. Advice for achieving a healthy life-style should be actively disseminated by all health professionals at every opportunity, the postnatal visit being no exception.

Interventions for cardiovascular health in later life

Intervention for women with a past history of pre-eclampsia could be focused on the perimenopausal years, a time when risk of vascular disease increases rapidly, or even earlier, as discussed above. Screening in these women would take the form of routine coronary heart disease assessment including measurement of blood pressure, fasting lipids (total cholesterol, triglycerides and HDL cholesterol) and glucose concentrations, and thus determination of coronary heart disease risk using widely available risk factor charts based on equations derived from the Framingham heart study. Guidelines for the primary prevention of coronary heart disease published jointly by the British Cardiac, Hypertension and Hyperlipidaemia Societies[11] recommend that the use of cholesterol-lowering drugs, antihypertensive agents and aspirin should be based on the individual's projected risk of developing coronary heart disease or cardiovascular disease, and not on measurements of serum cholesterol or blood pressure alone. For cholesterol-lowering treatment, individuals should have a projected risk of sustaining a coronary heart disease event in a 10-year period of ≥30%, but a risk of only ≥15% for initiating antihypertensive or aspirin treatment (Table 2).

To help to ensure that appropriate women are screened and given relevant health education, adverse pregnancy outcomes could be recorded in general practitioner computer databases for targeted health screening programs. Indeed, such interventions could start at the routine 6-week postpartum review, when these women could be made aware of their potentially increased risk for coronary heart disease and counselled appropriately regarding life-style factors such as diet and exercise. However, as yet, novel markers of cardiovascular risk such as impaired insulin sensitivity without glucose dysregulation, inflammation and endothelial dysfunction have not been incorporated into preventive strategies in the cardiovascular arena. Therefore, a past history of pre-eclampsia may only be used to flag up women where conventional risk assessment could be offered in early middle age. Novel markers of inflammation such as C-reactive protein (CRP) concentrations are being

Table 2 Summary of cardiovascular risk chart recommended by Joint British Cardiac Hyperlipidaemia and Hypertension Societies and endorsed by the British Diabetic Association[11]

Table	*Age (years)*	*Risk assessed*	*Risk factors included*	*Format*
Joint British guidelines	30–70 (in four decades)	10-year CHD risk, three bands: < 15%, 15–30%, > 30%	age, sex, smoking (Y/N), SBP (110–120 mmHg), diabetes (Y/N), total : HDL cholesterol ratio	graph of SBP vs. total : HDL cholesterol ratio; three risk categories color coded; separate graphs for each permutation of sex, diabetes, smoking and decade of age
Revised joint British guidelines	35–74 (in four decades)			

CHD, coronary heart disease; Y/N, yes/no; SBP, systolic blood pressure; DHL, high-density lipoprotein

evaluated, and in the USA, the American Heart Association has already recommended CRP measurements for coronary heart disease risk screening. In the context of pre-existing studies of statin therapy for abnormal lipid concentrations, those with highest CRP concentrations appear to benefit most from these drugs, perhaps secondary to their anti-inflammatory effect.

Implications for cardiovascular health in future pregnancies: maternal

The second implication of an association between maternal coronary heart disease risk and adverse pregnancy outcome is the potential for modification of risk factors in advance of a subsequent pregnancy or in early pregnancy. For example, increased physical activity in women who are sedentary may result in a better pregnancy outcome for both mother and child. Indeed, there are preliminary data to support this hypothesis; increasing exercise during pregnancy may increase birth weight[13] and reduce the risk of gestational diabetes[14]. Clearly, such data would suggest that complications are not simply genetically determined, but that life-style factors play a major role. At present, this remains speculative, and further research is ongoing to address this important issue.

Implications for cardiovascular health in future pregnancies: fetal?

Barker's hypothesis[15], and more recently the fetal insulin hypothesis[16], have suggested that impaired adult cardiovascular health may be programmed *in utero* by poor fetal nutrition, or by genetically determined attenuation of insulin-mediated fetal growth, resulting in a small infant. Undernutrition is now infrequent in developed societies, while sedentary life-style and poor diet leading to obesity and insulin resistance are alarmingly common, yet the direct effects of maternal obesity upon fetal programming have been neglected in favor of studies of maternal undernutrition. There is a body of direct and indirect evidence that may provide an insight into potential mechanisms linking maternal obesity, with resulting insulin resistance, to fetal vascular programming, irrespective of birth weight. For example, data from a population-based study conducted in Finland[17] provide direct evidence of a link between maternal obesity and cardiovascular disease in adult offspring. Barker's observation of higher adult death rates from coronary heart disease in men who were thin at birth was confirmed in this population. However, a positive relationship between maternal BMI on admission to the labor ward and future death rate from coronary heart disease in male offspring was also observed, and judged to be independent of, and additional to, the effect of low birth weight. This example is consistent but unusual in that, thus far, most researchers have neglected considering maternal BMI as a potential factor in pathways of fetal programming. One may hypothesize that maternal obesity and insulin resistance provide the fetus with an inappropriate nutrient mixture, particularly in terms of fatty acid fluxes, and, as fatty acids are components of cell membranes, fetal tissue and vascular architecture, programming may be impaired in children born to obese mothers. In the long term, these adverse effects could result in an increased risk of adult cardiovascular disease. Given that obesity and maternal insulin resistance are not only genetic but also acquired, then improvement of periconceptional maternal insulin sensitivity via exercise or diet may have an impact not only on a mother's health, but also on the future cardiovascular risk of her child. Again, this hypothesis remains speculative, and further research is needed to address this issue.

FUTURE RESEARCH

The majority of the above findings come from observational studies with relatively small numbers of cases or end-points, and so require confirmation in larger cohorts with longer periods of follow-up, adequate control groups and proper attention to confounding by smoking. These

studies should address whether established risk factors account for excess risk associated with pregnancy complications, or whether novel factors are potentially implicated. Simultaneously, large prospective longitudinal studies (of several thousand women) examining changes in conventional (lipids, blood pressure, hemostatic factors) and novel (inflammation, insulin resistance) risk factor pathways during and after pregnancy should be undertaken. Such studies lend themselves well to long-term follow-up, with the eventual aim of linking pregnancy outcome to maternal vascular risk factor status at the first antenatal visit in the short term, to post-pregnancy risk factor status in the medium term and to vascular and metabolic disease end-points in later life. Clearly, a variety of study designs are needed to confirm associations and to elaborate mechanisms and causality. In conclusion, there is an urgent need to explore vascular and metabolic risk factors and their association with adverse pregnancy outcome, since such knowledge has great potential to impact not only upon women's health but also upon the health of future generations.

CONCLUSION

In conclusion, therefore, these data suggest that the phenotype associated with pre-eclampsia is linked to mechanisms underlying coronary heart disease, and may explain in part the epidemiological association between pre-eclampsia and coronary heart disease. This association may be genetically or phenotypically determined, but evidence exists to suggest that life-style modifications such as weight loss may ameliorate this underlying cardiovascular disease risk.

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16

The prevention of pre-eclampsia

L. Takser and W. D. Fraser

INTRODUCTION

Pre-eclampsia, or proteinuric gestational hypertension, accounts for one-third of all maternal deaths in pregnancy. This trend has not changed since the 1970s. The majority of referrals to high-risk antenatal clinics are for high blood pressure[1], and a high proportion (24%) of hospital admissions in pregnancy are to manage women with hypertension[2]. Gestational hypertension, especially pre-eclampsia, is a primary cause of low birth weight (<2500 g) and perinatal deaths through both preterm delivery and intrauterine growth restriction[3]. Since delivery is the only known cure, gestational hypertension is a leading cause of indicated preterm delivery and accounts for 10–25% of all very-low-birth-weight infants. As many as 60% of these infants suffer learning disabilities[4], increasing the hidden costs of the disease. Thus, the identification of an effective strategy to prevent pre-eclampsia is a priority for research in obstetrics. Progress has been inhibited by the lack of understanding of the underlying etiology of the disease. However, recent advances in knowledge concerning disease pathophysiology have led to novel and promising hypotheses which are now being tested in the clinical setting.

Numerous clinical studies and randomized trials have assessed a broad range of strategies designed to reduce the incidence and severity of pre-eclampsia. The objective of this chapter is to provide an overview of the published literature concerning such strategies to prevent pre-eclampsia.

NUTRITIONAL INTERVENTIONS WITH SOME DEMONSTRATED BENEFITS

Calcium

The case of calcium provides an example of an astute empirical observation leading to a series of studies designed to reduce pre-eclampsia. The first observation of a possible relationship between calcium intake and the reduction in risk of pre-eclampsia was reported in Mayan Indians in Guatemala, who traditionally soak their corn in lime before cooking. These women had a high calcium intake and a low incidence of pre-eclampsia. A similar association was observed in Ethiopia where the diet also contained high levels of calcium. Subsequently the results of numerous clinical and epidemiological studies have supported the hypothesis that an increase in calcium intake during pregnancy may reduce the incidence of hypertension and pre-eclampsia, particularly among women with a low calcium intake.

Recently, a meta-analysis of the effect of calcium supplementation on pre-eclampsia was conducted. The study included 11 trials, involving over 7000 women. The overall effect was a 65% reduction in the risk of pre-eclampsia in both low- and high-risk women receiving supplementation. The effect was smallest in the largest trial, which studied low-risk women with adequate baseline calcium diet, and where the placebo group received routine calcium supplementation (50 mg vs. 2 g in the supplemented group)[5]. The results of the meta-analysis are strongly influenced by the latter trial, and reveal a statistically significant reduction in risk associated with the intervention among low-risk women (six trials, 6307 women: relative risk (RR) 0.49, 95% confidence interval (CI) 0.28–0.87). Such an effect was not seen in trials of women with adequate calcium intake (four trials, 5022 women: RR 0.62, 95% CI 0.32–1.20). The beneficial effects were even greater among high-risk women (five trials, 587 women: RR 0.22, 95% CI 0.12–0.42), as well as among those with low baseline calcium intake (six trials, 1842 women: RR 0.29, 95% CI 0.16–0.54)[6].

The results of two large trials demonstrate disparity with respect to the effect of calcium supplementation in relation to baseline calcium intake. The first study, which was conducted in Argentina, included 1167 women with low baseline calcium intake (mean intake of 650 mg/day). Women were randomly assigned to either 2 g/day of calcium supplementation or placebo. A significant reduction of the incidence of hypertensive disorders (pregnancy-induced hypertension (PIH) and pre-eclampsia) was observed among supplemented women[7] (RR 0.67, 95% CI 0.49–0.91). The effect was more pronounced in women with

low baseline urinary calcium excretion than in those with high baseline calcium intake. In a second large (*n*=4589) trial conducted in the USA, where the average baseline calcium intake was twice that measured in the Argentinian study, a much smaller overall effect was observed (RR 0.94, 95% CI 0.76–1.16)[5]. The effect was independent of baseline calcium intake, even though the threshold used to define an 'adequate' calcium intake was similar to that used in the Argentine study[7].

In addition, follow-up studies of children aged 5–9 years from the Argentinian trial observed lower mean systolic blood pressures among those whose mothers had received calcium supplementation during pregnancy. The effect was strongest among overweight children[8].

In summary, calcium supplementation appears to be most beneficial to women at high risk of gestational hypertension and in communities with low dietary calcium intake.

A large multicenter (seven centers in six countries) clinical trial is currently in progress to test the efficacy of 1500 mg/day of calcium from 20 weeks' gestation to delivery in 8500 nulliparous women. Two substudies will be conducted in low-calcium-intake women: one to assess the effect of calcium supplementation on maternal calcium metabolism, and the second to assess the effect of calcium supplementation on placental hemodynamics and fetal growth[9].

NUTRITIONAL INTERVENTIONS OF UNPROVEN BENEFIT

Magnesium

Magnesium is the second most abundant cation in intracellular fluid, and is an essential element involved in protein synthesis and in maintaining membrane potential. Of the seven published trials of magnesium supplementation in pregnancy, only two small trials reported pre-eclampsia as an outcome. One trial recruited women at increased risk of nutritional deficiency. The methodological quality of these studies is poor, particularly with respect to the methods of treatment allocation and blinding. A meta-analysis of these studies showed no apparent effect of magnesium supplementation (mean dose of 365 or 500 mg) on the risk of pre-eclampsia[10].

Fish oil

Fish oil, which is rich in *n*-3 polyunsaturated fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DCHA)), has been shown to interfere with prostaglandin metabolism resulting in a decrease in the thromboxane

(TX)/ prostacyclin (PI) ratio. The effect of fish oil on blood pressure has often been assumed to be due to this mechanism.

An early meta-analysis of controlled clinical trials of the effects of fish oil on blood pressure in untreated hypertensive non-pregnant subjects demonstrated a significant reduction in systolic and diastolic blood pressure[11]. This hypothesis was supported by the results of one controlled intervention study, in which fish oil, together with vitamins B, A, C and D, and minerals (ferrous iron, calcium, iodine, manganese and copper), was given to pregnant women. A reduction in the occurrence of pre-eclampsia was observed in the treatment group[12]. Subsequently, several clinical trials have attempted to confirm a possible prophylactic or therapeutic effect of fish oil supplementation in low- and high-risk pregnancies. However, they failed to detect any significant effect on blood pressure[13,14].

A recent, double-blind, multicenter clinical trial (total n=1447) compared fish oil with placebo in women at high risk of gestational hypertension and intrauterine growth restriction (IUGR). No statistically significant differences were found in the risk of pre-eclampsia[15].

One longitudinal study suggests that the altered essential fatty acid status in PIH is a late phenomenon, and is therefore unlikely to contribute to the pathogenesis of the disorder[16].

Iron

It has been suggested that disturbances in iron metabolism could play a role in the pathogenesis of pre-eclampsia, through the generation of oxidative stress. Iron released from red blood cells which have been damaged by free radicals of placental origin could exacerbate lipid peroxidation and promote endothelial cell injury. A significant decrease in serum-iron buffering was observed in women with pre-eclampsia, compared with healthy pregnant subjects, whereas serum iron concentration, ferritin and percentage saturation of ferritin were significantly higher[17].

To date, we are aware of no randomized trials that have assessed the effects of iron supplementation, as an isolated intervention, on the risk of pre-eclampsia.

Folate

Recently, homocysteine has been recognized as an independent risk factor for coronary artery disease, peripheral vascular disease, stroke and venous thrombosis. Homocysteine is a demethylated metabolite of the essential amino acid methionine, and high plasma levels have been associated with increased lipid peroxidation, high plasma triglycerides,

serum uric acid and alteration of the endothelial phenotype from anticoagulant to procoagulant. These vascular and metabolic changes are similar to those observed in pre-eclampsia. It has been suggested that folate and vitamin B_{12}, two major factors involved in the methionine-homocysteine pathway may be implicated in the development of placental diseases such as placental abruption, recurrent pregnancy loss and pre-eclampsia[18]. However, studies assessing the relationship between folate deficiency and pre-eclampsia are inconsistent in their results, ranging from the suggestion of an association of moderate strength[19] to a highly significant relationship[20]. The two latter reports were well-designed case-control studies, both of which adjusted for a number of potentially confounding variables. In one longitudinal study, the second-trimester levels of folates and homocysteine were not related to the occurrence of pre-eclampsia in a group of low-risk women[21].

Two small clinical trials on folate and iron supplementation included in a Cochrane meta-analysis were unable to detect any effect of the interventions on PIH[22].

Zinc

Several studies have suggested that maternal zinc status is associated with pre-eclampsia, but their results are inconsistent. Several investigators noted that women with pre-eclampsia had lower plasma and serum zinc concentrations, compared with normotensive women, but other studies have shown either increased levels or failed to find an association[23]. The majority of studies involved a case-control design with relatively small sample sizes. Moreover, there are considerable interethnic differences in zinc levels. As well, important between-tissue differences (extracellular fraction such as plasma or serum, versus erythrocyte or lymphocyte contents) have been observed. Of the seven clinical trials included in a Cochrane meta-analysis, varying doses of zinc supplementation were studied (from 20 to 90 mg/day), and only one trial focused on pre-eclampsia as an outcome[24]. The trial was carried out in 2000 low-risk women, recruited before 20 weeks of pregnancy, to whom either 44 mg/day of zinc or placebo was administered. No between-group differences were seen with respect to pre-eclampsia, PIH, preterm labor or fetal growth restriction[25].

Low-salt diet

A historical review of studies covering most of the last century[26], as well as a recent randomized trial[27], found no evidence to support the theory that salt restriction during pregnancy prevents or reduces the incidence of pre-eclampsia.

Energy/protein restriction

Epidemiological studies suggest that excess maternal weight is positively associated with pre-eclampsia. Two prospective population-based studies examined the relationship between nutrient intake and pre-eclampsia risk, with contradictory conclusions. In one study[28], where the intake of 23 nutrients was measured using a 24-h dietary recall, no significant association was demonstrated. In the second study[29], in which a semiquantitative frequency food questionnaire was employed, the incidence of pre-eclampsia was associated with a high intake of energy, sucrose and polyunsaturated fatty acids. Significant relationships persisted after adjustment for age, smoking and body mass index. Moreover, differences were reported when early-onset and late-onset pre-eclampsia were examined separately. These between-study differences may be partially explained by differences in methods: 24-h dietary recall is characterized by high day-to-day intraindividual variability; it may be inappropriate for epidemiological studies of pregnant women whose dietary habits change greatly over the course of pregnancy.

Three clinical trials of energy restriction are included in a Cochrane systematic review[30]. None of the trials specified the method of treatment allocation. Moreover, the intervention was initiated late in gestation (28–30 weeks). The limited evidence suggests that energy/protein restriction for pregnant women who are overweight or who exhibit high weight gain during pregnancy is unlikely to be beneficial and may be harmful to the developing fetus.

NUTRITIONAL INTERVENTIONS REQUIRING FURTHER INVESTIGATION

Antioxidant vitamins

Because of increasing evidence that oxidative stress, mediated in part by abnormal lipid metabolism, is a final common pathway in the pathophysiology of pre-eclampsia, the use of antioxidants has recently been suggested. The basis of these hypotheses is the increasingly recognized role of oxidative stress in the maternal syndrome of gestational hypertension, especially pre-eclampsia. Oxidative stress is an imbalance between pro-oxidant and antioxidant forces resulting in an overall pro-oxidant insult. In recent years, studies have provided compelling evidence for the involvement of oxygen free radicals in the principal disorders of pre-eclampsia. It has been proposed that the placenta of pre-eclamptic women releases circulating products that lead to oxidative stress[31,32]. In turn, pro-oxidant forces are proposed to

lead to maternal endothelial cell dysfunction. Markers of lipid peroxidation, such as isoprostanes and malondialdehyde, are increased in plasma, small arteries and the decidua basalis of women with pre-eclampsia, and lipid peroxides are implicated in failed trophoblast invasion. Numerous studies of cells and tissues have emphasized the deleterious effects of oxidized low-density lipoprotein (LDL) contributing to cellular toxicity, inflammation, vascular apoptosis and endothelial cell dysfunction. Oxidized LDL produces deleterious effects, including increased expression of inflammatory cytokines and vascular cell adhesion molecules (sVCAM-1). Furthermore, in pre-eclampsia, increased plasma levels of endothelial-type inhibitors of fibrinolysis (plasminogen activator inhibitor-1, (PAI-1)) and decreased plasma levels of placental-type inhibitors of fibrinolysis (PAI-2) may reflect a response to maternal hypertension or renal damage, combined with decreased placental function or mass. Low concentrations of water-soluble and lipid-soluble antioxidants have also been reported in plasma and placentas in women with pre-eclampsia[33,34]. Oxidative stress, mediated in part by abnormal lipid metabolism, is likely to contribute to maternal endothelial cell activation, a hallmark of the disease, and is believed to underlie the intense vasoconstriction and procoagulant state of pre-eclampsia. The capacity of the antioxidant system, reflected in its ability to protect against oxidative insult, influences the susceptibility of the endothelium to oxidative stress. Antioxidants are compounds that, by interacting with reactive oxygen species, protect against the harmful effects of reactions that can cause excessive oxidation.

The first randomized trial of antioxidants in women with severe pre-eclampsia (n=56) did not demonstrate a significant effect on pregnancy duration, lipid peroxide levels or neonatal outcomes[35].

A UK research group recently reported a randomized controlled trial of prophylactic vitamin C (1000 mg/day) and vitamin E (400 IU a-tocopherol/day) supplementation in a group of 283 pregnant women at increased risk of pre-eclampsia, as defined by abnormal uterine artery Doppler waveform or by past history of the disease[36]. The trial was designed to test the effect of antioxidants on biochemical markers of disease. Pre-eclampsia was the main clinical outcome measure. The ratio of PAI-1 (marker of endothelial cell activation)/PAI-2 (marker of placental function) was significantly decreased in the vitamin-treated group. By an 'intention to treat' analysis, there was a 54% reduction in pre-eclampsia in the active treatment group (RR 0.39; 95% CI 0.17–0.90). High-risk women who developed pre-eclampsia in the placebo group had lower plasma vitamin C concentrations compared with normal pregnant controls, and these returned to normal levels on supplementation. Plasma concentrations of isoprostane, 8-epiprostaglandin F_2a, a marker of lipid peroxidation, were raised in

the high-risk placebo group but fell to concentrations comparable to those in low-risk subjects after vitamins C and E supplementation. Importantly, this is the first study to have reported a clinical benefit of antioxidant supplementation together with supportive biochemical evidence of a reduction in oxidative stress and improvement in a range of markers of the disease process.

The above trial stimulated considerable interest from the obstetric and lay communities, with the question often raised as to whether vitamins C and E should routinely be administered to prevent gestational hypertension with or without proteinuria, and thereby to reduce maternal and infant morbidity and mortality. At present, this cannot be recommended for several reasons: confirmation of the results of this small study in high-risk women is required; the results cannot be extrapolated from high- to low-risk women; and neonatal safety has yet to be evaluated in sufficient numbers. As more than 50% of all cases of pre-eclampsia occur in nulliparous women with no known risk factors, it is also essential to assess the potential benefits of supplementation in these women. The original study was performed in high-risk women selected primarily on the basis of abnormal uterine Doppler analysis, a procedure which is not routine in the clinical management of low-risk women. The effect of the intervention may be different in clinical contexts where a high proportion of women routinely take multivitamin supplementation in pregnancy, as opposed to the UK where vitamin supplementation is practiced in only a small proportion of pregnant women.

Several multicenter trials of antioxidant prophylaxis are currently in progress. The Canadian Institutes of Health Research recently funded a study that is to be carried out in both low-risk (5000 per group) and high-risk (1250 per group) women. Women will be stratified according to the presence or absence of clinical risk factors (chronic hypertension, diabetes, multiple gestation, history of pre-eclampsia). The primary outcome for the trial is gestational hypertension with adverse conditions, as proposed by the Canadian consensus document on hypertensive disorders in pregnancy.

Selenium

The rationale for a role for selenium in the pathophysiology of pre-eclampsia is based on the hypothesis of a protective mechanism of selenium-dependent glutathione peroxidase against lipoperoxide damage in vascular epithelium. Only one study reports the effects of selenium supplementation on the incidence of gestational hypertension in high-risk women. A significant reduction in blood pressure was observed. Proteinuria was observed in four of 47 cases in the control group, but in none of 52 cases in the supplemented group. However, the method of

randomization, definition of the level of risk, method of assessment of outcome and gestational age at randomization were not reported[37].

MEDICATIONS

Antiplatelet agents and anticoagulants

The rationale for the use of aspirin to prevent pre-eclampsia is based on the understanding of mechanisms of TX/PI metabolism. Studies involving the culture of placental tissue from pre-eclamptic patients demonstrated an imbalance in the TX/PI ratio in pre-eclampsia patients[32]. It was hypothesized that aspirin could correct this TX/PI imbalance. Early systematic reviews of antiplatelet drugs included only small trials, and reported promising reductions in the risk of developing pre-eclampsia. However, large trials have failed to confirm a clinically important reduction in the risk of pre-eclampsia.

A recent meta-analysis which included 42 trials involving over 30000 women suggests that antiplatelet drugs are associated with a moderate (15%) reduction in the risk of pre-eclampsia, a 14% reduction in the risk of a stillbirth or neonatal death and an 8% reduction in the risk of preterm birth[38]. There was some evidence that there may be greater benefits for women treated before 20 weeks of pregnancy, particularly for reduction in risks of pre-eclampsia and stillbirth/neonatal death. The reduction of risk appears to be greater at aspirin doses of >75 mg, but the number of women in the subgroup analysis was small, resulting in a broad confidence interval.

A randomized clinical trial was carried out recently in France. In all, 3294 nulliparous women recruited before 20 weeks of gestation were treated with aspirin at a dose of 100 mg/day or placebo[39]. The aspirin and placebo groups did not differ with respect to the incidence of pre-eclampsia and/or gestational hypertension. Moreover, the incidence of maternal side-effects and the proportion of babies with birth weight below the third centile were significantly higher in the treated group.

In a South African study of 138 high-risk patients randomized before 20 weeks of gestation, the addition of 40 mg of ketanserin (selective serotonin-2-receptor blocker with some degree of a al-blocker activity) to 75 mg of aspirin was associated with a substantial decrease in the frequency of superimposed pre-eclampsia (9% vs. 52%), and an improvement of pregnancy outcomes (diastolic blood pressure, gestation duration, birth weight)[40].

It has recently been suggested that in pre-eclampsia, the coagulation system of the uteroplacental unit may be highly activated. However, most current data relating pre-eclampsia to hemostatic disorders are

derived from studies of the peripheral circulation. Very limited data are available on the hemostatic system within the uteroplacental unit. Pregnancy is a physiological state where the risk of venous thromboembolism is increased two- to four-fold[41]. This situation is aggravated in women who harbor risk factors such as antiphospholipid syndrome or essential thrombocytosis, or congenital factors such as antithrombin, protein C or protein S deficiencies, methylenetetrahydrofolate reductase (MTHFR) mutation, factor V Leiden mutation or G20210A prothrombin mutation, which are frequently associated with pre-eclampsia syndrome as well as with other obstetric complications. Factor V Leiden, G20210A mutation of prothrombin, C677T mutation of MTHFR and antithrombin III are the thrombophilic factors most studied in relation to pre-eclampsia. Findings are inconsistent across studies, with results ranging from a strong association of these factors with pre-eclampsia to an absence of association. There are considerable interethnic differences in mutation prevalence that may contribute to this diversity of results, but some methodological differences between studies, such as inclusion criteria, could also explain such differences[42].

It has been proposed that certain forms of pre-eclampsia must be considered as a thrombotic event, and that these women could benefit from anticoagulation. However, few studies have explored the possibility of preventive anticoagulant treatment of at-risk women. One small retrospective study (n=147) examined the benefits of combined heparin and aspirin therapy on the prevalence of pre-eclampsia in women with renal disease, and demonstrated a reduced prevalence of pre-eclampsia in the heparin (combined with low-dose aspirin or dipyridamole) group versus the placebo (odds ratio (OR) 0.06, 95% CI 0.01–0.30) and aspirin (OR 0.07, 95% CI 0.01–0.38) groups[43]. Another prospective case-control study found that in women (n=22) with a diagnosis of thrombophilia, heparin therapy increased the rate of live births and decreased the number of obstetric complications with or without abortions, compared with previous pregnancy without treatment[44]. Two clinical trials that are designed to assess the effect of heparin therapy in women with thrombophilic disorders and with other pregnancy complications are in progress in Canada.

OTHER INTERVENTIONS

Prenatal care

Several clinical studies have assessed the effects of the approach to prenatal care on pregnancy outcomes, including pre-eclampsia. They evaluated the effectiveness of 'evidence-based' antenatal care packages

as well as prenatal care managed by providers other than obstetricians. The results of a Cochrane meta-analysis (ten trials, 60000 women) show no differences between groups in the incidence of pre-eclampsia. In a study by Villar and colleagues, the rate for pre-eclampsia/eclampsia was slightly higher in the group receiving an evidence-based package of prenatal care (1.69% vs. 1.38%; OR 1.26 (95% CI 1.02–1.56))[45].

Physical activity

Recently it has been hypothesized that regular physical exercise training may decrease pre-eclampsia risk. Decreased plasma antioxidant capacity has been described as one of the characteristics of women with pre-eclampsia. Physical exercise is reported to induce a significant increase in antioxidant protection via superoxide dismutase, catalase and glutathione peroxidase activities, and has been proposed as a possible preventive measure especially in obese women[46]. To date, a single retrospective study reports an inverse relationship between leisure-time physical activity during the first 20 weeks of pregnancy and the risk of development of pre-eclampsia[47]. Furthermore, prospective studies are required to confirm the effect of regular exercise on the incidence of pre-eclampsia. Such a study is in progress at the Magee-Women's Research Institute[48]. Multiparous women who are 18 weeks' gestation or less and who had pre-eclampsia in a previous pregnancy are randomized to one of two groups. The intervention group will perform 30 min of moderate-intensity physical activity on 5 days/week, and short bouts of exercise (3–10-min episodes of walking). A sample size of 160 women per group is projected in this trial.

CONCLUSIONS

Based on this literature review, there is little evidence that either dietary advice or pharmacological intervention can be effectively applied to prevent pre-eclampsia. With regard to certain interventions, there is considerable disparity in results across studies, particularly with respect to the role of aspirin and calcium administration. These variations may be due to differences in population selection (low-risk or high-risk), gestational age at randomization and the dose used. Pre-eclampsia is a syndrome of uncertain etiology and pathophysiology. Very few nutritional questions in pre-eclampsia have been answered definitively. It seems unlikely that sodium restriction or supplementation with zinc, magnesium or fish oil will prove useful to reduce the risk of pre-eclampsia. Antioxidant vitamins C and E hold promise as an approach to reducing the risk of occurrence of pre-eclampsia, but further studies are required. It is also unclear whether

for interventions involving vitamins or certain essential elements the aim should be to correct inadequate nutritional intake or to produce a pharmacological effect. Given that pre-eclampsia is a disease of complex etiology, simple dietary interventions are unlikely to have a major impact on disease incidence. Further prospective investigations should focus on achieving a better understanding of risk factors involved in complex pathways and targeting the biochemical key mechanisms for developing new prevention strategies.

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Appendix

The North-West regional guidelines for the management of severe pre-eclampsia

NORTH-WEST PRE-ECLAMPSIA CORE GROUP MEMBERS

Consultant obstetricians

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Midwife

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This protocol was devised in November 2002 and will be revised by November 2005.

The core group members gratefully acknowledge the support and input of their colleagues from the Yorkshire region, particularly midwife Dawn Jankowicz.

PRE-ECLAMPSIA GUIDELINES

Indicators of severe disease

The criteria for managing a woman with these guidelines are subjective to a certain degree. However, the following are indicators of severe disease and justify close assessment and monitoring. They would not necessarily lead to delivery but may do so. They are also not the only entry requirements.

(1) Eclampsia

(2) Severe hypertension: systolic blood pressure over 170 mm Hg or diastolic blood pressure over 110 mmHg (mean arterial pressure 120–130 mmHg) with at least proteinuria of a+ or 1 g on a semiquantitative assessment (three blood pressure readings in a 15-min period)

(3) Moderate hypertension: systolic blood pressure over 140 mm Hg or diastolic blood pressure over 90 mmHg (mean arterial pressure > 110 mmHg) with at least proteinuria ++ or 3 g on a semiquantitative assessment (three blood pressure readings in a 45-min period) and any of:
- severe headache with visual disturbance
- epigastric pain
- signs of clonus
- papilledema
- liver tenderness
- platelet count falling to below 100×10^9/l
- alanine aminotransferase rising to above 50 IU/l
- creatinine >100 mmol/l

General measures

The woman should be managed in a quiet, well-lit room in a high-dependency care-type situation. Ideally, there should be one to one midwifery care. Initial assessment charts should be commenced to record all physiological monitoring and investigation results. All charts

should be for a continuous 24-h period of high-dependency care. A new chart should not be started until the previous one has a full 24-h assessment. All treatments should be recorded.

The Consultant Obstetrician and the Consultant Anesthetist should be informed in order that they can be involved at an early stage in management.

A large-bore intravenous cannula should always be inserted, but not necessarily used for infusing drugs or fluid until either an indication presents or a decision is made to deliver. If intravenous fluid is given, it should be by controlled volumetric pump.

Basic investigations

Blood should be sent for:

Serum electrolytes	(Na, K, urea, creatinine, urate)
Liver function tests	(albumin, ALT)
Full blood count	(Hb, WCC, Plts)
Clotting	(PT, KCCT±fibrinogen, FDPs)
Group and save serum	

All tests should be checked daily or more frequently if abnormal.

Measurement of blood pressure

While Dinamaps are good for measuring blood pressure trends, absolute measurements of blood pressure should be measured using sphygmomanometers. Karotkov phase 5 should be used to determine the diastolic pressure.

Monitoring

Blood pressure and pulse should be measured each 15 min until stabilized and then half hourly.

An indwelling catheter should be inserted and urine output measured hourly whenever intravenous fluids are given.

Oxygen saturation should be measured continuously and charted with the blood pressure. If saturation falls below 95% then medical review is essential.

Fluid balance should be monitored very carefully. Detailed input and output recordings should be charted.

Respiratory rate should be measured hourly.

Temperature should be measured 4 hourly.

When present CVP/arterial lines should be measured continuously and charted with the blood pressure.

Fetal well-being should be assessed carefully. In the initial stages this will be with a cardiotocograph but consideration should be given to assessing the fetus with a growth scan, liquor assessment and umbilical artery Doppler flow velocity waveform.

Thromboprophylaxis

All patients should have anti-embolic stockings and/or heparin while immobile. Following delivery or after insertion of an epidural either unfractionated heparin or a low-molecular-weight heparin (dose adjusted on early pregnancy weight) daily should be given until the patient is fully mobile. Low-molecular-weight heparin should not be given until 1 h after spinal anesthesia. An epidural catheter should be left in place for least 8 h after low-molecular-weight heparin administration.

ANTEPARTUM/INTRAPARTUM MANAGEMENT

Control of blood pressure

The aim of stabilization of blood pressure is to reduce the blood pressure to <160/105 mmHg in the first instance (mean arterial pressure <125 mmHg) and maintain the blood pressure at or below that level. This will necessitate medical staff remaining in attendance. Blood pressure may suddenly drop in response to treatment, thus treatment should be titrated gradually by the Obstetrician or Anesthetist.

First choice agent: labetalol

If the woman can tolerate oral therapy an initial 200 mg oral dose can be given. This can be done immediately before venous access is obtained and so can achieve as quick a result as an initial intravenous dose. This should lead to a reduction in blood pressure in about half an hour. A second oral dose can be given if needed.

If there is no initial response to oral therapy or if it cannot be tolerated, control should be by repeated boluses of labetalol followed by a labetalol infusion.

Bolus infusion is 50 mg (=10 ml of labetalol 5 mg/ml) given over at least 5 min. This should have an effect by 10 min and should be repeated if diastolic blood pressure has not been reduced (to <160/

105). This can be repeated in doses of 50 mg, to a maximum dose of 200 mg, at 10-min intervals.

Following this, or as initial treatment in moderate hypertension, a labetalol infusion should be commenced. An infusion of (neat) labetalol 5 mg/ml at a rate of 4 ml/h (20 mg/h) via a syringe pump should be started. The infusion rate should be doubled every half hour to a maximum of 32 ml/h (160 mg/h) until the blood pressure has dropped and then stabilized at an acceptable level.

Contraindication: severe asthma, use with caution in women with preexisting cardiac disease.

Second choice agent: hydralazine

If labetalol is contraindicated or fails to control the blood pressure then hydralazine is an alternative agent.

Bolus infusion is 10–20 mg over 10–20 min measuring the blood pressure every 5 min. This may be followed by an infusion of 40 mg of hydralzine in 40 ml of normal saline, which should run at 1–5 ml/h (1–5 mg/h).

Antenatal fluid management

Careful fluid balance is aimed at avoiding fluid overload. Total input should be limited to 80 ml/h. If syntocinon is used it should be at high concentration (30 IU in 500 ml, as per NICE guidelines) and the volume of fluid included in the total input. Oliguria at this point should not precipitate any specific intervention except to encourage early delivery.

DELIVERY GUIDELINES

'Planned delivery on the best day in the best way'

The delivery should be well planned, done on the best day, performed in the best place, by the best route and with the best support team. Timing affects the outcome for both mother and baby. If the mother is unstable then delivery is inappropriate and increases risk. Once stabilized with antihypertensive and possibly anticonvulsant drugs then a decision should be made. In the absence of convulsions prolonging the pregnancy may be possible to improve the outcome of a premature fetus but only if the mother remains stable. Continued close monitoring of mother and baby is needed. It seems ideal to achieve delivery, particularly of premature infants, during normal working hours.

H_2 antagonists should be given as per local guidelines.

Even a few hours may be helpful if it allows the neonatal unit to be more organized or to transfer a mother to a place where a cot is available, assuming the mother is stable before transfer (see Stabilization before transfer).

If the pregnancy can be prolonged in excess of 48 h, steroids help mature the fetal lungs. However, even if delivery is planned for within 24 h, steroids may be of benefit and should be given. Since the benefits to the fetus peak between 48 h and 6 days, then after 48 h further consideration should be given to delivery as further delay may not be advantageous to the baby or mother. In all situations, a planned elective delivery suiting all professionals is appropriate.

Delivery is not necessarily by Cesarean section but if gestation is under 32 weeks it is preferable. After 34 weeks' gestation, vaginal delivery should be considered in a cephalic presentation. The mode of delivery should be discussed with the Consultant Obstetrician. Vaginal prostaglandins will increase the chance of success. Antihypertensive treatment should be continued throughout assessment and labor.

If vaginal delivery is planned then the second stage should be short (15–30 min active second stage) with consideration given to elective operative vaginal delivery. An epidural will normally be used. The third stage should be managed with *5 units of i.v. SYNTOCINON* ***NOT*** ergometrine or syntometrine.

Regional blockade and fluids

Women with genuine pre-eclampsia tend to maintain their blood pressure, despite regional blockade. When this happens, fluid load is unnecessary and may complicate fluid balance. For this reason, *fluid loading in pre-eclampsia should always be controlled and should never be done prophylactically or routinely*. Hypotension, when it occurs, can be easily controlled with very small doses of ephedrine. General anesthesia can add to the risks of delivery since intubation and extubation can lead to increases in systolic and diastolic blood pressure, as well as heart rate, so should be avoided where possible.

Indications for central venous pressure monitoring

CVP lines can be misleading in women with pre-eclampsia. However, a CVP line may be indicated if blood loss is excessive:

(1) Particularly at Cesarean section; or

(2) If delivery is complicated by other factors such as abruptio placentae.

An intra-arterial pressure monitor may be indicated if:

(1) The woman is unstable;

(2) The blood pressure is very high;

(3) The woman is obese, when non-invasive measurements are unreliable;

(4) There is a hemorrhage of >1000 ml.

ANTICONVULSANT THERAPY

Prophylaxis

It is appropriate to treat cases of severe pre-eclampsia with magnesium sulfate. NO other agents are appropriate for prophylaxis.

MANAGEMENT OF ECLAMPSIA

Call appropriate personnel—including the resident Anesthetist. Remember ***ABC***

Give the loading dose of magnesium sulfate 4 g over 5–10 min intravenously and start an infusion of magnesium sulfate (see below).

Diazemuls may be administered if the fitting continues at the discretion of the Anesthetist 5–10 mg intravenously.

Once stabilized the woman should be delivered.

Oximetry should be instituted if not already in place.

Management of recurrent fits

Increase rate of infusion of magnesium to 1.5 g/h. Continue observations and consider the need for ventilation.

Consider other causes of seizures. It may be appropriate to organize a CT scan when the woman is stabilized.

MAGNESIUM SULFATE PROTOCOL

Magnesium sulfate is given as a loading dose followed by a continuous infusion for 24 h or until 24 h after delivery—whichever is the later.

Loading dose: 4 g magnesium sulfate i.v. over 5–10 min.

> For example: using six 50-ml syringes, in each draw up 8 ml of 50% magnesium sulfate with 22 ml dextrose 5%, to make a total volume of 30 ml. Administer using a syringe pump over 10 min at an infusion rate of 180 ml/h.

Maintenance dose: 4 g magnesium sulfate i.v. over 4 h 1 g/h.

> For example: use 50-ml syringe, draw 8 ml magnesium sulfate from a 50% solution (4 g) into six 50-ml syringes. Make up each syringe to 50 ml with 5% dextrose. Label each syringe as magnesium sulfate 4 g. Cap five syringes and store. The maximum storage time for prepared syringe is 24 h.

Important observations

Formal clinical review should occur at least every 4 h.

The following observations should be performed:

(1) Continuous pulse oximetry and ECG (alert Anesthetist if O_2 sat <95%);

(2) Hourly urine output;

(3) Hourly respiratory rate;

(4) Deep tendon reflexes (every 4 h);

(5) The ECG should be checked with each change of syringe by medical/anesthetic staff.

Cessation/reduction of the magnesium sulfate infusion should be considered if:

(1) The biceps reflex is not present;

(2) The respiratory rate is <12/min.

97% of magnesium is excreted in the urine and therefore the presence of oliguria can lead to toxic levels. In the presence of oliguria then further administration of magnesium sulfate should be reduced or withheld. If magnesium is not being excreted then the levels should not fall and no other anticonvulsant is needed. Magnesium should be re-introduced if urine output improves.

Side-effects

Motor paralysis, absent tendon reflexes, respiratory depression and cardiac arrhythmia (increased conduction time) can all occur but will be at a minimum if magnesium is administered slowly and the woman observed as above.

The antidote is 10 ml 10% calcium gluconate given slowly intravenously.

THERE IS NO NEED TO MEASURE MAGNESIUM LEVELS WITH THE ABOVE PROTOCOL

POSTPARTUM FLUID MANAGEMENT

Following delivery the woman should be fluid restricted in order to wait for the natural diuresis which occurs sometime around 36–48 h post-delivery. Total intravenous/oral fluid should be given at 80 ml/h: Hartmanns solution or equivalent plus other infusions of drugs.

Urine output should be recorded hourly and each 4-h block should be summated and recorded on the chart. Each 4-h block should total in excess of 80 ml. If two consecutive blocks fail to achieve 80 ml then further action is appropriate. This would either be:

A. If total input is more than 750 ml in excess of output in the last 24 h (or since starting the regime) then 20 mg of i.v. frusemide should be given. Colloid should then be given as below if a diuresis of >200 ml in the next hour occurs.

OR

B. If total input is less than 750 ml in excess of output in the last 24 h (or since starting the regime) then an infusion of 250 ml of colloid over 20 min should be given. The urine output should then be watched until the end of the next 4-h block. If the urine output is still low then 20 mg of i.v. frusemide should be given. If a diuresis in excess of 200 ml occurs in the next hour the fluid

should be replaced with 250 ml of gelofusine over 1 h in addition to baseline fluids.

If the urine output fails to respond to frusemide in either situation then a discussion with a Renal Physician or a member of the regional advisory panel would be appropriate.

Special problems

If persisting oliguria requiring fluid challenge or frusemide occurs then the electrolytes need to be carefully assessed and checked 6 hourly. If there is concern over a rising creatinine and/or potassium the case should be discussed with a Renal Physician or a member of the regional panel.

If the woman has a falling oxygen saturation, this is most likely to be due to fluid overload. Input and output should be assessed together with either clinical or invasive assessment of the fluid balance. However, the most appropriate treatment is likely to be frusemide and oxygen. If there is no diuresis and the oxygen saturation does not rise then renal referral should be considered.

STABILIZATION BEFORE TRANSFER

When the woman is ill and requires delivery, transfer for fetal reasons is often considered, although *ex utero* transfer may be more appropriate. If the woman requires transfer for delivery, it is of paramount importance that her condition is stabilized. The following are therefore recommended as a minimum requirement before transfer:

(1) When the woman is ventilated it is important to ensure ventilatory requirements are stable and oxygen saturations are being maintained;

(2) Blood pressure should be stabilized at <160/105 according to the above protocol;

(3) Transfer must be agreed on a consultant to consultant basis and the case should be discussed with all the relevant people at the receiving unit, e.g. the neonatal unit and neonatal medical staff, the resident obstetrician, the midwife in charge of delivery suite, intensive care and the intensive-care anesthetist (where appropriate).

(4) Appropriate personnel are available to transfer the woman. This will normally mean at least a senior midwife often with an anesthetist.

(5) All basic investigations should have been performed and the results clearly recorded in the accompanying notes or telephoned through as soon as available.

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Index