FOLATE METABOLISM AND PREECLAMPSIA

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INTRODUCTION

Preeclampsia (PE) is a multisystem disorder of human pregnancy, affecting about 6% of all pregnancies worldwide, and is one of the leading causes of maternal and infant morbidity and mortality. Despite decades of research into the pathogenesis of this complex disease, the underlying mechanisms remain unclear. As a result, the options for prevention and management of PE are limited. In recent years, there has been a growing body of evidence suggesting that folate deficiency is associated with PE, and folic acid supplementation may reduce the risk of developing PE in certain populations. Folate contributes to cell division and growth, and folate metabolism is involved in a large number of physiological and pathophysiological processes in human development. Sufficient supply of folate is therefore particularly important during pregnancy. Nevertheless, the exact mechanisms of folic acid deficiency increasing the risk of developing PE are still unclear. This article reviews what is understood about the aetiology of PE and the relationship between folate metabolism and PE so as to enhance further discussions on the subject.

PREECLAMPSIA

Preeclampsia, a syndrome characterized clinically by hypertension and proteinuria, is a leading cause of maternal and perinatal morbidity and mortality worldwide. It affects approximately 6% of all pregnancies resulting in about 8.37 million cases worldwide. The prevalence of PE varies in different populations and in different
ethnic groups. If left untreated, PE can develop into a convulsive state known as eclampsia. It has serious health consequences to the affected women and their offspring, and results in enormous economic impact to the society. Preeclampsia is associated with a significant increase in adverse perinatal outcomes including fetal growth restriction (FGR), stillbirth, preterm delivery and abruptio placentae. Nearly one-third of maternal deaths, ranking second among causes of pregnancy associated deaths, can be attributed to PE in Canada and other industrialized countries. Preeclampsia increases 3- to 25-fold the risk of other serious pregnancy complications such as thrombocytopenia, disseminated intravascular coagulation, pulmonary oedema, and aspiration pneumonia. Moreover, women with a history of PE continue to be at risk of future cardiovascular conditions. Despite being the subject of active research for many years, the aetiology of this disorder is still undefined.

Background

Preeclampsia was first recognized almost 2,000 years ago. In ancient Greece, there was already a description of the disease. Celsus described pregnant women with seizures that resolved after delivery. The disorder was termed eclampsia and was considered a pregnancy-specific seizure disorder. In the late 1800s, the association of initial proteinuria and later increased blood pressure with eclampsia was recognized. It was also recognized that increased blood pressure and urinary protein are present prior to seizures.

In 1901, a symposium on the aetiology of preeclampsia-eclampsia was held in Germany; all the delegates agreed that the cause of PE were blood toxins. The pregnancy toxaemia hypothesis was widely accepted, and the disease was once called “toxaemia of pregnancy” and “preeclamptic toxaemia”. Later researches failed to identify toxins in the blood of affected women. In the past 10 to 15 years, thoughts about this disorder have changed, but the concept was still based on the description of the clinical manifestations, such as “pregnancy-induced hypertension”, “preeclampsia-eclampsia” and “oedema, proteinuria, hypertension (EPH) syndrome”. All of these names were unsatisfactory and did not fulfill the description of all aspects of the disorder. However, PE has been the most popular name of the syndrome.

Concept of preeclampsia

No universal agreement exists on the definition of PE. Considering PE as distinct from preexisting hypertension is vitally important, the National High Blood Pressure Education Program (NHBPEP) defined several hypertension disorders including PE in 2000. Since then, there has been agreement between international working groups. The NHBPEP have defined PE, eclampsia, gestational hypertension and chronic
hypertension. PE is defined as a blood pressure (BP) of 140/90 mmHg or above on two
consequent exams 6 hours apart with ≥300 mg of protein in 24 hour urine collection or
≥1+ on urine dipstick. In women with PE, blood pressure usually returns to baseline
within days to weeks after delivery. The only real cure for PE is the birth of the
baby. Eclampsia is the occurrence, in a woman with PE, of seizures that cannot be
attributed to other causes. Gestational hypertension is defined as a blood pressure
elevation detected for the first time after midpregnancy and is distinguished from
PE by the absence of proteinuria. Gestational hypertension is a working diagnosis
only during pregnancy. If proteinuria develops and the hypertension resolves after
the pregnancy, the diagnosis is changed to PE. If elevated blood pressure persists,
chronic hypertension is diagnosed. In the absence of other factors, the diagnosis
is termed transient hypertension of pregnancy. Chronic hypertension refers to an
elevated blood pressure in the mother that predated the pregnancy. It can also be
diagnosed in retrospect when PE or gestational hypertension fails to normalize after
delivery. Thus, hypertension that has not normalized by 12 weeks postpartum is
considered to be chronic hypertension.

Clinical manifestations and diagnosis

The diagnosis of PE is inconsistent in research reports due to different classification
systems. PE is frequently subdivided into mild PE and severe PE, based on the severity
of hypertension and accompanying signs, symptoms and laboratory abnormalities.
More recently, the terms early onset (before 34 weeks) and late-onset (34 weeks or
more) PE are commonly used.

Mild PE

Mild PE is defined as hypertension developing during pregnancy, accompanied by the
onset of proteinuria. Proteinuria is defined as greater than or equal 300 mg/L in a 24
hour urine specimen (or greater than or equal 1+ urine protein by ‘dipstick’ on two
random specimens taken 6 or more hours apart). The presence of generalized oedema
(soft tissue fluid accumulation), or 5 lb or more weight gain (usually mostly fluid
retention) within a week, often accompany the hypertension and proteinuria, but are
not required for the strict diagnosis of PE. Moreover, if the criteria for hypertension
and proteinuria are met, a pregnant woman remains a ‘mild preeclamptic’ unless she
develops evidence of one of the more severe manifestations of PE.

Severe PE

Severe PE is based on one of the following findings: BP ≥160 mmHg systolic or
110 mmHg diastolic on two occasions 6 or more hours apart; proteinuria ≥ 5 g
in 24 hr (or 3–4+ on two dipstick specimens 4 or more hours apart); oliguria
Conditions that also help to define severe PE when present include: cerebral or visual disturbances (headache, blurred vision, scotomata, blindness, altered consciousness); pulmonary oedema; epigastric and right upper quadrant pain; eclampsia; FGR\textsuperscript{17}.

Specific subsets of severe PE, eclampsia and haemolysis, elevated liver enzyme levels and low platelet count (HELLP) syndrome, are major contributors to infant and maternal morbidity and mortality\textsuperscript{18}. Eclampsia is defined as seizures in a woman with PE in the absence of any other neurologic disorder. HELLP syndrome is one of the most enigmatic and potentially serious manifestations of PE. This condition is characterized by intense contraction of small blood vessels resulting in damage to endothelial cells and activation of the coagulation system along the blood vessel wall as the result of the exposure of sub-endothelial thrombogenic factors\textsuperscript{19}.

Complications of PE

Preeclampsia is also associated with a significant increase in adverse maternal-infant outcomes. Maternal complications of PE include uteroplacental insufficiency, placental abruption, premature delivery, cesarean section, seizure, cerebral haemorrhage, disseminated intravascular coagulation (DIC), thrombocytopenia, renal failure, hepatic rupture or failure and pulmonary oedema. For the fetus, PE may lead to placental infarction and/or abruption, intrapartum fetal distress, stillbirth, asymmetric and symmetric small for gestational age (SGA), FGR and oligohydramnios\textsuperscript{20}.

Pathogenesis of preeclampsia

The current generally acceptable pathophysiology of PE is that it is a two-stage disorder: at stage I (most likely during the late first trimester or early second trimester), there is a decrease in placental perfusion, which is secondary to abnormal migration of trophoblasts into maternal spiral arteries; of stage II (most likely during the early third trimester) of the maternal syndrome of PE, is secondary to systemic endothelial dysfunction (e.g., impairments in the nitric oxide system, overactivity of the autonomic nervous and/or renin-angiotensin systems, activation of a systemic inflammatory response, and activation of circulating proteins that interfere with angiogenesis)\textsuperscript{21,22}. The link between reduced perfusion and the maternal syndrome is believed to be increased oxidative stress and/or an increased inflammatory response\textsuperscript{23}.

Some modifications of this two-stage model have recently been proposed. Roberts et al\textsuperscript{24,25} suggested that reduced perfusion, posited as secondary to failed remodeling of the maternal vessels supplying the intervillus space, was not sufficient to cause PE. Maternal constitutional, genetic, behavioral and environmental factors were necessary to interact with reduced placental perfusion to lead to the maternal
abnormalities of PE. Roberts et al\textsuperscript{24,25} also considered that there was more than one linkage between stage 1 and stage 2 and subtypes of PE existed that might be identified by the linker.

**Placentation abnormalities (Stage I)**

The origin of PE is still poorly understood. No single candidate mechanism exists to explain the complex pathogenesis. Since reduced placental perfusion is the end result of complex molecular interactions between the placenta and the female body, reflecting genetic and immunological processes\textsuperscript{26}. That is, polygenic inheritance may reduce the maternal recognition of fetal trophoblast membrane antigens, weaken the protective immune response and enhance the rejection reaction, resulting in trophoblast damage, reduced infiltration and shallow placental implantation. Consequently, placental ischaemia and hypoxia, local cellular immune response enhancement and partial placental oxidative stress will occur\textsuperscript{27}.

**Immunological aspects of implantation.** Pregnancy is a natural successful semi-allogeneic graft, depending on the maternal-fetal immune balance. Disturbance on this balance may lead to immune rejection, resulting in pathological pregnancy. There is strong evidence linking immunologic mechanisms with impaired development of spiral arterioles (ie, shallow placental implantation) and acute atherosclerotic lesions in the wall of spiral arterioles. Maternal-fetal immune imbalance may be related to the following factors:

1. Allogeneic antigen overload may impact on the development of placental vascular bed and the remodeling process, resulting in immaturity of the trophoblast. Antigenicity of immature trophoblast cells is much stronger than that of the mature cell\textsuperscript{27}.

2. Maternal-fetal immune dysequilibrium and blocking antibody-1 (Ab-I) deficiency may result in a weakened protective effect of TCX antigen produced by trophoblast leading to a local placental immune response.

3. Human leucocyte antigen (HLA): Qiao et al\textsuperscript{27} suggested that HLA-DR4, may act as immune genes reducing maternal-fetal antigen-presenting and identification functions, resulting in blocking antibody deficiency and disease virulence gene linkage dysequilibrium.

4. HLA-E, HLA-G gene polymorphism: HLA-E and HLA-G, newly discovered non-classical HLA-I molecules, may play an important role in immune tolerance during pregnancy.

HLA-G products, which are mainly distributed on extravillous trophoblast cells, can combine with natural killer (NK) cell inhibitory receptors, natural killer cell Ig-like receptor (KIR), to inhibit NK cell killing. HLA-G may also induce Th2-type cytokine secretion, providing a protective function during pregnancy.
HLA-G may also inhibit peripheral blood NK cells and T cytotoxicity. The enhanced placental local immune rejection may lead to vascular endothelial cell damage and dysfunction\textsuperscript{28}. Goldman et al\textsuperscript{29} suggested that decreased or absent HLA-G expression on trophoblast in women with PE led to attack by the maternal immune system resulting in failure of invasion of spiral arteries, defective vascular remodeling, shallow placental implantation and placental ischaemia and hypoxia\textsuperscript{29}. Recent studies have shown that PE is associated with HLA-G gene polymorphism. Bermingham et al\textsuperscript{30} demonstrated that polymorphism frequencies of HLA-G exon 8 insertion or deletion were altered in offspring of PE patients. O’Brien et al\textsuperscript{31} also reported that reduced transcription of HLA-G3 was associated with polymorphism of exon 3 in pregnant rats with PE. Homozygotes with deletion mutation of the HLA-G exon cannot express HLA-G1, and have an increased risk of PE. However, several studies have reported no HLA-G gene defects in PE.

5 Complement activation: Complement activation is more common in PE patients. Austgulen\textsuperscript{32} showed that complement component 3 (C3) and complement component 4 (C4) were reduced secondary to complement activation. The activated complement further activated leukocytes in the placental circulation\textsuperscript{32}.

6 Abnormalities of cellular and humoural immunity: The T helper 1 (Th1)/T helper 2 (Th2) ratio is reported to be less than 1 in normal pregnant women, while in PE patients the ratio shows a tendency to be more than 1. With the increase in Th1 cell number, cytotoxic factors also increased including tumour necrosis factor α (TNF-α), interferon-γ (IFN-γ), interleukin-1 (IL-1), interleukin-10 (IL-10) and interleukin-6 (IL-6). These cytokines may induce fat cell degradation, damage liver fatty acid oxidation and interfere with synthesis of prostacyclin and nitric oxide\textsuperscript{33}.

Genetic causes of PE. A number of susceptibility genes have been associated with the pathogenesis of PE. At present, susceptibility genes are found in endothelial nitric oxide synthase (eNOS) gene, renin -angiotensin -aldosterone system genes, Fas / FasL gene, V Leiden gene, prothrombin gene, prothrombin-regulated protein (TM), methylenetetrahydrofolate reductase (MTHFR) gene, mitochondrial deoxyribonucleic acid (DNA) mutation, lipoprotein lipase gene (LPL), apolipoprotein E gene, TNF-α gene, HLA-G, and HLA-DR4 genes\textsuperscript{27}.

The fetus could be seen as a semi-homotransplant with half of the paternal genes. In recent years, the role of the paternal factor in the pathogenesis of PE has received increasing attention. Paternal HLA-C and TGF-β expressed by invasive trophoblast cells could stimulate NK cells in the maternal decidua. Excessive activation of the maternal inflammatory response to these factors could affect the normal chorioplacental formation by both maternal and paternal genetic conflict. Broughton-Pipkin\textsuperscript{35} reported that the risk of PE in women with a history of PE was reduced 30% after changing partner; however, women with a normal blood pressure during pregnancy who changed partner, had a 30% higher risk than those with the same partner.
The maternal syndrome (stage II)

Systemic endothelial cell dysfunction appears to be the key event in the diverse clinical manifestations of PE\(^3\). Chelbi and Vaiman\(^3\) proposed that in PE insufficient utero-placental blood flow leads to placental hypoxia, oxidative stress, and consequently the release in the maternal blood circulation of placental factors that disrupt normal endothelial barrier function and induce increased endothelial permeability\(^3\). Fms-like tyroine kinase 1 (sFlt-1), a vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) antagonist, is now thought to play a significant role in the development of PE. Maynard et al\(^3\) reported that placental hypoxia resulting from inadequate perfusion upregulated sFlt-1, leading to damaged maternal endothelium and restriction of placental growth. In addition, Venkatesha et al\(^3\) reported that endoglin, a TGF-beta antagonist, was elevated in pregnant women who developed PE. Soluble endoglin (sEng) is likely upregulated by the placenta in response to upregulation of cell-surface endoglin produced by the maternal immune system, although there is also the potential that sEng is produced by the maternal endothelium\(^4\). Levels of both sFlt-1 and sEng increase as severity of disease increases, with levels of sEng surpassing levels of sFlt-1 in HELLP syndrome\(^4\).

Both cytotoxic and inflammatory mediators, including oxygen free radicals, lipid peroxides, tumour necrosis factor, IL-6 and very low density lipoprotein (LDL), may cause vascular endothelium impairment. Once the vascular endothelial cell is impaired, endothelium derived relaxing factor (EDRF), nitric oxide (NO), as well as prostacyclin (PGI\(2\)) decrease, while the vascular endothelial contracting factor, thromboxane A\(2\) (TXA\(2\)) will increase\(^4\). This results in systolic and diastolic hypertension, as well as a series of pathological changes.

Epidemiological Studies

Risk factors for PE

Although some epidemiological studies have identified several risk factors for PE, including maternal, paternal, pre-conceptional, pregnancy-associated and environmental factors, current understanding of the cause of PE is still limited\(^4\).

Nulliparity

Nulliparity has been confirmed as a risk factor for PE in both large-scale epidemiologic studies and detailed clinical studies\(^4\). Skjaerven et al\(^4\) reported that PE rates were 3.9%, 1.7%, 1.8%, respectively, in the first, second and third time pregnant women. A number of epidemiological studies have also shown multiparous women who changed partner had an increased risk of PE in the following pregnancy compared with multiparous women with the same partner\(^4\).
Body mass index (BMI)

Women with a BMI greater than 30 early in pregnancy are more likely to become PE than normal BMI women, with reported odds ratios (OR) between 3 and 547. Hrazdilova et al48 reported that pre-pregnancy BMI was associated with PE and women with a very low BMI had a decreased risk of PE.

Age

PE is more common at the extremes of maternal age (<18 years or >35 years old). Skaznik et al49 reported that pregnant women aged ≥ 35 years old are at increased risk of PE. In a review of risk factors for PE, women older than 40 years had almost twice the risk of developing PE compared with younger women. Demir et al50 reported that 14.5% of pregnant women aged less than 19 years developed PE50.

History of chronic hypertension

Women with preexisting chronic hypertension also have an increased risk of PE. Brown et al51 showed that white women who have hypertension alone during pregnancy (gestational hypertension) are more likely to have chronic hypertension in later life than those who have both hypertension and proteinuria during pregnancy. Conversely, Jonsdottir et al52 reported women who had PE were more likely to develop ischaemic heart disease in later life than those who had gestational hypertension alone. Broughton-Pipkin et al53 also reported that women who remain normotensive during all pregnancies are less likely than women in the general population to develop hypertension in later life.

Insulin resistance, diabetes, lipid abnormalities

Cundy et al54 found that the overall rate of PE was similar in women with type 1 diabetes and type 2 diabetes, (41% and 45% respectively)54. Type 2 diabetic women were more susceptible to chronic hypertension.

Environmental factors

Environmental factors may also contribute to the development of PE. For example, the high incidence of PE in many poor countries suggests that an inadequate diet may be a risk factor for PE. Dietary inadequacies that have been proposed as relevant include folic acid, calcium, zinc, vitamins C and E, and essential fatty acids53.
Genetic factors

The large number of epidemiologic studies carried out on PE patients indicate that it is a heritable disorder; daughters of women with a history of PE are much more likely to develop the disease than daughters-in-law\textsuperscript{55}. Based on data from approximately 1.7 million births in Norway, Lie et al\textsuperscript{56} found that a woman who becomes pregnant by a man who has already had a child with a different woman who developed PE during pregnancy, had a risk of PE that was nearly twice as high as that of a woman whose partner did not have such a history. In addition, Esplin et al\textsuperscript{57} reported that men who were themselves born of pregnancies complicated by PE were twice as likely to have a child who was the product of a pregnancy complicated by PE as men who were born after a normal pregnancy. There thus seems to be both a maternally transmitted and a paternally transmitted genetic predisposition to PE. However, the ability to study this predisposition is hampered by the obvious fact that PE is a disease of pregnancy and no markers for the disorder have yet been identified in non-pregnant women, let alone men.

An increased risk of PE may be associated with gene polymorphisms. A tendency to hypercoagulability predisposes women to PE\textsuperscript{58}. It is reported that a polymorphism in the angiotensinogen gene is more frequent in women with PE than in normotensive pregnant women\textsuperscript{59}. Several polymorphisms in genes that control the clotting cascade have also been identified, although the data linking particular polymorphisms to PE are contradictory, presumably due to the differences in the populations studied.

Other factors

Other factors, such as pregnant women with low birth weight, polycystic ovarian disease, previous history of PE, multiple pregnancy, chronic nephritis, antiphospholipid syndrome, obesity, malnutrition, low socio-economic conditions, pregnancy intervals, assisted reproductive technology, may also be associated with the development of PE\textsuperscript{60}.

Protective factors

Antioxidants

Markers of oxidative stress have been reported in women with established PE. Accordingly, these findings support an expected beneficial effect of antioxidant therapy in the prevention of PE and other pregnancy-related disorders\textsuperscript{61}. Numerous studies have been carried out to investigate prophylactic and/or therapeutic prevention of oxidative stress and the impact on PE and its perinatal complications\textsuperscript{62}. Despite the logic behind using antioxidant vitamins, several studies show that Vitamin C/E supplementation is of no benefit and may in fact cause harm\textsuperscript{63}. 
Aspirin supplementation

Low dose aspirin supplementation provides a slight preventative benefit in women at high risk. However, significant research has been done on aspirin and the results thus far are unimpressive\textsuperscript{64}.

Vitamin D

Low levels of vitamin D are considered a risk factor for PE. However, Trumbo et al\textsuperscript{65,66} reported that calcium supplementation in women with low-calcium diets resulted in no change in PE rates although there was a decrease in the rate of severe PE complications. Moreover, a negative correlation between calcium intake and incidence of PE has recently been detected in Guatemala, Colombia and India\textsuperscript{67}.

Smoking

Since a study which was published in the New England Journal of Medicine in 1999, indicated that the risk of developing PE was 32% lower in women who smoked than in nonsmokers\textsuperscript{68}, other studies have reported similar observations. A systemic review identified 48 epidemiologic studies on the association between smoking and PE from 1959 to 2006. The review concluded that smoking during pregnancy reduces the risk of PE by up to 50% with a dose-response pattern. A protective effect was consistently found in both nulliparas and multiparas, singleton and multifetal pregnancies\textsuperscript{68}.

The mechanism for the protective effect of cigarette smoking on PE is unknown but cannot be explained by nicotine, confounding factors, and changes in placental morphologic or histopathologic characteristics. Some investigators have hypothesized that carbon monoxide (CO) produced by cigarette smoking may be the substance that underlies the negative association\textsuperscript{68}. However, Engel et al\textsuperscript{69} found that smoking is only protective against PE in women without pre-gestational hypertension, and even then principally among younger women.

Folic acid

Some studies have shown that the folic acid supplementation has protective effects on PE. Wen et al\textsuperscript{70} found that folate supplementation in the early second trimester reduced the risk of PE in Ottawa and Kingston (Oak) Birth Cohort data.

FOLATE METABOLISM

Folic acid, or folate, is one of the B vitamins \(B_9\). Man cannot synthesize folate, and thus depends on a variety of dietary sources or dietary supplements for folate supply. Rich dietary sources of folic acid include leafy vegetables, fruits and berries, beans,
Folate metabolism and preeclampsia

**Abbreviations:**
- DHF, dihydrofolate; DHFR, dihydrofolate reductase; dUMP, deoxyuridine monophosphate; GAR, glycaminide ribonucleotide; GART, glycaminide ribonucleotide transformylase; FR, folate receptor; MS, methionine synthase; MT, methyltransferases; MTHFR, 5,10-methylenetetrahydrofolate reductase; RFC, reduced folate carrier; SAH (AdoHcy), S-adenosylhomocysteine; SAM (AdoMet), S-adenosylmethionine; THF, tetrahydrofolate; dTMP, deoxythymidine monophosphate; TS, thymidylate synthase; X, a variety of substrates for methylation.

Whole grain products and liver, nuts, whole-meal bread, and fortified breakfast cereals are also good sources of folate.

**Folic acid metabolism**

Once absorbed, folate plays an essential role in several complex metabolic pathways, including transfer of one-carbon units, leading to the synthesis of DNA and RNA and participation in methylation reactions, such as the methylation of DNA or other substrates (by use of S-adenosylmethionine, SAM, which is concurrently converted to S-adenosylhomocysteine) (see Figure 1).

Tetrahydrofolate (THF), the metabolically active form of folate, is central to normal one-carbon metabolism. Accepting or donating one-carbon units are the principal functions of folate coenzymes in key metabolic pathways. The crucial first step in the cycle is conversion of THF to 5,10-methylene-THF with the 3-carbon of serine as a major carbon source. This one-carbon unit is transferred to THF via pyridoxal...
phosphate (PLP)-dependent serine hydroxymethyltransferase (SHMT) to generate 5,10-methylene-THF and glycine. Methylene tetrahydrofolate reductase (MTHFR) then catalyses the irreversible conversion of 5,10-methylene-THF to 5-methyl-THF. The MTHFR substrate, 5,10-methylene-THF, is also a substrate for the thymidylate synthase (TS) enzyme in the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). The N-5 methyl group of 5-methyl-THF can only be used metabolically for transfer to homocysteine. About 50% to 80% of the homocysteine generated is remethylated, depending on the dietary content of methionine and choline. In the methionine synthase reaction, a methyl group is removed from 5-methyl-THF, which serves as a substrate, and is sequentially transferred to the vitamin B-12 coenzyme, thus generating methionine. In addition to protein synthesis, methionine serves as a methyl group donor through conversion to S-adenosylmethionine (SAM), which is a key methyl group donor in methylation reactions, whereas SAM inhibits the MTHFR enzyme providing a negative feedback loop.

The methionine synthase reaction also regenerates THF required for the generation of 5,10-methylene-THF and 10-formyl-THF that are used in thymidylate and purine synthesis, respectively. In the thymidylate synthase reaction, 5,10-methylene-THF also donates its CH₂ unit.

**Hyperhomocysteine and preeclampsia**

Homocysteine plays an important role in folic acid metabolism. Reduced folate intake or genetic abnormalities of folate metabolism are associated with increased serum homocysteine concentration. It has been demonstrated that one of the risk factors in the pregnant women for PE is hyperhomocysteinemia, which can result in endothelial dysfunction and increase oxidative stress. Numerous studies have demonstrated a direct relationship between hyperhomocysteinemia and PE. Leeda et al. found the same incidence of hyperhomocysteinemia in preeclamptic patients as Dekker et al. (17.7%)⁷⁴. De Vries et al.⁸² also reported that nearly 24% of pregnant women with PE had hyperhomocysteinemia. However, Kang et al.⁸³ found similar concentrations of homocysteine in pregnant women with PE and non-pregnant women. All this could be explained by a greater sensitivity of the endothelium during pregnancy to lower concentrations of homocysteine.⁸⁴ Leeda et al.⁸¹ and De la Calle.⁸⁵ demonstrated that the presence of hyperhomocysteinemia in patients with PE was associated with reduced levels of folic acid in comparison with a control group. In addition, it is reported that patients treated with folic acid during the second and third trimester of pregnancy develop less severe PE than untreated patients.⁸⁵ For this reason, 30% of women with severe PE will have a recurrence in subsequent pregnancies; Sibai et al.⁸¹,⁸⁵ believe that it is important to identify hyperhomocysteinemia in order to administer appropriate treatment with folic acid during later pregnancies. However, Leeda et al.⁸¹ indicated that there were as many as 50% relapses in subsequent pregnancies of patients with prior PE despite treatment with folate.
Gene polymorphism studies in folate metabolism and risk of preeclampsia

There are four key enzymes involved in the folate-homocysteine metabolic pathway: 5,10-methylentetrahydrofolate reductase (MTHFR), cystathionine β-synthase (CBS), methionine synthase (MTR), and methionine synthase reductase (MTRR). Polymorphisms in the genes coding for these enzymes have also been described and studied in relation to hyperhomocysteinemia associated with PE.

5,10-methylentetrahydrofolate reductase (MTHFR)

MTHFR plays a very important role in folate metabolism. It can deoxidize the N5, N10-methylenetetrahydrofolate into N5-methyltetrahydrofolate, resulting in homocysteine being methylated into methionine. Thus MTHFR defects impede the transformation from homocysteine to methionine.

The most common polymorphism associated with hyperhomocysteinemia is the 677C>T variant of the MTHFR gene. The C667T mutation was shown to induce an enzyme with thermolabile properties and with decreased activity, resulting in elevated plasma homocysteine concentrations. Further, the effect of the C667T mutation could be reversed by additional folic acid intake. There are three types of MTHFR: C/C, C/T, T/T. Studies found the 677C>T polymorphism was associated with several pregnancy complications, but the relationship with PE is controversial. Although Grandone et al. and Sohda et al. were the first to propose the MTHFR 677C>T allele as a genetic risk for PE, most studies have failed to confirm this association. Recently, a second common polymorphism in the MTHFR gene has been identified. The 1298A>C polymorphism in the MTHFR gene may decrease the enzyme activity. However, the effect of the A1928C mutation on MTHFR activity and on homocysteine concentration is less than that observed for the C667T mutation. There are few studies investigating the effect of the 1298A>C polymorphism in the MTHFR gene on the risk of PE. Homozygosity for the C667T mutation and combined heterozygosity for the C667T and A1298C mutation may result in reduction in MTHFR activity, elevating plasma homocysteine and redistribution of folate. However, Kaiser et al. have not confirmed an association between 1298A>C (alone or in combination with 677C>T mutation) and PE in an Australian population. Lachmeijer et al. also excluded it in a Dutch population. Eva Also-Rall et al. did not find an association between this polymorphism or the combined heterozygosity for the two polymorphisms (677C>T and 1298A>C) and hyperhomocysteinemia or PE.

Cystathionineβ-synthase (CBS)

CBS is one of the key enzymes in the process of folic acid metabolism. It catalyzes homocysteine and serine into cystathionine. The transsulphuration pathway transfers the sulphur atom, originally derived from methionine, via homocysteine to cysteine (Figure 1). This pathway is the main route of disposal of methionine and thus of
homocysteine. Homocysteine may combine with serine to develop the thioether cystathionine by the Vitamin B₆ (pyridoxine) dependent CBS enzyme. Increased homocysteine concentrations after methionine loading may indicate impaired transsulphuration. Gene mutations in some sites can lead to reduced enzyme activity and increased homocysteine in vivo.

Some polymorphisms of the CBS gene had also been associated with mild hyperhomocysteinemia. Sebastio et al and Tsai et al reported the 844ins68 polymorphism in the CBS gene was associated with homocysteine levels. Kim et al did not find an increased risk of PE in pregnant women with this insertion. Eva et al found no association between the 844ins68 CBS polymorphism and PE. There were other two polymorphisms of the CBS gene: 699C > T and 1080C > T. Both were studied by Eva et al, however, no association between the two polymorphisms and the development of PE was found.

**Methionine synthase (MTR)**

The vitamin B₁₂-dependent MTR may catalyze methionine biosynthesis from homocysteine. In this reaction, a methyl-group from methyl-THF is transferred to cobalamin to generate methylcobalamin and THF. The methyl-group of methylcobalamin is then transferred to homocysteine to produce methionine and regenerate cobalamin.

A polymorphism at bp 2756 in the MTR gene my converts an aspartate to a glycine residue. This variant, however, is much less common than the MTHFR variants, with homozygosity frequencies of approx 4%. Eva et al demonstrated that the 2756A > G polymorphism of the MTR gene had no association with PE, which had been evaluated in neural tube defects (NTD) before.

**Methionine synthase reductase (MTRR)**

MTRR also plays an important role in folate-homocysteine metabolism. The prevalence of the 66G > A polymorphism of MTRR has been evaluated in NTD. Eva et al found that the 66G > A polymorphism of MTRR had no association with PE. Moreover, two new polymorphisms in the MTRR gene, IVS1+766G > A and IVS1+754A > C, were also reported to have no association with PE.

In conclusion there is limited evidence confirming a relationship between gene polymorphisms and PE. However, further prospective studies are required before firm conclusions can be made.

**FOLIC ACID SUPPLEMENTATION AND PREECLAMPSIA**

**Protective effects of folate supplementation on preeclampsia**

In recent years, several studies had reported the protective effects of folate supplementation on PE. Merchant et al conducted a randomized trial to evaluate
the effect of multivitamin (20 mg thiamine, 20 mg riboflavin, 25 mg B6, 50 microg B12, 500 mg C, 30 mg E, and 0.8 mg folic acid) and vitamin A supplements (30 mg beta-carotene plus 5000 IU preformed vitamin A) on hypertension in pregnancy (systolic BP \( \geq 140 \) mm Hg or diastolic BP \( \geq 90 \) mm Hg at any time during pregnancy) in 955 human immunodeficiency virus (HIV)-positive pregnant Tanzanian women. They found that women who received multivitamins were 38\% less likely to develop hypertension during pregnancy than those who received placebo (RR = 0.62, 95\% CI 0.40–0.94) while no such effect was found in women who received vitamin A (RR = 1.00, 95\% CI 0.66–1.51). The outcome measure in this RCT was very heterogeneous and included all forms of hypertension in pregnancy. The trial intervention included other vitamins not just folic acid, and there was a baseline supplementation of 5 mg folic acid in both trial and placebo arms which may have diluted the effect. Despite these limitations, the results of this RCT of folic acid as a co-intervention in HIV-positive patients, were consistent with findings from observational studies.

Epidemiological studies found that supplementation of multivitamins containing folic acid were associated with reduced risk of PE. Bodnar et al\(^{114}\) examined the association between regular use of multivitamins containing folic acid at <16 weeks’ gestation and the risk of PE in 1,835 women in Pittsburgh, Pennsylvania between 1997–2001. They found that regular use of multivitamins containing folic acid was associated with a 45\% reduction in PE risk compared with nonusers (OR = 0.55, 95\% CI 0.32, 0.95). Using data collected from 2,100 women with non-malformed infants in a case-control study conducted in the United States and Canada who participated in the Sloan Epidemiology Center Birth Defects Study between 1993 and 2000, Hernandez-Diaz et al\(^{115}\) observed that the multivariate-adjusted relative risk of developing gestational hypertension during the month after supplementation of multivitamins containing folic acid, compared with not using folic acid during that same month, was 0.55 (95\% CI 0.39, 0.79). In the OaK Birth Cohort data, Wen et al\(^{70}\) found that supplementation with \( \geq 1.0 \) mg folic acid or multivitamins containing \( \geq 1.0 \) mg folic acid in the early second trimester was associated with increased serum folate, lowered plasma homocysteine, and a 63\% reduction in the risk of PE\(^{70}\).

Most women in these studies had supplementation of multivitamins containing folic acid. There are three reasons to suggest that folic acid may have played a more important role in preventing PE than other vitamins. First, recent randomized controlled trials (RTC) found that supplementation with vitamin C and E during pregnancy, at doses many times higher than those used in our study, had no protective effect on PE\(^{116,117}\). Second, in the clinical trial of supplementation of multivitamins containing 0.8 mg folic acid in HIV-positive women, a 38\% reduction in the rate of gestational hypertension (including PE) in the intervention group was observed\(^{113}\). Third, in a sub-group of women who had supplementation of folic acid alone (\( n = 421 \)), Wen et al\(^{70}\) observed a statistically non-significant trend towards a protective effect on PE.

In another study, Wen et al\(^{70}\) compared the occurrence of PE in pregnant women exposed to folic acid antagonists and non-exposed women (matched by year of
childbirth, type of institute at birth, and mother’s residence (postal code), using the 1980 to 2000 Canadian province of Saskatchewan databases. A total of 14,982 exposed women and 59,825 non-exposed women were included in the analysis. The risks of PE (adjusted OR 1.52, 95% CI: 1.39, 1.66) and severe PE (OR: 1.77, 95% CI: 1.38, 2.28) were increased in mothers with folic acid antagonists exposure. Supplementary analyses by tight matching with propensity scores, restricting study subjects to first and second trimester exposure and to specific categories of folic acid antagonists yielded similar results. Folic acid antagonists include a broad spectrum of drugs with various clinical indications ranging from epilepsy and mood disorders to urinary tract infections, with a common mechanism of depleting maternal folate and impairing maternal folate metabolism. Findings that maternal exposure to folic acid antagonists may increase the risk of PE adds weight to the evidence that folic acid supplementation may decrease the risk of PE.

Folic acid supplementation has been recommended at a dose of 4.0 mg for women with a previous pregnancy complicated by neural tube defects by the federal government of Canada\textsuperscript{118}. The Society of Obstetricians and Gynaecologists of Canada (SOGC) recommend (5.0 mg) in targeted populations (including women with epilepsy, relevant family history, high-risk ethnic groups, or women without obviously increased risk but with poor compliance to life-style changes for healthy pregnancy. The SOGC also recommend the federal agency change their policy on food fortification (with an increase from 140 mg/100 g to 300 mg/100g folic acid flour fortification)\textsuperscript{119}. No short term adverse outcome associated with folic acid supplementation in pregnancy at the recommended dosage has been reported. Czeizel and Tomcsik\textsuperscript{120,121} demonstrated no adverse outcome in women who took very high doses of folic acid in suicide attempts\textsuperscript{120,121}. As folic acid is an essential coenzyme in purine and thymine nucleotide biosynthesis and hence DNA and RNA metabolism, there have been concerns that folic acid may stimulate initiation or promotion of cancers such as colorectal cancer. Findings from animal experiments and human studies of the relationship between folic acid supplementation and colorectal cancer are controversial; some studies reporting a protective effect while other studies show a potential causative effect\textsuperscript{122,124}. The effect of long term folic acid supplementation for cancer prevention (usually many years) may be quite different from the effects of short term folic acid supplementation for PE prevention (usually a few months). Charles et al\textsuperscript{125} followed up participants from a clinical trial of folic acid supplementation in pregnancy and found a non-significant increase in the risk of breast cancer deaths in the two supplementation groups (0.2 and 5.0 mg folic acid/day) as compared with the placebo group. This is a short report with little description of the study population and research methodology. The number of deaths was small, the confidence intervals were wide, and the authors had not pre-specified the hypothesis that taking folic acid supplementation in pregnancy would increase the risk of cancer. In the accompanying commentary, Oakley and Mandel\textsuperscript{126} suggested that the most likely explanation for the reported association was chance. In contrast, a number of other studies have reported that folic acid supplementation was associated with lower risk of breast cancer\textsuperscript{126,127}. 
Figure 2  Schematic of different proposed mechanisms of action by which folic acid may decrease the risk of developing preeclampsia

Rationale of preeclampsia prevention by folic acid supplementation

There are several biological mechanisms that may explain the protective effect of folic acid on PE [Figure 2]. The current paradigm of the pathophysiology of PE is one of a two-stage disorder. Supplementation of large doses of folic acid in early gestation may work at both stages of PE development.

Folic acid may improve early placentation

The placenta develops from a single cell to a complex organ with a weight of about 500 grams. Adequate cellular folate supply, which is essential for DNA and protein synthesis, plays an important role in the implantation and development of the placenta. Folic acid from food intake and routine supplementation may be sufficient during the periconception period. Larger dose of folic acid may be needed in early gestation [late first trimester or early second trimester] when the growth and development of the placenta are at their peak, especially for women at higher risk of developing PE\textsuperscript{128}.

Folic acid may improve systemic endothelial function

Folate may reduce the risk of developing PE by improving systemic endothelial function. Nitric oxide (NO) is an important protective molecule in the vasculature and endothelial NO synthase (eNOS) is responsible for most of the vascular NO produced. Laboratory investigations have shown that folic acid can improve eNOS coupling and decrease superoxide and peroxynitrite production\textsuperscript{129}. Folic acid deficiency, even when mild, is proatherosclerotic\textsuperscript{130}. Diabetes is associated with endothelial dysfunction,
which in part may be related to uncoupling of the endothelial NO synthase enzyme, thus reducing the availability of NO. Folic acid supplementation normalizes endothelial dysfunction in patients with diabetes.\textsuperscript{131,132}.

\textit{Folic acid reduces plasma homocysteine}

Another possible mechanism of folic acid reducing the risk of developing PE is through lowering blood homocysteine. Hyperhomocysteinemia is a known risk factor for adult vascular disease\textsuperscript{133} and there are similarities between systemic vascular dysfunction and placental vascular dysfunction\textsuperscript{134}. Endothelial dysfunction is demonstrable within the myometrial arteries of women with PE, and the incubation of healthy vessels with plasma from women with PE induces similar endothelial changes\textsuperscript{135}. Young women with hyperhomocysteinemia may be prone not only to systemic endothelial dysfunction but also placental endovasculature dysfunction\textsuperscript{102}. Folic acid supplementation can substantially lower blood homocysteine levels in healthy subjects and patients with kidney disease\textsuperscript{136–139}.

\textit{Women at high risk for PE may have greatest benefit from folic acid supplementation}

Wen et al\textsuperscript{70} demonstrated a clear dose-response relationship between folic acid supplementation and PE risk in women with additional risk factors in their preliminary analysis of OaK Birth Cohort data. The results suggested that a high dose may be needed for these women because of existing placental, endothelial, and metabolic defects (including those of folate metabolism).

\section*{CONCLUSION}

Preeclampsia is a multisystem disorder of pregnancy that occurs at a high incidence in all populations. It is caused by a combination of factors. A 2-stage model of pathophysiology is generally accepted: the first stage relates to reduced placental perfusion leading to the maternal syndrome in stage 2. However, a complete explanation for the aetiology and pathogenesis is still missing. Further studies on immune imbalance, susceptibility genes, endothelial cell impairment and the relationship between placental trophoblast ischaemia and oxidative stress are needed.

Although gene polymorphisms in the four key enzymes involved in the folate-homocysteine metabolic pathway (MTHFR, CBS, MTR and MTTR) have been studied in terms of the relationship with PE, there is no persuasive evidence suggesting a clear association. Further investigations in this area also need to be conducted in the future.

Several epidemiological studies suggest that folic acid supplementation has protective effects against PE, while the exact mechanisms remain unclear. These most likely involve an improvement in early placentation and systemic endothelial function, and a reduction in plasma homocysteine. Animal models provide little
insight into the relationship between folic acid and PE, because of their limited applicability to the human disease. Therefore, large scale randomized controlled trials are needed to prove (or disapprove) the potential protective effect of folic acid supplementation on PE.

REFERENCES


