A prospective study evaluating the association of specific risk factors with the development of preeclampsia.

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Declaration

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ABSTRACT

OBJECTIVE:

To determine the association, if any, between certain risk factors for preeclampsia (PE) and the subsequent development of PE in a local cohort of South African women.

DESIGN:

A prospective study of women with any risk factor for PE for the duration of their pregnancy.

METHOD:

A prospective study was performed on selected women referred to Tygerberg Hospital, High-risk antenatal clinic, with specific risk factors for the development of PE. A history-based questionnaire was completed by participants at enrolment and biophysical parameters were recorded at each subsequent antenatal visit, admission and at confinement. All data was captured according to the attached data sheet. All information was kept confidential and was strictly for the purposes of this study.

STATISTICS:

Statistics were performed using the SPSS statistics software version 17.0. Pearson Chi-square test of independence was used for correlating maternal historical factors, mean arterial pressures (MAP) and cardio-metabolic risk with the development of PE in the index pregnancy. p values were considered significant at values < 0.05.

RESULTS:

The incidence of PE in the cohort was 11.4%. The age of the patient, parity, history of diabetes mellitus, MAP at booking and MAP at 24 weeks showed no significant association with the development of PE at any gestational age. There was a significant association between a history of PE in a previous pregnancy and the development of PE at any gestational age in the index pregnancy ($p < 0.05$). There were a significantly higher number of women with a history of chronic hypertension that developed PE in the index pregnancy ($p < 0.0001$). There were also a significantly greater number of obese women that developed PE at any gestational age in the index pregnancy ($p < 0.05$). The presence of any one or, a combination of cardio-metabolic risk factors was significantly associated with the development of PE at any gestational age ($p < 0.005$).

CONCLUSION:

The changing cardio-metabolic milieu, paralleling the pandemic in obesity, may be impacting on the development of PE. Screening for PE in South Africa may mean; validation of historical factors and MAP in prediction of PE in the local population, and if validated, incorporating it with the cardio-metabolic risk profile to triage women to appropriate levels of care. The pre-pregnancy, antenatal, postpartum and inter-pregnancy intervals can be used to optimise cardio-metabolic risk factors and these women, at the end of their reproductive careers, may benefit from life-long surveillance for cardiovascular disease.

Opsomming

Doel:

Om die assosiasie, indien enige, te bepaal tussen sekere risikofaktore vir pre-eklampsie (PE)[1] en die daaropvolgende ontwikkeling van PE in ‘n lokale kohort Suid-Afrikaanse vroue.

Ontwerp:

‘n Prospektiewe studie van vroue met enige risikofaktor vir PE vir die duur van hul swangerskap.

Metode:

‘n Prospektiewe studie is uitgevoer op geselekteerde vroue wat na Tygerberg Hospitaal hoë risiko voorgeboortekliniek verwys is met spesifieke risikofaktore vir PE. ‘n Geskiedenis gebaseerde vraelys is met werwing voltooi en biofisiese parameters is met elke daaropvolgende besoek, toelating en verlossing voltooi. Alle data is volgens die datablad vasgelê. Alle inligting is vertroulik hanteer en was spesifiek vir die doeleindes van die studie.

Statistiek:

Statistiese ontleding is met SPSS sagteware weergawe 17 uitgevoer. Pearson chi-kwadraat toets is gebruik om moederlike historiese faktore, gemiddelde arteriële druk (MAP) en kardio-metaboliese risiko met die ontwikkeling van PE in die indeks swangerskap te korrelleer. p-waardes < 0.05 is as betekenisvol beskou.

[1] PE = pre-eklampsie

Resultate:

Die insidensie van PE in die kohort was 11.4%. Die ouderdom van die pasiënt, pariteit, geskiedenis van diabetes mellitus, MAP met bespreking en MAP teen 24 weke swangerskapsduurte had geen betekenisvolle assosiasie met PE op enige swangerskapsduurte nie. Daar was ‘n betekenisvolle assosiasie tussen ‘n geskiedenis van PE in ‘n vorige swangerskap en die ontwikkeling van PE op enige swangerskapsduurte in die indeksswangerskap ($p < 0.05$). Daar was betekenisvol meer vroue met ‘n geskiedenis van chroniese hipertensie wat PE ontwikkel het in die indeksswangerskap ($p < 0.0001$). Daar was ook betekenisvol meer obees vroue wat PE in die indeksswangerskap ontwikkel het ($p < 0.05$). Die teenwoordigheid van een, of ‘n kombinasie van kardio-metaboliese risikofaktore was betekenisvol geassosieer met die ontwikkeling van PE op enige swangerskapsduurte. ($p < 0.05$).

Gevolgtrekking:

Die veranderende kardio-metaboliese milieu, met die pandemie van obesiteit, mag impak op die ontwikkeling van PE. Sifting vir PE in Suid-Afrika mag beteken: bevestiging van historiese faktore en MAP in die voorspelling van PE in die plaaslike bevolking en indien bevestig, die insluiting van kardio-metaboliese risikoprofiel om vroue na toepaslike vlakke van sorg te stuur. Die pre-swangerskap, voorgeboortelike, postpartum, en inter-swangerskap intervalle kan gebruik word om kardio-metaboliese risikofaktore te optimaliseer en hierdie vroue mag aan die einde van hul reproduktiewe lewe baat by lewenslange opvolg vir kardiovaskulêe siekte.

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CHAPTER 1

INTRODUCTION

The hypertensive disorders of pregnancy (HDP) are common. According to population-based data, 7–9% of pregnancies are complicated by hypertension[1]. Preeclampsia (PE) complicates approximately 3-5% of pregnancies worldwide[2].

HDP continues to rank among the top five causes of maternal death in South Africa (SA). HDP has been identified as the most common disorder causing morbidity, accounting for 26% of severe acute maternal morbidity[3]. In addition, reports have confirmed that hypertension is the commonest reason for obstetric referral to an intensive care unit[4,5]. A review of all stillbirths and early neonatal deaths reported by the World Health Organisation (WHO) Calcium supplementation trial for the prevention of PE, conducted at centres in seven developing countries, showed hypertension to be the second commonest obstetric event leading to perinatal deaths, accounting for 23.6% of all perinatal deaths[6]. HDP, and in particular PE, remain a leading cause of adverse maternal and fetal outcomes[7-9].

HDP is heterogeneous in its classification and diagnostic criteria. There has never been consensus on the classification and diagnostic criteria for HDP. This has made comparison of data from different centres difficult and has negatively impacted on the body of evidence available to guide clinical management. The International Society for the Study of Hypertension in Pregnancy (ISSHP) attempted to ameliorate the problem by appointing a committee that reviewed available classifications and endorsed and published an international recommendation for how these disorders should be classified and diagnosed in pregnancy[10]. Since publishing this classification in 2001, much has changed in the realm of PE; its

proposed aetio-pathogeneses, models for screening and proposed preventative strategies. The ISSHP has since revised the classification, diagnosis and management of HDP[11].

Despite on-going research in the field of PE, the aetio-pathogeneses of this disease remains incompletely understood. Many theories have been proposed including; impaired trophoblastic differentiation and invasion, placental and endothelial dysfunction, immune maladaptation to paternal antigens and an exaggerated systemic inflammatory response[12]. PE, due to its heterogeneous nature, makes elucidation of the precise aetio-pathogeneses complex. The absence of precise aetio-pathogeneses has negatively impacted on the formulation of effective preventative strategies against the development of PE. Current interventions are being used in an attempt to correct theoretical pathophysiological abnormalities. Two such interventions are maternal dietary calcium supplementation and low-dose aspirin (LDA) prophylaxis. The reduction in the burden of disease with these prophylactic measures can be described as modest.

We have also come to learn that PE has more than one phenotype and the disease has recently been described as 'Pre-eclampsia: one name, two conditions'[13]. A case has been made that PE presenting earlier in gestation differs in several ways compared to that which presents later in the pregnancy and it may actually be different disease entities. Evidence from epidemiological observations, clinical trials and biological studies seem to suggest that there may be different origins to PE presenting at different gestational ages with the early form of PE being associated with poor placentation and the late form of the disease being linked to an unfavourable maternal metabolic milieu[13,14].

Having proposed different origins and phenotypes for PE it is very likely that prediction models, preventative measures and management strategies of the early versus the late forms of the disease may also differ.

The disease entity remains unchanged but our fundamental understanding of the condition has changed and this requires a change in our mind-set to better manage this condition.

CHAPTER 2

DEFINITIONS AND CLASSIFICATIONS OF HDP

As previously alluded to; one of many factors contributing to the heterogeneity of HDP, and in particular PE, are the varying definitions and classification systems of the disease. The lack of consensus in this regard has retarded efforts to make research on the disease universally comparable and applicable. Steegers *et al.* reviewed various classification frameworks and found the main differences were: (1) inclusion or exclusion of complicated non-proteinuric gestational hypertension (GH) as PE; (2) differentiation between clinical and research definitions; (3) use of early-onset PE as a severity criterion; (4) clinical importance of assessing white-coat hypertension; and (5) definition of severe hypertension[8].

The ISSHP in an effort to reach universal consensus on the definition and classification of HDP has updated its previous recommendation on how these disorders should be classified in pregnancy[10]. It has taken into account various existing classification systems, changes in clinical practice, recent developments and the growing knowledge of the aetio-pathogeneses of the disorder.

The revised classification of HDP according to the ISSHP is as follows:

1. Chronic hypertension,

2. Gestational hypertension,

3. Preeclampsia – de novo or superimposed on chronic hypertension,

4. White coat hypertension[11].

Definitions:

PE and gestational hypertension are characterised by the new onset of hypertension (>140 mmHg systolic or >90 mmHg diastolic) after 20 weeks gestation[15.]

GH is defined as the de novo development of high blood pressure after 20 weeks gestation, without any of the abnormalities that define PE, as listed below[11].

Chronic hypertension refers to high blood pressure predating the pregnancy[11].

PE refers to de novo hypertension present after 20 weeks gestation and the coexistence of one or more of the following new-onset conditions:

1. Proteinuria (spot urine protein/creatinine >30 mg/mmol [0.3 mg/mg] or >300 mg/day or at least 1 g/L[‘2 + ’] on dipstick testing)
2. Other maternal organ dysfunction:
    - renal insufficiency (creatinine >90 µmol/L)
    - liver involvement (elevated transaminases – at least twice upper limit of normal ± right upper quadrant or epigastric abdominal pain)
    - neurological complications (examples include eclampsia, altered mental status, blindness, stroke, or more commonly hyper-reflexia when accompanied by clonus, severe headaches when accompanied by hyper-reflexia, persistent visual scotomata)
    - haematological complications (thrombocytopenia –platelet count below 150,000/dL, DIC, haemolysis)
3. Utero-placental dysfunction
    - Fetal growth restriction[11].

Pertinent points to emphasise about this classification are:

- the demonstration of significant proteinuria is not a prerequisite for the diagnosis of PE,
- there is no grading of hypertension into severe and non-severe forms,
- hypertension in a patient who initiates antenatal care after 20 weeks of gestation with no previous blood pressure recordings shall be managed as GH or PE and investigations undertaken postpartum to exclude pre-existing chronic hypertension,
- fetal growth restriction represents placental dysfunction and therefore forms part of the spectrum disorder of PE and,
- the diagnosis of PE requires selected laboratory investigations. This may not be readily available in low resource settings which may result in the overburdening of higher levels of health care with referrals that in retrospect may have been unnecessary (particularly pertinent in a South African setting).

This classification is by no means complete and unfortunately global consensus on a classification framework may never be achieved in the face of an evolving understanding of the disease process and dynamic research into prediction models, prophylaxis and optimal treatment strategies.

CHAPTER 3

AETIO-PATHOGENESES

Central to the understanding of current theories of the aetio-pathogeneses of PE is the appreciation that there is more than one phenotype of the disease. The evidence from biological, clinical and epidemiological studies[16-23] support the view of essentially two phenotypes: placental, which usually occurs early in pregnancy and is associated with poor early placentation of different severity manifesting as a clinical spectrum of PE and/or restricted fetal growth; and maternogenic, which generally occurs late in pregnancy and it is not related to placental insufficiency and fetal growth restriction. Endothelial dysfunction is the final common pathway of the two postulated entities. The literature distinguishes early from late disease based on the gestational age at diagnosis, or, in some cases, gestational age at delivery with 34 weeks being the most used cut-off[8].

a) **Early PE**

1. Normal pathophysiology

1.1. The intervillus circulation

The implantation of the embryo into the decidua occurs in a fairly avascular environment[24]. During this period extensive cytotrophoblastic proliferation occurs as opposed to cytotrophoblastic differentiation which is thought to be a process dependent on high oxygen tension[25]. The increased proliferative activity is thought to result in villus plugging of the terminal spiral arteries, inhibiting inter-villus perfusion. Remodelling of the maternal spiral arteries occurs between 8-10 weeks of

gestation and results in cytotrophoblastic unplugging and perfusion of the intervillus space. Increased oxygen delivery results in increased oxygen tension with consequences of oxidative stress and conversion of the cytotrophoblast into an invasive phenotype[24,25].

1.2. Remodelling of the Spiral Arteries

Pre-pregnancy the spiral arteries are characteristically small, muscular arteries, richly innervated and sensitive to humoral and neural signals[23]. Between 10 and 20 weeks of gestation, these vessels are normally transformed, with a 5-10 fold increase in their intra-luminal diameter and elimination of the musculo-elastic components of the vessel wall resulting in flaccid and dilated vessels[26,27]. They lose all sphincteric function and their ability to respond to humoral and neural signals are blunted[23,28].

2. Proposed pathophysiology in early PE

2.1 Remodelling of the spiral arteries.

In women with early PE the spiral arteries fail to remodel and appear to maintain their pre-pregnancy characteristics. The consequences of this may be failure of the cytotrophoblastic unplugging with hypo-perfusion of the intervillus space but, more importantly, lack of spiral artery remodelling may fail to reduce the velocity of blood delivered to the intervillus space[23]. Burton *et al.* describe the major impact of the terminal dilatation of the spiral arteries is to reduce the velocity of the blood delivered to the intervillus space. This reduced velocity is essential to protect the delicate placental villi, which in the hemochorial placenta are in direct contact with the spiral artery blood flow. The reduced velocity of perfused blood is also necessary to allow time for the oxygen extraction. The potential consequence of failed terminal dilatation

is villus damage with an increased shedding of villus fragments into the maternal circulation and reduced oxygen extraction from the intervillus space. The villus damage is also proposed to lead to the release of procoagulants into the intervillus space with activation of the coagulation cascade, vascular occlusion, and placental infarction[23]. It has been postulated that the reduced oxygen extraction with the failed vascular remodelling, makes it highly possible that with an ischemic placenta, the blood in the intervillus space may actually be hyperoxic. The abnormalities in response to failed remodelling also result in oxidative stress secondary to a hypoxic reperfusion scenario[29].

What is the aetiology of lack of remodelling of the spiral arteries? This question remains unanswered. One theory is based on the finding of reduced trophoblastic invasion together with failed spiral artery remodelling, making it likely that spiral artery remodelling is dependent on material produced by the trophoblast[26]. Another theory is based on the maternal-fetal immunological interaction.

The hemochorial placentation brings the genetically different mother and fetus in intimate vascular contact with each when the trophoblastic cells invade the maternal spiral arteries[26]. This creates a potential immunological conflict between the mother and fetus. This theory is supported by the well-recognized protective effect of maternal exposure to paternal antigen prior to pregnancy. This theory would explain why primigravidae without prior exposure to fetal (paternal) antigens as part of the normal pregnancy associated intermingling of maternal and fetal (paternal) tissues, pregnancies occurring after only a brief exposure to paternal antigen, and the use of barrier contraception are associated with increased rates of PE[30].

The case for angiogenic and anti-angiogenic factors playing a role in the defective trophoblastic invasion, differentiation and spiral artery remodelling is poorly supported. It is proposed that the angiogenic factors, placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), act to stimulate remodelling of the spiral arteries. It may superficially appear to play a causal role in defective early placentation, implicated in the pathogenesis of PE, as a relationship has been demonstrated between low PlGF concentrations and high concentrations of the decoy receptor for VEGF and PlGF, soluble fms-like tyrosine kinase 1 (s-Flt1), in PE, however the chronological sequence does not fit well to support this hypothesis. The higher concentration of s-Flt1 is not present until after the remodelling of the spiral arteries is proposed to take place[31], while the concentrations of PlGF are minimally different at this time[32].

Other suggested mediators of vascular remodelling and trophoblastic invasion based largely on associations and in vitro experiments include uric acid and several cytokines and cytokine antagonists[33,34]. Ultimately, the pathogenesis of defective early placentation in PE remains to be fully understood.

2.2 The effects of defective early placentation

The main result of the defective placentation described above is that it renders the placenta ischaemic. There are both local and/or systemic consequences that ensue.

2.2.1 Local Effects

2.2.1.1 Oxidative Stress

As alluded to above, blood in the intervillus space is relatively hyperoxic, which exposes the fetal surface of the placenta to increased oxygen concentration, while at the same time, reduced extraction renders the majority of the placenta hypoxic. It is proposed that this combination and the residual responsiveness of the incompletely remodelled spiral arteries result in the generation of oxidative stress[35]. Oxidative stress occurs when the concentration of reactive oxygen species exceeds the buffering capacity of available antioxidants[36]. As a result of oxidative stress, the concentration of free radicals increases and free radicals associated with reactive oxygen species are extremely toxic and damage protein, lipids, and nucleic acids[36,37]

2.2.1.2 Endoplasmic reticulum (ER) stress

ER stress is a pathophysiological phenomenon closely linked with oxidative stress[38]. ER stress is a cellular mechanism to reduce protein synthesis in settings in which nutrient and oxygen delivery are insufficient to fully process proteins, resulting in a characteristic response, the "unfolded protein response" (UFR). With UFR, protein synthesis stops, the mechanism proposed for the genesis of small placentas with fetal growth restriction[37]. Under conditions of extreme stress, UFR can result in cellular apoptotic death. The release of inflammatory mediators, generation of reactive oxygen species, reduced protein synthesis, and the induction of apoptotic cell death have local as well as a systemic impact[38].

2.2.2. Systemic Effects

The two-stage model of PE is that early defective placentation associated with failed spiral artery remodelling results in the release of placental products into the maternal circulation, which induces the pathophysiological features of PE. Oxidized lipids formed as a result of oxidative stress are incorporated into the membrane of the syncytiotrophoblast. The associated apoptotic effect of ER stress with the necrotic effects of oxidative stress and hypoxia augment structural changes secondary to oxidation to facilitate shedding of syncytiotrophoblast microparticles (STBM) into the maternal circulation. The STBM from the placenta associated with failed spiral artery remodelling contain oxidized lipids that are proposed to transfer oxidative stress systemically. In addition, in PE, an increased number of the particles that are released are necrotic rather than apoptotic and have toxic cellular effects that are not present with apoptotic particles[41,42].The large number of STBM induce a more pronounced inflammatory response exacerbated by the qualitative changes of the particles that may also have additional toxic properties. Angiogenic and anti-angiogenic factors produced by the placenta are also influenced by hypoxia and oxidative stress[43].The anti-angiogenic factor- s-Flt1 is increased with hypoxia[43]. This decoy receptor sequesters the angiogenic factors, VEGF and PlGF, preventing interaction with their receptors[44]. It is also likely the PlGF production is also reduced since the circulating concentration of PlGF is lower in women destined to be pre-eclamptic prior to the increase in s-Flt1[45].

b) **Late PE**

Normal and preeclamptic pregnancies have been shown to be systemic pro-inflammatory states with features similar to those seen in sepsis[18,46]. Endothelial cells play a key role in intravascular inflammation by producing pro-inflammatory cytokines and stimulating leukocytes, platelets and other humoral components[47,48]. PE represents the exacerbation of these phenomena and the physiological changes that are present at term gestation closely resemble those seen in women with PE[18]. Factors proposed to mediate the inflammatory changes include: sFlt1 and STMP among others. They can be detected in the plasma of normal pregnant women and in even higher concentrations in the plasma of women with early PE[49], but not usually those with late PE.

What mediates the exaggerated systemic inflammatory response in late PE? It has been hypothesized that the presence of low-intensity pro-inflammatory factors derived from the placenta may exacerbate pre-existing low-grade chronic systemic inflammation caused by obesity or other conditions, resulting in a clinical condition termed maternogenic (late) PE[18]. Borzychowski *et al*., describe a concept of maternal PE as hypertension and proteinuria occurring in "women with normal placenta, susceptible to systemic inflammation"[18].

How does obesity and other components of the metabolic syndrome predispose to a systemic pro-inflammatory state? Adipose tissue is an active endocrine organ involved in nutrient homeostasis, immune response, blood pressure control, thyroid and reproductive function[19]. Adipocytes produce and release numerous biologically active substances, such as leptin, adiponectin, interleukins, tumor necrosis factor (TNF), interferon-γ and monocyte chemo-attractant protein (MCP)-1, collectively termed adipokines[50]. Leptin and adiponectin, produced by white adipose tissue, have an opposite effect: adiponectin, has anti-inflammatory properties, and is reduced in obesity[51], while leptin, a pro-inflammatory mediator, is

increased in obesity. As a result, cytokines, such as a MCP-1 and leptin, are secreted into the systemic circulation and act as strong chemo-attractant factors for the macrophages[52]. Macrophages present around adipocytes trigger a paracrine interaction loop with adipose cells[53]. Ultimately, this interaction will aggravate inflammatory changes in the adipose tissue and will further contribute to the release of pro-inflammatory cytokines in the circulation[54] resulting in endothelial activation and a systemic inflammatory response. Thus it is postulated that obesity and the metabolic syndrome can result in endothelial dysfunction and a hypertensive disorder, without or with minimal placental involvement. There may be overlap in the preeclamptic phenotypes with one or more of the pathogeneses implicated in a particular patient.

CHAPTER 4

PREDICTION OF PE

Having described the phenotypes of early and late PE and postulated different pathogeneses for the two entities, it would seem logical that the prediction of both phenotypes would also differ. Indeed this is the case, as I shall describe below, but the discussion on the prediction of PE would also be incomplete if the merit of screening for the condition is not elaborated upon.

In 2004, Conde-Agudelo et al.[55] conducted a large systematic review of predictive tests on behalf of the WHO. They identified over 50 screening tests in the published literature, of which many could be discounted because of low predictive values. The remainder were included in a meta-analysis that found that only uterine artery Doppler, anti-phospholipid antibodies and urinary kallikrein were of moderate predictive value in low risk women. The authors concluded that, as of 2004, there was 'no clinically useful screening test to predict pre-eclampsia'. They established desirable criteria for any future screening test:

Simple,

Rapid,

Non-invasive,

Inexpensive,

Easy to carry out early in gestation,

Imposes minimal discomfort or risk,

Widely available technology,

Valid,

Reliable,

Reproducible,

High likelihood ratio for a positive result (>10) and

Low likelihood ratio for a negative result (<0.1)[55].

Since the establishment of these criteria there have been several tests and models proposed for the prediction of PE, but few that satisfy the above criteria and I shall therefore only review the most promising predictive tests in recent published literature.

1. Should we offer universal screening for PE?

   A major role of antenatal care is to detect incipient PE and to prevent its progression[56]. The criteria presented above, proposed for any future screening test for PE, can only be met for screening performed in the first trimester. It is in this gestational window that the disease is in a latent form and is still amenable to the beneficial effects of available prophylactic measures[57-61].

   Proponents for universal screening argue that screening in the first trimester includes a personalized risk estimate that is available to the entire obstetric population, with a 97.5–99.8% certainty that PE will not occur in screen-negative women[62] and the ability to evaluate the impact of preventive therapies on the observed-to-expected PE rate, thus improving the statistical and clinical validity of intervention trials[63-65].
   Opponents to universal screening for PE accede that first trimester screening is useful for the prediction of those who will ultimately progress to PE and require delivery at < 34 weeks of gestation[66]. They cite the following factors in counter argument to universal screening: the screening algorithms have low positive predictive values, ranging between 6% and 10%[66,67] and limited validity in external populations[62,68],

they preferentially predict early-onset disease with severe hypertensive features[69] and there is an apparent absence of effective preventive interventions[66,67].

Baschat proposes that we improve the utility of the first trimester screen by using it to identify treatable conditions in screen-positive women, 'as pre-eclampsia results from the convergence of multiple risk factors, evaluating the characteristics of women in whom prevention fails may point towards additional risk factors that require attention.'[70].

2. Proposed screening strategies

2.1. Maternal historical factors

Factors that affect the risk of developing PE have been recognised since the beginning of the last century and include parity[71], family history of PE[56,60], diabetes mellitus[72], chronic hypertension[73], maternal age[56,74], BMI[75], ethnicity[76] and socioeconomic status[56]. However it has only recently been possible to statistically estimate the individual risk that is attributable to each of the factors.[60,77,78]. The National Institute for Health and Clinical Excellence (NICE) guideline on routine antenatal care, recommends that at the booking visit a woman's level of risk for PE, based on factors in her history, should be determined and the subsequent intensity of antenatal care should be based on this risk[79]. However, there is no reference on the performance of such a recommended screening strategy, which treats each of the risk factors, as separate screening tests with additive detection and false positive rates[80]. Poon et al. showed that this strategy of treating each of the risk factors as separate screening tests would falsely classify two thirds of the obstetric population as high risk and in need of intensive monitoring[80]. They further showed that 'meaningful

screening for hypertensive disorders in pregnancy by maternal history necessitates the use of algorithms derived by logistic regression analysis' and that with such a strategy the estimated detection rates for early-PE, late-PE and GH are about 47%, 41% and 31%, respectively, at a 10% false-positive rate[80]. This study suggested a methodology for deriving the a priori risk for early PE, late PE and GH based on maternal age, BMI, racial origin, history of PE and chronic hypertension and method of conception[80].

2.2. First trimester uterine artery Doppler assessment

As alluded to earlier, one of the key theories in the aetio-pathogenesis of early PE is defective placentation with impaired remodelling of the spiral arteries. Assessment of impedance to uterine artery blood flow by Doppler ultrasound, as first described by Campbell et al.[81] serves as a surrogate marker of the degree to which successful spiral artery remodelling has occurred. Doppler studies of the uterine arteries at 11–13 weeks have demonstrated that impedance to flow is increased in pregnancies that subsequently develop hypertensive disorders and that the increase is particularly marked for early PE[82-84]. The best performance of screening is provided by assessing the uterine artery with the lowest pulsatility index (L-PI)[84]. The estimated detection rate, at a 10% false-positive rate, in screening by a combination of the maternal factor-derived *a-priori* risk with uterine artery L-PI was 81% for early PE, 45% for late PE and 35% for GH[84]. It is as expected that this screening strategy would perform poorly for late PE based on the different aetiopathogenesis postulated for this phenotype.

2.3. Mean arterial blood pressure (MAP)

Various screening strategies for PE have been evaluated using various clinical parameters including diastolic blood pressure, systolic blood pressure, 24 hour ambulatory blood pressure and MAP. Cnossen et al.[85] subjected these methods to a systematic review and found that MAP was a better predictor for PE than systolic blood pressure, diastolic blood pressure or an increase of blood pressure. Second trimester MAP of 90 mmHg or above showed a positive likelihood ratio of 3.5 (95% confidence interval [CI] 2 to 5) and a negative likelihood ratio of 0.46 (0.16 to 0.75)[85]; falling far short of the desirable likelihood ratios for a predictive test[55]. The elevated MAP in pregnancies destined to develop PE may be an indicator of the cardiovascular risk profile of the patient. Once again, for maximum benefit from available preventative strategies for PE, a proposed screening test would have to be performed in the first trimester to be of value clinically. Poon et al.[86] evaluated the performance of MAP at 11 weeks and 13 weeks and 6 days in combination with maternal variables in the prediction of PE and found that testing could, for a 10% false positive rate, identify 62.5% of those who would develop PE and 40% of those who would develop GH[86]. Unlike uterine artery Doppler, no difference was found in the ability of MAP to predict early PE over term disease[86]. More recently Gallo at et al.[87] showed that the performance of screening for PE by MAP is best when measurements are taken at both 11-13 and 20-24 weeks' gestation than at only one of these gestational ranges. In screening at both gestational ages the DR at a FPR of 10%, were 84.3, 65.7 and 52.5%; the DR at a FPR of 5% were 60.0, 49.7 and 37.6%, respectively[87].

2.4. Biomarkers

No individual biomarker has a predictive value sufficient for clinical prediction of PE[88]. Panels of tests have been shown to have better performance in the prediction of PE[88].

2.4.1. Markers of placental function

Serum levels of pregnancy-associated plasma protein A (PAPP-A) and plasma protein 13 (PP-13) are have been shown to be reduced in the first trimester in women destined to develop PE[89-91]. PAPP-A is a protease derived from the syncytiotrophoblast and reduced serum levels may suggest either impaired function or a decreased cellular mass of this component of the placenta. PAPP-A also acts to cleave insulin-like growth factors (IGF) from their specific binding proteins, thereby increasing their mitogenic effect. The IGF family of proteins are believed to play a significant role in extravillus trophoblast migration and invasion, and therefore a reduction in the level of PAPP-A may reduce unbound available IGF at a cellular level and have functional effects[92]. PP 13, also produced by the syncytiotrophoblast, binds to proteins on the extracellular matrix between the placenta and the endometrium and is thought to play a role in placental implantation and maternal artery remodeling[93]. Both markers are of predictive value; however, no additive effect was found in using both, probably because they both assess aspects of placental function[90]. In a combined screening model, PAPP-A in the first trimester was found to be significantly lower in early and late-onset PE, but not GH and contributed to the predictive ability of the model[94].

2.4.2 Angiogenic factors

PlGF and VEGF are angiogenic factors. They play a central role in normal vasculogenesis in early pregnancy and later in normal maternal endothelial function[95,96]. They are inhibited by sFlt1. sFlt1 is a circulating anti-angiogenic protein that adheres to the receptor binding domains of PlGF and VEGF, thus preventing interaction with endothelial cell receptors and leading to endothelial dysfunction[96]. Endoglin (sEng) acts in synergy with sFlt1 to exert their anti-angiogenic action[88]. PlGF levels are lower in the first trimester in women who subsequently develop PE[99]. Free VEGF levels in serum are low as it has a higher affinity than PlGF for binding to sFlt1 making its detection technically difficult and discounting its clinical use as a predictive test[99]. Most studies have not shown any increase in sEng or sFlt1 levels before 16 weeks in women who subsequently develop PE[31,45,97,100].

2.5. Combined screening models

There are many proposed combined screening models described in the literature. I shall elaborate on the most well studied and accepted. Poon et al.[94] evaluated a large population (7797) of women with singleton, first-trimester pregnancies attending routine antenatal care, with an overall incidence of PE of 2%. The predictive model included: maternal variables (e.g. BMI, nulliparity or prior history of PE, ethnic origin), uterine artery Doppler, maternal MAP, PAPP-A and PlGF. For a 5% false-positive rate, the sensitivity and specificity for early PE were 94.1% and 94.3%, respectively. The likelihood ratio for a positive test was 16.5 and the negative likelihood ratio was 0.06, meeting the screening criteria outlined previously.

More recently, Akolekar et al.[101] investigated a model for prediction of PE based on maternal characteristics, biophysical and biochemical markers at 11–13 weeks' gestation in which the gestation at the time of delivery for PE is treated as a continuous variable. They showed that screening by maternal characteristics, biophysical and biochemical markers detected 96% of cases of PE requiring delivery before 34 weeks and 54% of all cases of PE at a fixed false-positive rate of 10%[102]. Wright et al.[60] investigated a screening model for the prediction of PE based on maternal characteristics and biophysical markers at 11–13 weeks' gestation in which gestation at the time of delivery for PE is treated as a continuous variable. They found that screening by maternal characteristics, uterine artery PI and MAP detected 90% of PE cases requiring delivery before 34 weeks and 57% of all PE cases at a fixed false-positive rate of 10%[60].

In summary, it appears as if screening tests need to be performed early in gestation to be amenable to preventative strategies, they perform poorly individually and are better predictors of disease when combined screening strategies are employed, however; their positive predictive values remain low and they are generally better at predicting early than late disease. Sibai, in a recent commentary[66] states that, 'given the poor PPV for PE at < 34 weeks, and the poor detection rates for all cases of GH/PE the clinical implications of a PE screen test in the first trimester remain unclear.'.

Baschat, in a recent editorial[70], offers a novel approach to increasing the clinical utility of the first trimester screening test by not only using it to predict the personalised risk for PE but also to identify treatable conditions in screen positive women.

In the editorial[70], Baschat refers to a study in which the rate of PE, despite the early initiation of LDA, was 9.3%[57], much higher than the general obstetric population. He agrees that LDA does have limited efficiency but postulates that women may be developing PE because they have additional risk factors that are not addressed by LDA. There are studies that indicate that women at high risk for PE, and in whom LDA fails, are more likely to have chronic hypertension, with a higher blood pressure at enrolment, pre-existing diabetes and an increased BMI[64,102]. It would appear that the cardiovascular and metabolic risks for PE are not addressed by current prophylactic measures, as expected from the proposed aetio-pathogeneses, and that these women go on to develop PE.

2.6. Cardiovascular, metabolic and prothrombotic risk profiles

Scholten et al.[103] evaluated the prevalence of cardiovascular, metabolic and prothrombotic risk profiles in women with a prior history of PE. They identified that 77% of women had at least one risk profile. Of these, the cardiovascular risk profile was most prevalent (66.1%), followed by hyperhomocysteinemia (18.7%), metabolic syndrome (15.4%) and thrombophilia (10.8%).

The components included in the prediction rules of first trimester multivariable predictive models are typically categorised as maternal historic factors, maternal physical characteristics, uterine artery Doppler studies and biomarkers[60,67,77,101]. Within these categories multivariate prediction models identify maternal BMI, hypertension, prior PE, MAP, uterine artery Doppler and biomarkers among the top ten independent predictors of PE[101,104-111]. Baschat[70], proposes an alternate way to categorize these screening variables is by their representation of risk profiles. A prior history of hypertension, renal disease and elevated blood pressure can be considered as representative of a cardiovascular risk profile, while increased BMI, prior diabetes

or gestational diabetes represent a metabolic risk profile and a history of thrombophilia represents a prothrombotic one[70]. He suggests that this categorization has the advantage of allowing estimation of the contribution of treatable conditions to a woman's personalized PE[70].

2.6.1. The metabolic risk profile

Pregnancy is a state of relative insulin resistance and each additional component of the metabolic syndrome has been shown to increase the risk of PE by 30-40%[112,113]. Metabolic syndrome features that persist after pregnancy, increases the recurrence of PE up to three-fold with each additional component of the metabolic syndrome present, with hypertension and hyperinsulinemia as the leading risk factors[114]. Theoretically, optimisation of the metabolic profile pre-pregnancy or early in the gestation may decrease the risk of the development of PE and also have long term health benefits for the patient.

2.6.2. The cardiovascular risk profile

As alluded to earlier, several first trimester screening algorithms identify hypertension in the first trimester as the most prevalent and consistently demonstrated, independent risk factor for the development of PE. Thresholds for the treatment of hypertension in the first trimester remain controversial. First-trimester, observational data indicate that high-risk women who are normotensive in the first trimester have a 50% reduction of PE, while those with pre-hypertension or hypertension have a greater than two-fold increased risk[64]. Addressing chronic hypertension prepregnancy or in early pregnancy may be the key to significantly impacting on the development of PE.

CHAPTER 5

PREVENTATIVE STRATEGIES

There have been a host of therapeutic interventions proposed over the decades for the prevention of PE, most with disappointing outcomes. The hope of elucidation of the precise aetiopathogenesis and a better understanding of the disease process holds the hope of discovering more promising prophylactic strategies.

I shall further elaborate on the most successful prophylactic measures to date and also provide an insight into what lies ahead in this field.

1. Low Dose Aspirin

   As previously alluded to, pathologic findings associated with the PE include defective remodelling of the spiral arteries, activation of the clotting system, and imbalances of prostacyclin and thromboxane[115]. Based on these findings, LDA, an antiplatelet agent, thought to restore prostacyclin and thromboxane levels and prevent vasoconstriction, has been targeted as an intervention to reduce PE in at-risk women[116,117]. Large RCTs investigating the benefit of aspirin prophylaxis have demonstrated either a very modest reduction in PE risk[116] or more commonly no significant benefit at all[115, 118-123]. The PARIS Collaboration utilized individual patient data in a meta-analysis that demonstrated a statistically significant 10% reduction in risk[124] and this has been supported by similar findings in a Cochrane review[125]. The validity of meta-analyses on this topic has come into question as it is thought to be limited by the inclusion of small reports subject to publication bias that may over estimate effect size, if present[124]. Bujold et al.[57], in a subgroup meta-analysis, investigated the benefit of

LDA started at 16 weeks or earlier and showed a major benefit with up to 50% reduction in PE and fetal growth restriction. Once again one needs to be wary of bias in subgroup meta-analyses[126].

2. Calcium

Low dietary calcium intake has been associated with an increased risk of developing PE[127]. A large RCT found no benefit from calcium supplementation in terms of risk reduction of the incidence or severity of PE. There were no differences in the number of preterm deliveries, small-for-gestational-age births, or timing of onset of hypertensive diseases[128]. This is in contrast to the RCT conducted by the WHO which showed that in nulliparous women with low dietary calcium intake, calcium supplementation did not reduce the primary outcome of incidence of PE however it did reduce some secondary outcome measures including maternal and neonatal morbidity[129]. It can be concluded that calcium supplementation for PE prophylaxis is not indicated in calcium replete populations but there may be some benefit derived, in terms of reduced maternal and neonatal morbidity, from calcium supplementation in calcium deplete populations.

3. Vitamin C and E

Vitamin C and E have been investigated for the prevention of PE, based on their known antioxidant properties and the proposed theory that PE may arise as a result of a process of oxidative stress. The VIP trial evaluated the supplementation of vitamins C and E in the prevention of PE and found no risk reduction associated with vitamin supplementation[128]. These findings were subsequently confirmed by large RCTs[129,130].

4. Prophylactic interventions under investigation

4.1. Statins

Statins were listed as Food and Drug Administration Category X primarily because of theoretical concerns regarding inhibition of cholesterol formation during fetal development and, in addition, until now there have been no specific indications for use of this class of drugs in pregnancy. However, subsequent data on pravastatin, lovastatin, and simvastatin have not demonstrated these drugs to be associated with increased risk for congenital malformations or adverse outcomes[131]. Statins have demonstrated effectiveness in counteracting the angiogenic imbalance, endothelial damage, oxidative stress, and inflammation implicated in the pathogenesis of PE[132] and this has been supported from animal model research into PE[133,134]. Currently a double-blind, placebo-controlled pilot study is underway to collect safety and pharmacokinetic data for pravastatin use during high-risk pregnancies[135].

4.2. Metformin

The issues surrounding hyperinsulinaemia and insulin resistance could possibly be addressed by the administration of Metformin. A recent meta-analysis of women with polycystic ovary syndrome who continued therapy after conception showed that metformin reduced PE by almost 50%, with a pooled odds ratio of 0.53 (95% CI, 0.30–0.95)[136]. Metformin may also have benefits of an improved uteroplacental circulation as demonstrated in the reduced uterine artery impedance in the second and third trimesters in women taking metformin during pregnancy[137].

4.3.Folate

Higher plasma homocysteine levels, in early pregnancy, increase the risk of PE three to four-fold[138,139]. Increased homocysteine levels are significantly more common in women who develop PE and low dietary folate is an important contributor to this[140]. Modification of homocysteine levels requires high-dose folate and a randomized trial evaluating a daily folate dose of 4 mg is currently underway[141,142].

4.4.Lifestyle modification

Optimisation of risk factors preconception may hold the key to optimising outcomes in pregnancy. In women with diabetes and one factor of maternal vascular disease, there is a 25% risk of PE and fetal growth restriction; good glycaemic control modifies this risk[143]. Obesity is known to be a risk for both PE and gestational hypertension; with morbid obesity (BMI of > 35) the odds ratio for PE is 7.2[144]. Weight loss in overweight and obese women before pregnancy may modify these risks but remains to be demonstrated in well-designed studies.

CHAPTER 6

SCREENING FOR PE IN A SOUTH AFRICAN SETTING

PE is now better conceptualised as a spectrum disorder as it may clinically manifest as more than one phenotype and sometimes with overlap among the various phenotypes. There are currently many theories on the aetio-pathogeneses of PE and again, they vary according the different phenotypes. Many predictive tests have been described but few that meet recommended criteria for a screening test for PE. There has been a gradual move toward combined screening algorithms, to increase detection rates, early in pregnancy, when prophylactic intervention may still have meaningful impact. There are many arguments in favour of, and against the routine screening for PE in pregnancy, but one that stands out is the low positive predictive values of individual and/or combined screening algorithms. Increasing the utility of first trimester screening tests by incorporating cardio-metabolic and thrombotic risk profiling has been proposed. This may have advantages of being able to modify risk factors early in gestation, deciding on the appropriate level of care for the woman, and may translate into optimal health care for long-term cardiovascular health surveillance and early detection and treatment of disease. Unfortunately, there is little in the way of PE prophylaxis available. LDA, and perhaps calcium in certain populations, show the most promising results to date. There are however many trials on-going, looking especially at ways of ameliorating the cardio-metabolic risk associated with the development of PE.

Many factors need to be incorporated when thinking of screening for PE in SA, among them:

- Competing interests for limited resources
- The poor socio-economic make-up of the majority of the population

- The changing face of PE in the background of a population in which 42% of women have been recorded as obese[145].
- The rising cardiovascular and metabolic related morbidities paralleling the rising rate of obesity and impacting on the incidence and phenotype of PE and affecting long term health.

The existing screening models have not been validated in local populations and all the components of suggested screening models may not be feasible in a resource constrained setting. Common practice in many SA health institutions is to refer any patient with historical risk factors for PE to a higher level of care for further ante-natal care. The implication of this practice is that the majority of women will not go on to develop PE resulting in the overburdening of already strained health care services, usually these women are expected to have many health care visits placing greater financial burden on the women and the unnecessary medicalization of a large number of pregnancies. What factors, in a local population of pregnant women, are associated with the development of PE?

We performed a prospective study of women referred to the antenatal clinic at Tygerberg Hospital (a level 2 hospital in the Western Cape, SA), following a positive historical screen for PE, and determined specific pregnancy outcomes based on certain risk factors for PE.

CHAPTER 7

STUDY: A PROSPECTIVE STUDY EVALUATING THE ASSOCIATION OF SPECIFIC RISK FACTORS WITH THE DEVELOPMENT OF PREECLAMPSIA.

Aim:

To determine the association, if any, between certain risk factors for PE and the subsequent development of PE in a local cohort of South African women.

PATIENTS AND METHODS

After institutional ethical approval was obtained, all women referred to Tygerberg Hospital High Risk antenatal clinic, following a positive maternal historical screen for PE, were screened for eligibility for the study. Inclusion criteria were a singleton pregnancy with a positive maternal historical screen for PE (a positive screen included any one of the following: age < 18 years old, age > 37 years old, nulliparity, grande-multiparity, history of PE, chronic hypertension, chronic renal disease, gestational diabetes mellitus (GDM), pre-gestational diabetes mellitus (DM) or BMI ≥ 30) and a gestational age of < 20 weeks (by last menstrual or an early ultrasound). All women with a non-viable pregnancy, early pregnancy loss or intra-uterine fetal death diagnosed at the first antenatal visit were excluded. Women meeting the criteria were informed about the study and written consent was obtained prior to enrolment. All women enrolled in the study (n=299) over the 2 year period were assisted in completing a questionnaire (Appendix) at enrolment regarding their family, social and obstetric history. Their scheduled frequencies of visits to the antenatal clinic were as per standard protocol, and specific data were recorded on a data sheet (Appendix) at each antenatal visit until discharge from hospital, post-delivery.

Data were entered into a database and analysed retrospectively. Data included maternal age, race, parity, history of gestational diseases (PE, GDM), chronic diseases (hypertension, renal disease, DM, and autoimmune diseases), smoking history, BMI on first antenatal visit, MAP at first antenatal visit and at 24 weeks gestation and pregnancy outcomes (development of early PE (onset <34 weeks gestation) and late PE (onset ≥ 34 weeks gestation).

Points studied were the association of maternal historical factors, MAP and cardio-metabolic risk (chronic hypertension and/or chronic renal disease and/or diabetes mellitus and/or obesity) with the development of early PE , late PE and PE at any gestational age in the index pregnancy.

Statistics were performed using the SPSS statistics software version 17.0. Pearson Chi-square test of independence was used for correlating maternal historical factors, MAPs and cardio-metabolic risk with the development of PE in the index pregnancy. *p* values were considered significant at values < 0.05.

CHAPTER 8

RESULTS

The mean age of all women was 31 +/- 6.7 years. Women under the age of 18 years made up 2% of the cohort, while women from 18 years to 37 years of age and women > 37 years of age made up 77% and 21% of the cohort, respectively. The ethnic make-up of the cohort was predominantly Coloured (90.3%) and African (8%). Nulliparous women accounted for 23% of the cohort, women with a parity of 1 – 4 comprised 72% of the cohort and 5% of women had a parity of ≥ 5. Of all women, 21.4% had a history of PE, 17.4% had a history of chronic hypertension and 13.4% of the women were known with DM. There were only 2 women in the cohort with chronic renal disease. 30% of the women had a positive smoking history.

The average gestational age at booking, in the index pregnancy, was 11 weeks and 4 days. The average BMI was 30.38 +/-7.8 kg/m^2, with 49.2% of women being obese (BMI ≥ 30). The proportional representation of women in the various BMI categories is presented in Figure 1 (Appendix). The incidence of PE in the cohort was 11.4% of which 6.7% was early and 4.7% was late PE.

Data of ethnic groups and history of chronic renal disease were not analysed as the specific categories were over and under-represented, in the cohort, respectively. The age of the patient, parity, history of PE, chronic hypertension, DM, obesity, MAP at booking and MAP at 24 weeks gestation showed no significant association with the development of either, early or late PE. The age of the patient, parity, history of DM, MAP at booking and MAP at 24 weeks showed no significant association with the development of PE at any gestational age.

There was a significant association between a history of PE in a previous pregnancy and the development of PE at any gestational age in the index pregnancy ($p < 0.05$). There were a significantly higher number of women with a history of chronic hypertension that developed PE in the index pregnancy ($p < 0.0001$). There were also a significantly greater number of obese women that developed PE at any gestational age in the index pregnancy ($p < 0.05$). Of note is that obese women were more likely to be diagnosed with chronic hypertension in the index pregnancy (n = 24, $p < 0.0001$). The presence of any one or a combination of cardio-metabolic risk factors was significantly associated with the development of PE at any gestational age ($p < 0.005$). Results are presented in Table 1 (Appendix) and Figure 2 (Appendix).

CHAPTER 9

DISCUSSION

A major role of antenatal care is to detect incipient PE and to prevent its progression[56]. In SA, a community-based study found a 12% incidence of HDP in KwaZulu-Natal[146], while a tertiary facility-based study reported a rate of prevalence of 18%[147]. Our study found an incidence of 11.4%, specifically for PE. Most would agree that this represents an important health problem to warrant screening and early intervention. A major challenge in a developing country, like SA, is what would be meaningful, yet feasible, screening and preventative strategies to employ? Major factors to be incorporated when thinking of PE screening strategies in SA are; competing interests for limited resources, poor socio-economic make-up of the majority of the population, the impact of the obesity pandemic on PE (42% of South African women have been found to be obese[145]) and the rising cardiovascular and metabolic related morbidities, paralleling the rising rate of obesity, impacting on the incidence and phenotype of PE and affecting long term health.

The existing screening models have not been validated in local populations and all the components of suggested screening models may not be feasible in a resource constrained setting. Realistically, components of the suggested first trimester PE screen that may be feasible, in the South African public sector, are the maternal historical factors and MAP assessment. Serum biomarkers and uterine artery Doppler screening can be discounted as screening tests locally, mainly due to the high cost implications and need for a large number of expertly trained personel in the field. Pregnancy, for many women in SA, may be their only opportunity to be screened for disease and so apart from using pregnancy to screen for

PE, the screen may serve a greater purpose; to optimise risk factors for the index pregnancy, future pregnancies and for the short and long term health of women in general.

Our study, although not aimed to validate maternal historical factors in predicting the development of PE, does seem to suggest that, with the exception of a history of previous PE, maternal historical factors may not be the most important factors in risk categorising women in our population. This would however need validation in a larger study before any firm conclusions can be drawn. Is obesity with its attendant co-morbidities becoming the prime predictor for adverse pregnancy outcome in our population? Our study underscores the impact of obesity and cardio-metabolic risk on the development of PE. The study has demonstrated significant associations between the presence of one or more cardio-metabolic risk factors and the development of PE. Taking into consideration the pathophysiological mechanism whereby the cardio-metabolic milieu is proposed to influence the development of PE, one may reason that current preventative strategies (LDA and Calcium) may not be as effective in a population like ours and that there are perhaps other risks that need to be addressed. Our study also highlights the burden of cardio-metabolic risk in our antenatal clinic (overweight and obese women, together, making up 72 % of the cohort) and together with its significant association with the development of PE, a setting like ours, may serve as fertile ground for research into disease modifying interventions, like the use of statins, metformin and lifestyle modification.

It is not particularly surprising that none of the risk factors were significantly associated with the development of either early or late PE, when examined as separate entities. With the high prevalence of cardio-metabolic risk factors in the cohort, it was not uncommon to find that women often, had more than one risk profile and often a high risk profile for the development

of early and late PE was present in the same patient. This apparent overlapping of risk profiles would make the prediction of early versus late PE difficult and whether this knowledge (risk of developing early vs. late PE) would contribute further to the care of women in a developing country is debatable, as the women would, in any case, have been triaged to a higher level of care based on her initial positive screen for PE.

CHAPTER 10

CONCLUSION

In conclusion, screening for PE in SA may mean; validation of historical factors and MAP in prediction of PE in the local population, and if validated, incorporating it with the cardio-metabolic risk profile to triage women to appropriate levels of care. Women categorised as high risk can receive LDA and calcium prophylaxis, have their cardio-metabolic risk optimised, and have increased surveillance for the development of PE. The postpartum and inter-pregnancy intervals can be used to further optimise cardio-metabolic risk factors and these women at the end of their reproductive careers may benefit from life-long surveillance for cardiovascular and metabolic disease. We need integrated programs aimed at schooling-going children to prevent obesity and in reproductive aged women to modify their cardio-metabolic risk which may positively impact on the development of PE and its complications, and improve the long-term cardiovascular health of women in general.

APPENDIX

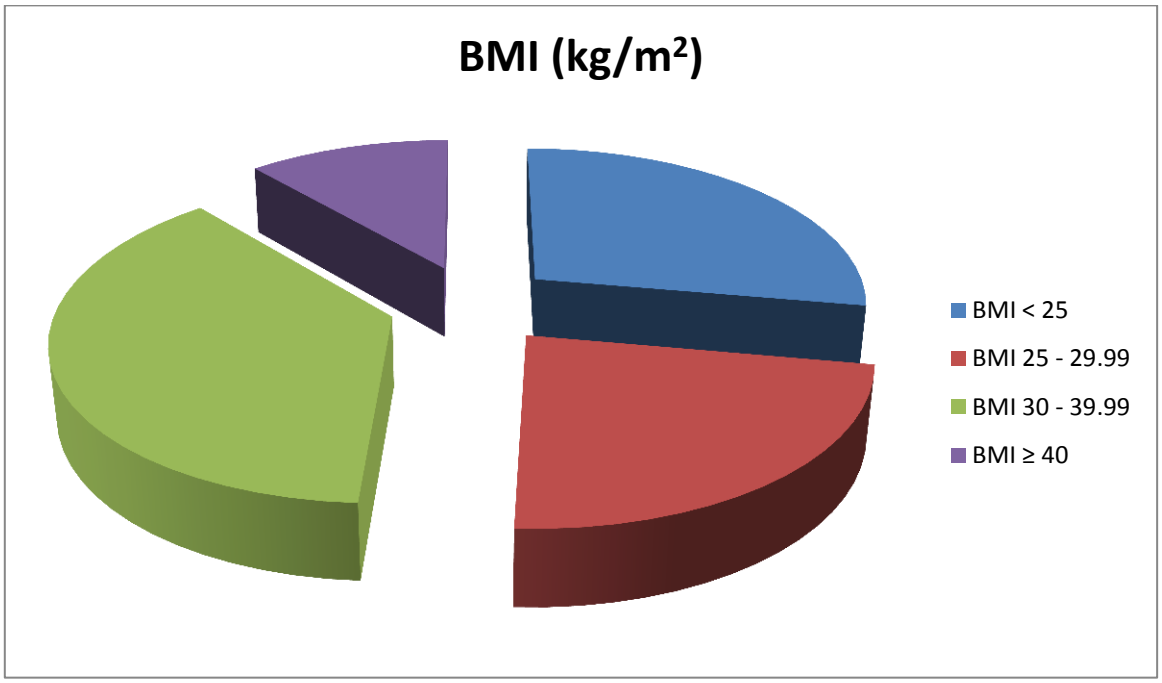

Figure 1.

Proportional representation of BMI categories in the cohort of women.

	Total number of patients (n)	Number who developed PE in the index pregnancy (n)	*p* value
History of PE	64	13	0.01
Chronic hypertension	48	14	0.000
Obesity	147	23	0.02
1 or more cardiometabolic risk factors	177	27	0.003

Table 1.

Association between specific risk factors and the development of PE in the index pregnancy.

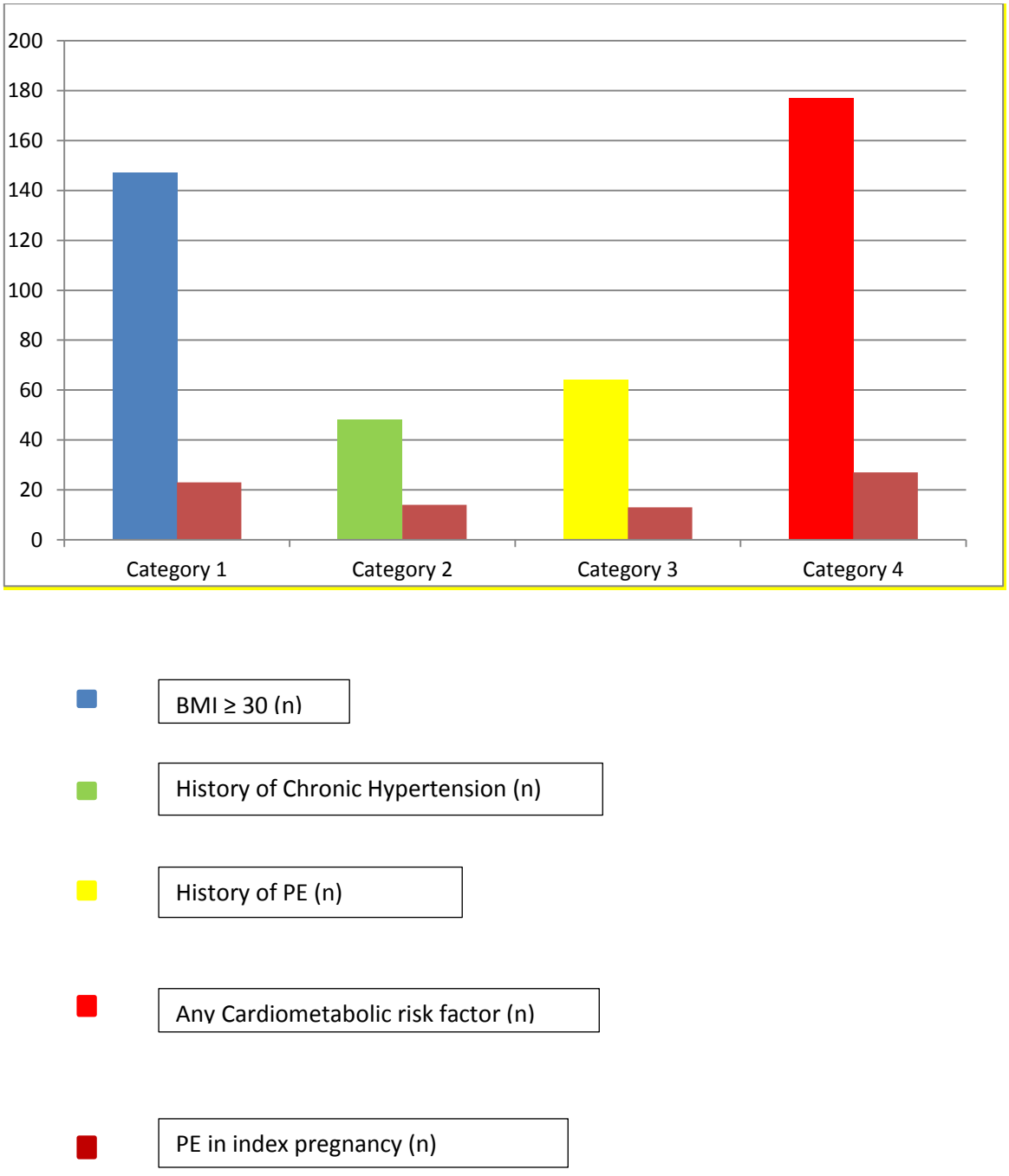

Figure 2.

Proportion of women with specific risk factors that developed PE in the index pregnancy.

A PROSPECTIVE STUDY EVALUATING THE ASSOCIATION OF SPECIFIC RISK FACTORS WITH THE DEVELOPMENT OF PREECLAMPSIA (PE).

Questionnaire

PLACE PATIENT STICKER HERE

Risk factor …….

Gravidity……. Parity…….

Previous Pregnancies

	Pregnancy 1	Pregnancy 2	Pregnancy 3	Pregnancy 4	Pregnancy 5
Year					
Gestation at delivery					
Baby Wt (kg)					
Mode of Delivery					
Complications					
Outcome					
Consort number					

Describe any complications:

……
……

Medical History:

……
……
……
…….

Medication:

……
……
……
……....

Surgical history:

……
……
……
……

Allergies:

……
……

Smoker (cigarettes/day): ……. Cans of cooldrink/day: ……

Personal History

Birth place: …….

Any complications with your birth?

……
……

Menarche (age)…….. Menarche (year) …….

LNMP…… No. of days……...

Duration of relationship with partner…….

Previous children with other partner(s)……..

Contraception (Y/N)……

When did you stop before current pregnancy……..

Types of contraceptives and duration……..

Ever use condoms?..... How long?.....

Until when? ……

History of father of this pregnancy

DOB:…… Birth place……

Any health problems:

……

Any children with another partner?.....

Any complications with the birth of those children?.....

History of your mother

How old is your mother?.....

Any illnesses?.....

Medications:…….

Operations:…….....

Weight (kg)…… Height (m)…….

Number of pregnancies…….. Live births ……

Miscarriages……..

Problems with pregnancies

……

……

History of your sisters

Number of sisters?..... How many were ever pregnant?.....

How many had hypertension in pregnancy?.....

How many had miscarriages?.....

How many had still born babies?.....

Details……

……

Do your sisters have any illnesses ?

.....

.....

Do they use any medication ?

……

……

DATA SHEET

Medical examination on booking:

……
……
……

Weight (kg) ……. Height (m) ……

Routine tests:

RPR:…….. Blood group: ……

Urine MCS: …… PAP smear: ……..

HIV: ……... Hb: ……..

Other tests:

……
……

Sonar:

Date: ……. Gestational age: ……

Placenta: ……

Other:

…….....
.....
.....
.....

Antenatal consultations:

Date						
SFH						
SBP						
DBP						
Proteinuria						
Glucosuria						
Oedema						
FBC						
Hb						
Complaints						
Admission						
Doppler						
Other						

Describe any complaints or complications

……
……
……
……
……
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……
……
……
……
……

Patient admitted before onset of labour (Y/N) ……

Reason for admission:

……
……..

Confinement:

Date: …… Gestation: ……

Weight (g) ……. Sex: …….

APGAR ……. …… …….

Mode of delivery …… Reason…….

SROM (Y/N) …… Gestation ……..

IOL (Y/N) ……. Method ……..

Indication ……..

Abruptio Placentae (Y/N)……..

Placental mass (g) …….

Other significant info.

……

Baby outcome (alive, SB, miscarriage, neonatal death)……

Days in hospital:…… Admiission to NICU (Y/N) …….

Complications during pregnancy:……

……..

PE (Y/N)…… Grade: …….

GA at diagnosis ……

Admission to Special Care (Y/N) …….

Hypertension without PE (Y/N) ……

Anti-hypertensive medication

……

……

Any other complications:

……

……

……

……

REFERENCES

1. Saftlas A, Olson D, Franks A, Atrash H, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States, 1979–1986. International Journal of Gynecology & Obstetrics [Internet]. Elsevier BV; 1991 Jun;35(2):192. [http://dx.doi.org/10.1016/0020-7292(91)90830-x]

2. Wang A, Rana S, Karumanchi SA. Preeclampsia: The Role of Angiogenic Factors in Its Pathogenesis. Physiology [Internet]. American Physiological Society; 2009 Jun 1;24(3):147–58. [http://dx.doi.org/10.1152/physiol.00043.2008]

3. Waterstone M. Incidence and predictors of severe obstetric morbidity: case-control study Commentary: Obstetric morbidity data and the need to evaluate thromboembolic disease. BMJ [Internet]. BMJ; 2001 May 5;322(7294):1089–94. [http://dx.doi.org/10.1136/bmj.322.7294.1089]

4. Baskett TF, Sternadel J. Maternal intensive care and near-miss mortality in obstetrics. BJOG:An international journal of O&G [Internet]. Wiley-Blackwell; 1998 Sep;105(9):981–4. [http://dx.doi.org/10.1111/j.1471-0528.1998.tb10261.x]

5. Mahutte N. Obstetric admissions to the intensive care unit. Obstetrics & Gynecology [Internet]. Ovid Technologies (Wolters Kluwer Health); 1999 Aug;94(2):263–6. [http://dx.doi.org/10.1016/s0029-7844(99)00274-4]

6. Nguyen Ngoc NT. Causes of stillbirths and early neonatal deaths: data from 7993 pregnancies in six developing countries. Bulletin of the World Health Organization [Internet]. WHO Press; 2006 Sep 1;84(9):699–705. [http://dx.doi.org/10.2471/blt.05.027300]

7. Duley L. The Global Impact of Pre-eclampsia and Eclampsia. Seminars in Perinatology [Internet]. Elsevier BV; 2009 Jun;33(3):130–7. [http://dx.doi.org/10.1053/j.semperi.2009.02.010]

8. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. The Lancet [Internet]. Elsevier BV; 2010 Aug;376(9741):631–44. [http://dx.doi.org/10.1016/s0140-6736(10)60279-6]

9. Wallis AB, Saftlas AF, Hsia J, Atrash HK. Secular Trends in the Rates of Preeclampsia, Eclampsia, and Gestational Hypertension, United States, 1987-2004. American Journal of Hypertension [Internet]. Oxford University Press (OUP); 2008 May 1;21(5):521–6. [http://dx.doi.org/10.1038/ajh.2008.20]

10. Brown MA, Lindheimer MD, de Swiet M, Assche AV, Moutquin J-M. The Classification and Diagnosis of the Hypertensive Disorders of Pregnancy: Statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy [Internet]. Informa Healthcare; 2001 Jan;20(1):ix–xiv. [http://dx.doi.org/10.3109/10641950109152635]

11. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health [Internet]. Elsevier BV; 2014 Apr;4(2):97–104 [http://dx.doi.org/10.1016/j.preghy.2014.02.001]

12. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. The Lancet [Internet]. Elsevier BV; 2005 Feb;365(9461):785–99. [http://dx.doi.org/10.1016/s0140-6736(05)17987-2]

13. VALENSISE H, NOVELLI GP, VASAPOLLO B. PRE-ECLAMPSIA: ONE NAME, TWO CONDITIONS – THE CASE FOR EARLY AND LATE DISEASE BEING DIFFERENT. Fetal and Maternal Medicine Review [Internet]. Cambridge University Press (CUP); 2013 Feb;24(01):32–7. [http://dx.doi.org/10.1017/s0965539513000016]

14. FERRAZZI E, STAMPALIJA T, AUPONT JE. THE EVIDENCE FOR LATE-ONSET PRE-ECLAMPSIA AS A MATERNOGENIC DISEASE OF PREGNANCY. Fetal and Maternal Medicine Review [Internet]. Cambridge University Press (CUP); 2013 Feb;24(01):18–31 [http://dx.doi.org/10.1017/s0965539513000028]

15. Redman CWG, Jacobson S-L, Russell R. Hypertension in Pregnancy. de Swiet's Medical Disorders in Obstetric Practice [Internet]. Wiley-Blackwell; 2010 Jun 18;153–81. [http://dx.doi.org/10.1002/9781444323016.ch6]

16. Mbah A, Kornosky J, Kristensen S, August E, Alio A, Marty P, et al. Super-obesity and risk for early and late pre-eclampsia. BJOG: An International Journal of Obstetrics & Gynaecology [Internet]. Wiley-Blackwell; 2010 May 19;117(8):997–1004. [http://dx.doi.org/10.1111/j.1471-0528.2010.02593.x]

17. Wood L. Review article: Obesity, waist-hip ratio and hunter-gatherers. BJOG: An International Journal of Obstetrics & Gynaecology [Internet]. Wiley-Blackwell; 2006 Sep 13;113(10):1110–6. [http://dx.doi.org/10.1111/j.1471-0528.2006.01070.x]

18. Borzychowski AM, Sargent IL, Redman CWG. Inflammation and pre-eclampsia. Seminars in Fetal and Neonatal Medicine [Internet]. Elsevier BV; 2006 Oct;11(5):309–16. [http://dx.doi.org/10.1016/j.siny.2006.04.001]

19. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature [Internet]. Nature Publishing Group; 2006 Dec 14;444(7121):847–53. [http://dx.doi.org/10.1038/nature05483]

20. Crispi F, Llurba E, Domínguez C, Martín-Gallán P, Cabero L, Gratacós E. Predictive value of angiogenic factors and uterine artery Doppler for early- versus late-onset pre-eclampsia and intrauterine growth restriction. Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2008;31(3):303–9. [http://dx.doi.org/10.1002/uog.5184]

21. Mayhew T., Ohadike C, Baker P., Crocker I., Mitchell C, Ong S. Stereological Investigation of Placental Morphology in Pregnancies Complicated by Pre-eclampsia with and without Intrauterine Growth Restriction. Placenta [Internet]. Elsevier BV; 2003 Feb;24(2-3):219–26. [http://dx.doi.org/10.1053/plac.2002.0900]

22. Egbor M, Ansari T, Morris N, Green C, Sibbons P. Maternal medicine: Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. BJOG: An International Journal of Obstetrics & Gynaecology [Internet]. Wiley-Blackwell; 2006 Mar 27;113(5):580–9. [http://dx.doi.org/10.1111/j.1471-0528.2006.00882.x]

23. Burton GJ, Woods AW, Jauniaux E, Kingdom JCP. Rheological and Physiological Consequences of Conversion of the Maternal Spiral Arteries for Uteroplacental Blood Flow during Human Pregnancy. Placenta [Internet]. Elsevier BV; 2009 Jun;30(6):473–82. [http://dx.doi.org/10.1016/j.placenta.2009.02.009]

24. Burton GJ, Jauniaux E, Charnock-Jones DS. The influence of the intrauterine environment on human placental development. Int J Dev Biol [Internet]. UBC Press; 2010;54(2-3):303–12. [http://dx.doi.org/10.1387/ijdb.082764gb]

25. Genbacev O. Regulation of Human Placental Development by Oxygen Tension. Science [Internet]. American Association for the Advancement of Science (AAAS); 1997 Sep 12;277(5332):1669–72 [http://dx.doi.org/10.1126/science.277.5332.1669]

26. Pijnenborg R, Vercruysse L, Hanssens M. The Uterine Spiral Arteries In Human Pregnancy: Facts and Controversies. Placenta [Internet]. Elsevier BV; 2006 Sep;27(9-10):939–58. [http://dx.doi.org/10.1016/j.placenta.2005.12.006]

27. BROSENS I. A STUDY OF THE SPIRAL ARTERIES OF THE DECIDUA BASALIS IN NORMOTENSIVE AND HYPERTENSIVE PREGNANCIES. Obstetrical & Gynecological Survey [Internet]. Ovid Technologies (Wolters Kluwer Health); 1964;19(5):736–8. [http://dx.doi.org/10.1097/00006254-196410000-00003]

28. Brosens JJ, Pijnenborg R, Brosens IA. The myometrial junctional zone spiral arteries in normal and abnormal pregnancies. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 2002 Nov;187(5):1416–23. [http://dx.doi.org/10.1067/mob.2002.127305]

29. Roberts JM. Pathophysiology of ischemic placental disease. Seminars in Perinatology [Internet]. Elsevier BV; 2014 Apr;38(3):139–45. [http://dx.doi.org/10.1053/j.semperi.2014.03.005]

30. Roberts JM, Funai EF. Pregnancy-Related Hypertension. Creasy and Resnik's Maternal-Fetal Medicine: Principles and Practice [Internet]. Elsevier BV; 2009;651–88. [http://dx.doi.org/10.1016/b978-1-4160-4224-2.50038-7]

31. Powers RW, Roberts JM, Cooper KM, Gallaher MJ, Frank MP, Harger GF, et al. Maternal serum soluble fms-like tyrosine kinase 1 concentrations are not increased in early pregnancy and decrease more slowly postpartum in women who develop preeclampsia. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 2005 Jul;193(1):185–91. [http://dx.doi.org/10.1016/j.ajog.2004.11.038]

32. Powers RW, Roberts JM, Plymire DA, Pucci D, Datwyler SA, Laird DM, et al. Low Placental Growth Factor Across Pregnancy Identifies a Subset of Women With Preterm Preeclampsia: Type 1 Versus Type 2 Preeclampsia? Hypertension [Internet]. Ovid Technologies (Wolters Kluwer Health); 2012 May 29;60(1):239–46. [http://dx.doi.org/10.1161/hypertensionaha.112.191213]

33. Bell MJ, Roberts JM, Founds SA, Jeyabalan A, Terhorst L, Conley YP. Variation in endoglin pathway genes is associated with preeclampsia: a case–control candidate gene association study. BMC Pregnancy Childbirth [Internet]. Springer Science + Business Media; 2013;13(1):82. [http://dx.doi.org/10.1186/1471-2393-13-82]

34. Anton L, Brown AG, Parry S, Elovitz MA. Lipopolysaccharide induces cytokine production and decreases extravillous trophoblast invasion through a mitogen-activated protein kinase-mediated pathway: possible mechanisms of first trimester placental dysfunction. Human Reproduction [Internet]. Oxford University Press (OUP); 2011 Nov 3;27(1):61–72. [http://dx.doi.org/10.1093/humrep/der362]

35. Poston L, Raijmakers MTM. Trophoblast Oxidative Stress, Antioxidants and Pregnancy Outcome—A Review. Placenta [Internet]. Elsevier BV; 2004 Apr;25:S72–S78. [http://dx.doi.org/10.1016/j.placenta.2004.01.003]

36. Schulz E, Gori T, Münzel T. Oxidative stress and endothelial dysfunction in hypertension. Hypertension Research [Internet]. Nature Publishing Group; 2011 Apr 21;34(6):665–73. [http://dx.doi.org/10.1038/hr.2011.39]

37. Burton GJ, Jauniaux E. Oxidative stress. Best Practice & Research Clinical Obstetrics & Gynaecology [Internet]. Elsevier BV; 2011 Jun;25(3):287–99. [http://dx.doi.org/10.1016/j.bpobgyn.2010.10.016]

38. Wang S, Kaufman RJ. The impact of the unfolded protein response on human disease. The Journal of Cell Biology [Internet]. Rockefeller University Press; 2012 Jun 25;197(7):857–67. [http://dx.doi.org/10.1083/jcb.201110131]

39. Roberts JM, Hubel CA. The Two Stage Model of Preeclampsia: Variations on the Theme. Placenta [Internet]. Elsevier BV; 2009 Mar;30:32–7. [http://dx.doi.org/10.1016/j.placenta.2008.11.009]

40. Redman CWG. Pre-eclampsia and the placenta. Placenta [Internet]. Elsevier BV; 1991 Jul;12(4):301–8. [http://dx.doi.org/10.1016/0143-4004(91)90339-h]

41. Burton GJ, Jones CJP. Syncytial Knots, Sprouts, Apoptosis, and Trophoblast Deportation from the Human Placenta. Taiwanese Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 2009 Mar;48(1):28–37 [http://dx.doi.org/10.1016/s1028-4559(09)60032-2]

42. Huppertz B, Kingdom J, Caniggia I, Desoye G, Black S, Korr H, et al. Hypoxia Favours Necrotic Versus Apoptotic Shedding of Placental Syncytiotrophoblast into the Maternal Circulation. Placenta [Internet]. Elsevier BV; 2003 Feb;24(2-3):181–90. [http://dx.doi.org/10.1053/plac.2002.0903]

43. Mutter WP, Karumanchi SA. Molecular mechanisms of preeclampsia. Microvascular Research [Internet]. Elsevier BV; 2008 Jan;75(1):1–8. [http://dx.doi.org/10.1016/j.mvr.2007.04.009]

44. Andraweera PH, Dekker GA, Roberts CT. The vascular endothelial growth factor family in adverse pregnancy outcomes. Human Reproduction Update [Internet]. Oxford University Press (OUP); 2012 Apr 11;18(4):436–57. [http://dx.doi.org/10.1093/humupd/dms011]

45. Levine RJ, Maynard SE, Qian C, Lim K-H, England LJ, Yu KF, et al. Circulating Angiogenic Factors and the Risk of Preeclampsia. N Engl J Med [Internet]. New England Journal of Medicine (NEJM/MMS); 2004 Feb 12;350(7):672–83. [http://dx.doi.org/10.1056/nejmoa031884]

46. Sacks GP, Studena K, Sargent IL, Redman CWG. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 1998 Jul;179(1):80–6 [http://dx.doi.org/10.1016/s0002-9378(98)70254-6]

47. Smarason AK, Gunnarsson A, Alfredsson JH, Valdimarsson H. Monocytosis and monocytic infiltration of decidua in early pregnancy. J Clin Lab Immunol 1986; 21 (1): 1–5. Epub 1986/09/01.

48. Austgulen R, Lien E, Liabakk N-B, Jacobsen G, Arntzen KJ. Increased levels of cytokines and cytokine activity modifiers in normal pregnancy. European Journal of Obstetrics & Gynecology and Reproductive Biology [Internet]. Elsevier BV; 1994 Dec;57(3):149–55. [http://dx.doi.org/10.1016/0028-2243(94)90291-7]

49. Maynard SE, Min J-Y, Merchan J, Lim K-H, Li J, Mondal S, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest [Internet]. American Society for Clinical Investigation; 2003 Mar 1;111(5):649–58 [http://dx.doi.org/10.1172/jci17189]

50. MATSUZAWA Y, FUNAHASHI T, NAKAMURA T. Molecular Mechanism of Metabolic Syndrome X: Contribution of Adipocytokines . Adipocyte-derived Bioactive Substances. Annals NY Acad Sci [Internet]. Wiley-Blackwell; 1999 Nov;892(1 THE METABOLIC):146–54 [http://dx.doi.org/10.1111/j.1749-6632.1999.tb07793.x]

51. Côté M, Mauriège P, Bergeron J, Alméras N, Tremblay A, Lemieux I, et al. Adiponectinemia in Visceral Obesity: Impact on Glucose Tolerance and Plasma Lipoprotein and Lipid Levels in Men. The Journal of Clinical Endocrinology & Metabolism [Internet]. The Endocrine Society; 2005 Mar;90(3):1434–9. [http://dx.doi.org/10.1210/jc.2004-1711]

52. Suganami T. A Paracrine Loop Between Adipocytes and Macrophages Aggravates Inflammatory Changes: Role of Free Fatty Acids and Tumor Necrosis Factor . Arteriosclerosis, Thrombosis, and Vascular Biology [Internet]. Ovid Technologies (Wolters Kluwer Health); 2005 Oct 1;25(10):2062–8. [http://dx.doi.org/10.1161/01.atv.0000183883.72263.13]

53. Cancello R, Clément K. Review article: Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue. BJOG: An International Journal of Obstetrics & Gynaecology [Internet]. Wiley-Blackwell; 2006 Aug 10;113(10):1141–7. [http://dx.doi.org/10.1111/j.1471-0528.2006.01004.x]

54. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest [Internet]. American Society for Clinical Investigation; 2003 Dec 15;112(12):1821–30. [http://dx.doi.org/10.1172/jci19451]

55. Conde-Agudelo A, Villar J, Lindheimer M. World Health Organization Systematic Review of Screening Tests for Preeclampsia. Obstetrics & Gynecology [Internet]. Ovid Technologies (Wolters Kluwer Health); 2004;104(6):1367–91 [http://dx.doi.org/10.1097/01.aog.0000147599.47713.5d]

56. Chesley LC. History and Epidemiology of Preeclampsia - Eclampsia. Clinical Obstetrics and Gynecology [Internet]. Ovid Technologies (Wolters Kluwer Health); 1984;27(4):801–20. [http://dx.doi.org/10.1097/00003081-198412000-00004]

57. Bujold E, Roberge S, Lacasse Y, Bureau M, Audibert F, Marcoux S, et al. Prevention of Preeclampsia and Intrauterine Growth Restriction With Aspirin Started in Early Pregnancy. Obstetrics & Gynecology [Internet]. Ovid Technologies (Wolters Kluwer Health); 2010;116(2, Part 1):402–14. [http://dx.doi.org/10.1097/aog.0b013e3181e9322a]

58. Vadillo-Ortega F, Perichart-Perera O, Espino S, Avila-Vergara MA, Ibarra I, Ahued R, et al. Effect of supplementation during pregnancy with L-arginine and antioxidant vitamins in medical food on pre-eclampsia in high risk population: randomised controlled trial. BMJ [Internet]. BMJ; 2011 May 19;342(may19 1):d2901–d2901. [http://dx.doi.org/10.1136/bmj.d2901]

59. Ayala DE, Hermida RC. Ambulatory Blood Pressure Monitoring for the Early Identification of Hypertension in Pregnancy. Chronobiol Int [Internet]. Informa Healthcare; 2013 Mar;30(1-2):233–59. [http://dx.doi.org/10.3109/07420528.2012.714687]

60. Wright D, Akolekar R, Syngelaki A, Poon LCY, Nicolaides KH. A Competing Risks Model in Early Screening for Preeclampsia. Fetal Diagn Ther [Internet]. S. Karger AG; 2012;32(3):171–8. [http://dx.doi.org/10.1159/000338470]

61. Easterling TR, Benedetti TJ, Schmucker BC, Millard SP. Maternal haemodynamics in normal and pre-eclamptic pregnancies: A longitudinal study. Obstet Gynecol 1990; 76:1061–1069.

62. Oliveira N, Magder LS, Blitzer MG, Baschat AA. First-trimester prediction of pre-eclampsia: external validity of algorithms in a prospectively enrolled cohort. Ultrasound in Obstetrics & Gynecology [Internet]. Wiley-Blackwell; 2014 Aug 13;44(3):279–85. [http://dx.doi.org/10.1002/uog.13435]

63. Baschat A, Poon LY, Blitzer M, Nicolaides K, Harman C. OC08.05: Impact of 1 st trimester aspirin on population prevalence of pre-eclampsia . Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2009 Sep;34(S1):14–14. [http://dx.doi.org/10.1002/uog.6499]

64. Block-Abraham DM, Turan OM, Doyle LE, Kopelman JN, Atlas RO, Jenkins CB, et al. First-Trimester Risk Factors for Preeclampsia Development in Women Initiating Aspirin by 16 Weeks of Gestation. Obstetrics & Gynecology [Internet]. Ovid Technologies (Wolters Kluwer Health); 2014;123(3):611–7 [http://dx.doi.org/10.1097/aog.0000000000000118]

65. Leslie K, Thilaganathan B, Papageorghiou A. Early prediction and prevention of pre-eclampsia. Best Practice & Research Clinical Obstetrics & Gynaecology [Internet]. Elsevier BV; 2011 Jun;25(3):343–54. [http://dx.doi.org/10.1016/j.bpobgyn.2011.01.002]

66. Sibai B. First-trimester screening with combined maternal clinical factors, biophysical and biomarkers to predict preterm pre-eclampsia and hypertensive disorders: are they ready for clinical use? BJOG: An International Journal of Obstetrics & Gynaecology [Internet]. Wiley-Blackwell; 2014 Aug 27;122(3):282–3. [http://dx.doi.org/10.1111/1471-0528.13052]

67. Kane SC, Da Silva Costa F, Brennecke SP. New directions in the prediction of pre-eclampsia. Australian and New Zealand Journal of Obstetrics and Gynaecology [Internet]. Wiley-Blackwell; 2013 Dec 23;54(2):101–7. [http://dx.doi.org/10.1111/ajo.12151]

68. Farina A, Rapacchia G, Sterrantino AF, Pula G, Morano D, Rizzo N. Prospective evaluation of ultrasound and biochemical-based multivariable models for the prediction of late pre-eclampsia. Prenatal Diagnosis [Internet]. Wiley-Blackwell; 2011 Oct;n/a–n/a. [http://dx.doi.org/10.1002/pd.2849]

69. Oliveira N, Doyle LE, Atlas RO, Jenkins CB, Blitzer MG, Baschat AA. External validity of first-trimester algorithms in the prediction of pre-eclampsia disease severity. Ultrasound in Obstetrics & Gynecology [Internet]. Wiley-Blackwell; 2014 Aug 7;44(3):286–92 [http://dx.doi.org/10.1002/uog.13433]

70. Baschat AA. First-trimester screening for pre-eclampsia: moving from personalized risk prediction to prevention. Ultrasound in Obstetrics & Gynecology [Internet]. Wiley-Blackwell; 2015 Jan 27;45(2):119–29. [http://dx.doi.org/10.1002/uog.14770]

71. Hinselmann H. In Die Eklampsie. Frederick Cohen: Bonn, 1924; 27–33.

72. White PW. Diabetes complicating pregnancy. Am J Obstet Gynecol 1938; 33: 380–385.

73. Seitz L. Die Schwangerschafts toxikosen. In Lehrbuch der Geburtshilfe, Stoeckel (2nd edn), Gustav-Fischer Jena 1923; 508–520.

74. Lehman K. Eklampsien i Danmark i aarene 1918–1927. Copenhagen: Busck, 1933.

75. Bublitschenko L. Zur Frage u¨ber gewisse konstitutionelle Eigentu¨mlichkeiten bei Eklamptischen. Monatsschrift fu¨ r Geburtshilfe und Gyn¨ akologie 1925; 69: 139.

76. Davies AM. Geographic and Ethnic Differences in Incidence of the Pregnancy Toxemias. Pathologia et Microbiologia [Internet]. S. Karger AG; 1970;35(1-3):210–4. [http://dx.doi.org/10.1159/000162231]

77. Cuckle HS. Screening for Pre-eclampsia–Lessons from Aneuploidy Screening. Placenta [Internet]. Elsevier BV; 2011 Feb;32:S42–S48. [http://dx.doi.org/10.1016/j.placenta.2010.07.015]

78. Friedman J, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. J Stat Softw 2010; 33: 1–22.

79. National Collaborating Centre for Women's and Children's Health. Antenatal care: routine care for the healthy pregnant woman. Clinical Guideline. Commissioned by the National Institute for Health and Clinical Excellence. RCOG Press: London, 2008.

80. Poon LCY, Kametas NA, Chelemen T, Leal A, Nicolaides KH. Maternal risk factors for hypertensive disorders in pregnancy: a multivariate approach. Journal of Human Hypertension [Internet]. Nature Publishing Group; 2009 Jun 11;24(2):104–10. [http://dx.doi.org/10.1038/jhh.2009.45]

81. CAMPBELL S, GRIFFIN DR, PEARCE JM, DIAZ-RECASENS J, COHEN-OVERBEEK TE, WILLSON K, et al. New Doppler Technique for Assessing Uteroplacental Blood Flow. Obstetrical & Gynecological Survey [Internet]. Ovid Technologies (Wolters Kluwer Health); 1983;38(9):548–9. [http://dx.doi.org/10.1097/00006254-198309000-00004]

82. Martin AM, Bindra R, Curcio P, Cicero S, Nicolaides KH. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler at 11-14 weeks of gestation. Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2001 Dec;18(6):583–6. [http://dx.doi.org/10.1046/j.0960-7692.2001.00594.x]

83. Plasencia W, Maiz N, Bonino S, Kaihura C, Nicolaides KH. Uterine artery Doppler at 11 + 0 to 13 + 6 weeks in the prediction of pre-eclampsia. Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2007 Sep 26;30(5):742–9. [http://dx.doi.org/10.1002/uog.5157]

84. Poon LCY, Staboulidou I, Maiz N, Plasencia W, Nicolaides KH. Hypertensive disorders in pregnancy: screening by uterine artery Doppler at 11-13 weeks. Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2009 Aug;34(2):142–8. [http://dx.doi.org/10.1002/uog.6452]

85. Cnossen JS, Vollebregt KC, Vrieze N d., Riet G t., Mol BWJ, Franx A, et al. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. BMJ [Internet]. BMJ; 2008 May 17;336(7653):1117–20. [http://dx.doi.org/10.1136/bmj.39540.522049.be]

86. Poon LCY, Kametas NA, Pandeva I, Valencia C, Nicolaides KH. Mean Arterial Pressure at 11+0 to 13+6 Weeks in the Prediction of Preeclampsia. Hypertension [Internet]. Ovid Technologies (Wolters Kluwer Health); 2008 Feb 7;51(4):1027–33. [http://dx.doi.org/10.1161/hypertensionaha.107.104646]

87. Gallo D, Poon LC, Fernandez M, Wright D, Nicolaides KH. Prediction of preeclampsia by mean arterial pressure at 11-13 and 20-24 weeks' gestation. Fetal Diagn Ther 2014;36:28-37 [http://dx.doi.org/10.1159/000360287]

88. Zhong Y, Tuuli M, Odibo AO. First-trimester assessment of placenta function and the prediction of preeclampsia and intrauterine growth restriction. Prenat Diagn [Internet]. Wiley-Blackwell; 2010;n/a–n/a. [http://dx.doi.org/10.1002/pd.2475]

89. Spencer K, Yu CKH, Cowans NJ, Otigbah C, Nicolaides KH. Prediction of pregnancy complications by first-trimester maternal serum PAPP-A and free β-hCG and with second-trimester uterine artery Doppler. Prenat Diagn [Internet]. Wiley-Blackwell; 2005;25(10):949–53. [http://dx.doi.org/10.1002/pd.1251]

90. Spencer K, Cowans NJ, Chefetz I, Tal J, Meiri H. First-trimester maternal serum PP-13, PAPP-A and second-trimester uterine artery Doppler pulsatility index as markers of pre-eclampsia. Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2007;29(2):128–34. [http://dx.doi.org/10.1002/uog.3876]

91. Khalil A, Cowans NJ, Spencer K, Goichman S, Meiri H, Harrington K. First trimester maternal serum placental protein 13 for the prediction of pre-eclampsia in women with a priori high risk . Prenat Diagn [Internet]. Wiley-Blackwell; 2009 Aug;29(8):781–9. [http://dx.doi.org/10.1002/pd.2287]

92. Bale LK. Disruption of insulin-like growth factor-II imprinting during embryonic development rescues the dwarf phenotype of mice null for pregnancy-associated plasma protein-A. Journal of Endocrinology [Internet]. BioScientifica; 2005 Aug 1;186(2):325–31. [http://dx.doi.org/10.1677/joe.1.06259]

93. Nicolaides KH, Bindra R, Turan OM, Chefetz I, Sammar M, Meiri H, et al. A novel approach to first-trimester screening for early pre-eclampsia combining serum PP-13 and Doppler ultrasound. Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2005 Dec 22;27(1):13–7. [http://dx.doi.org/10.1002/uog.2686]

94. Poon LCY, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-Trimester Prediction of Hypertensive Disorders in Pregnancy. Hypertension [Internet]. Ovid Technologies (Wolters Kluwer Health); 2009 Mar 9;53(5):812–8. [http://dx.doi.org/10.1161/hypertensionaha.108.127977]

95. Tjwa M, Luttun A, Autiero M, Carmeliet P. VEGF and PlGF: two pleiotropic growth factors with distinct roles in development and homeostasis. Cell and Tissue Research [Internet]. Springer Science + Business Media; 2003 Oct 1;314(1):5–14 [http://dx.doi.org/10.1007/s00441-003-0776-3]

96. Autiero M, Luttun A, Tjwa M, Carmeliet P. Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. Journal of Thrombosis and Haemostasis [Internet]. Wiley-Blackwell; 2003 Jul;1(7):1356–70. [http://dx.doi.org/10.1046/j.1538-7836.2003.00263.x]

97. Rana S, Karumanchi SA, Levine RJ, Venkatesha S, Rauh-Hain JA, Tamez H, et al. Sequential Changes in Antiangiogenic Factors in Early Pregnancy and Risk of Developing Preeclampsia. Hypertension [Internet]. Ovid Technologies (Wolters Kluwer Health); 2007 May 21;50(1):137–42. [http://dx.doi.org/10.1161/hypertensionaha.107.087700]

98. Akolekar R, Zaragoza E, Poon LCY, Pepes S, Nicolaides KH. Maternal serum placental growth factor at 11 + 0 to 13 + 6 weeks of gestation in the prediction of pre-eclampsia. Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2008 Nov;32(6):732–9. [http://dx.doi.org/10.1002/uog.6244]

99. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, et al. First Trimester Placental Growth Factor and Soluble Fms-Like Tyrosine Kinase 1 and Risk for Preeclampsia. The Journal of Clinical Endocrinology & Metabolism [Internet]. The Endocrine Society; 2004 Feb;89(2):770–5. [http://dx.doi.org/10.1210/jc.2003-031244]

100. Smith GCS, Crossley JA, Aitken DA, Jenkins N, Lyall F, Cameron AD, et al. Circulating Angiogenic Factors in Early Pregnancy and the Risk of Preeclampsia, Intrauterine Growth Restriction, Spontaneous Preterm Birth, and Stillbirth. Obstetrics & Gynecology [Internet]. Ovid Technologies (Wolters Kluwer Health); 2007;109(6):1316–24. [http://dx.doi.org/10.1097/01.aog.0000265804.09161.0d]

101. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing Risks Model in Early Screening for Preeclampsia by Biophysical and Biochemical Markers. Fetal Diagn Ther [Internet]. S. Karger AG; 2013;33(1):8–15. [http://dx.doi.org/10.1159/000341264]

102. Villa P, Kajantie E, Räikkönen K, Pesonen A-K, Hämäläinen E, Vainio M, et al. Aspirin in the prevention of pre-eclampsia in high-risk women: a randomised placebo-controlled PREDO Trial and a meta-analysis of randomised trials. BJOG: An International Journal of Obstetrics & Gynaecology [Internet]. Wiley-Blackwell; 2012 Nov 6;120(1):64–74. [http://dx.doi.org/10.1111/j.1471-0528.2012.03493.x]

103. Scholten RR, Hopman MT, Sweep FC, Van de Vlugt MJ, Van Dijk AP, Oyen WJ, Lotgering FK, Spaanderman ME. Co-occurrence of cardiovascular and prothrombotic risk factors in women with a history of pre-eclampsia. Obstet Gynecol 2013; 121: 97–105. [http://dx.doi.org/10.1002/uog.14770]

104. Audibert F, Boucoiran I, An N, Aleksandrov N, Delvin E, Bujold E, et al. Screening for preeclampsia using first-trimester serum markers and uterine artery Doppler in nulliparous women. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 2010 Oct;203(4):383.e1–383.e8. [http://dx.doi.org/10.1016/j.ajog.2010.06.014]

105. Parra-Cordero M, Rodrigo R, Barja P, Bosco C, Rencoret G, Sepúlveda-Martinez A, et al. Prediction of early and late pre-eclampsia from maternal characteristics, uterine artery Doppler and markers of vasculogenesis during first trimester of pregnancy. Ultrasound in Obstetrics & Gynecology [Internet]. Wiley-Blackwell; 2013 Apr 22;41(5):538–44. [http://dx.doi.org/10.1002/uog.12264]

106. Herraiz I, Arbue´s J, Caman˜ o I, Go´mez-Montes E, Gran˜ eras A, Galindo A. Application of a first-trimester prediction model for pre-eclampsia based on uterine arteries and maternal history in high-risk pregnancies. Prenat Diagn [Internet]. Wiley-Blackwell; 2009 Dec;29(12):1123–9. [http://dx.doi.org/10.1002/pd.2383]

107. Scazzocchio E, Figueras F, Crispi F, Meler E, Masoller N, Mula R, et al. Performance of a first-trimester screening of preeclampsia in a routine care low-risk setting. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 2013 Mar;208(3):203.e1–203.e10. [http://dx.doi.org/10.1016/j.ajog.2012.12.016]

108. Baschat AA, Magder LS, Doyle LE, Atlas RO, Jenkins CB, Blitzer MG. Prediction of preeclampsia utilizing the first trimester screening examination. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 2014 Nov;211(5):514.e1–514.e7. [http://dx.doi.org/10.1016/j.ajog.2014.04.018]

109. Odibo AO, Zhong Y, Goetzinger KR, Odibo L, Bick JL, Bower CR, et al. First-trimester placental protein 13, PAPP-A, uterine artery Doppler and maternal characteristics in the prediction of pre-eclampsia. Placenta [Internet]. Elsevier BV; 2011 Aug;32(8):598–602. [http://dx.doi.org/10.1016/j.placenta.2011.05.006]

110. Kuc S, Koster MPH, Franx A, Schielen PCJI, Visser GHA. Maternal Characteristics, Mean Arterial Pressure and Serum Markers in Early Prediction of Preeclampsia. Oudejans C, editor. PLoS ONE [Internet]. Public Library of Science (PLoS); 2013 May 22;8(5):e63546. [http://dx.doi.org/10.1371/journal.pone.0063546]

111. Caradeux J, Serra R, Nien J-K, Pérez-Sepulveda A, Schepeler M, Guerra F, et al. First trimester prediction of early onset preeclampsia using demographic, clinical, and sonographic data: a cohort study. Prenatal Diagnosis [Internet]. Wiley-Blackwell; 2013 May 3;33(8):732–6. [http://dx.doi.org/10.1002/pd.4113]

112. Ray JG, Vermeulen MJ, Schull MJ, McDonald S, Redelmeier DA.Metabolic syndrome and the risk of placental dysfunction. J Obstet Gynaecol Can 2005; 27: 1095–1101.

113. Srinivas SK, Srinivas SK, Sammel MD, Bastek J, Ofori E, Andrela CM, et al. Evaluating the association between all components of the metabolic syndrome and pre-eclampsia. Journal of Maternal-Fetal and Neonatal Medicine [Internet]. Informa UK Limited; 2009 Jan;22(6):501–9. [http://dx.doi.org/10.1080/14767050902794642]

114. Stekkinger E, Scholten R, van der Vlugt M, van Dijk A, Janssen M, Spaanderman M. Metabolic syndrome and the risk for recurrent pre-eclampsia: a retrospective cohort study. BJOG: An International Journal of Obstetrics & Gynaecology [Internet]. Wiley-Blackwell; 2013 Mar 6;120(8):979–86. [http://dx.doi.org/10.1111/1471-0528.12189]

115. CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. The Lancet [Internet]. Elsevier BV; 1994 Mar;343(8898):619–29. [http://dx.doi.org/10.1016/s0140-6736(94)92633-6]

116. Sibai BM, Caritis SN, Thom E, Klebanoff M, McNellis D, Rocco L, et al. Prevention of Preeclampsia with Low-Dose Aspirin in Healthy, Nulliparous Pregnant Women. N Engl J Med [Internet]. New England Journal of Medicine (NEJM/MMS); 1993 Oct 21;329(17):1213–8. [http://dx.doi.org/10.1056/nejm199310213291701]

117. Benigni A, Gregorini G, Frusca T, Chiabrando C, Ballerini S, Valcamonico A, et al. Effect of Low-Dose Aspirin on Fetal and Maternal Generation of Thromboxane by Platelets in Women at Risk for Pregnancy-Induced Hypertension. N Engl J Med [Internet]. New England Journal of Medicine (NEJM/MMS); 1989 Aug 10;321(6):357–62. [http://dx.doi.org/10.1056/nejm198908103210604]

118. ITALIANSTUDYOFASPIRININPREGNA. Low-dose aspirin in prevention and treatment of intrauterine growth retardation and pregnancy-induced hypertension. The Lancet [Internet]. Elsevier BV; 1993 Feb;341(8842). [http://dx.doi.org/10.1016/0140-6736(93)92988-6]

119. ECPPA: randomised trial of low dose aspirin for the prevention of maternal and fetal complications in high risk pregnant women. BJOG:An international journal of O&G [Internet]. Wiley-Blackwell; 1996 Jan;103(1):39–47. [http://dx.doi.org/10.1111/j.1471-0528.1996.tb09513.x]

120. Caritis S, Sibai B, Hauth J, Lindheimer MD, Klebanoff M, Thom E, et al. Low-Dose Aspirin to Prevent Preeclampsia in Women at High Risk. N Engl J Med [Internet]. New England Journal of Medicine (NEJM/MMS); 1998 Mar 12;338(11):701–5. [http://dx.doi.org/10.1056/nejm199803123381101]

121. Golding J. A randomised trial of low dose aspirin for primiparae in pregnancy. BJOG:An international journal of O&G [Internet]. Wiley-Blackwell; 1998 Mar;105(3):293–9. [http://dx.doi.org/10.1111/j.1471-0528.1998.tb10089.x]

122. Rotchell YE, Cruickshank JK, Phillips Gay M, Griffiths J, Stewart A, Farrell B, et al. Barbados Low Dose Aspirin Study in Pregnancy (BLASP): a randomised trial for the prevention of pre-eclampsia and its complications. BJOG:An international journal of O&G [Internet]. Wiley-Blackwell; 1998 Mar;105(3):286–92. [http://dx.doi.org/10.1111/j.1471-0528.1998.tb10088.x]

123. Subtil D, Goeusse P, Puech F, Lequien P, Biausque S, Breart G, et al. Aspirin (100 mg) used for prevention of pre-eclampsia in nulliparous women: the Essai Regional Aspirine Mere-Enfant study (Part 1). BJOG: An Internal Journal of Obs Gyn [Internet]. Wiley-Blackwell; 2003 May;110(5):475–84. [http://dx.doi.org/10.1046/j.1471-0528.2003.02096.x]

124. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA. Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. The Lancet [Internet]. Elsevier BV; 2007 May;369(9575):1791–8. [http://dx.doi.org/10.1016/s0140-6736(07)60712-0]

125. Duley L, Henderson-Smart D, Knight M, King J. Antiplatelet agents for preventing pre-eclampsia and its complications. Duley L, editor. Protocols [Internet]. Wiley-Blackwell; 1996 Sep 1 [http://dx.doi.org/10.1002/14651858.cd004659]

126. Meher S, Alfirevic Z. Aspirin for pre-eclampsia: beware of subgroup meta-analysis. Ultrasound in Obstetrics & Gynecology [Internet]. Wiley-Blackwell; 2013 Apr 22;41(5):479–85. [http://dx.doi.org/10.1002/uog.12470]

127. Belizán J, Villar J, Repke J. The relationship between calcium intake and pregnancy-induced hypertension: Up-to-date evidence. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 1988 Apr;158(4):898–902. [http://dx.doi.org/10.1016/0002-9378(88)90091-9]

128. Poston L, Briley A, Seed P, Kelly F, Shennan A. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. The Lancet [Internet]. Elsevier BV; 2006 Apr;367(9517):1145–54. [http://dx.doi.org/10.1016/s0140-6736(06)68433-x]

129. Villar J, Purwar M, Merialdi M, Zavaleta N, thi Nhu Ngoc N, Anthony J, et al. World Health Organisation multicentre randomised trial of supplementation with vitamins C and E among pregnant women at high risk for pre-eclampsia in populations of low nutritional status from developing countries. BJOG: An International Journal of Obstetrics & Gynaecology [Internet]. Wiley-Blackwell; 2009 Apr 23;116(6):780–8. [http://dx.doi.org/10.1111/j.1471-0528.2009.02158.x]

130. Roberts JM, Myatt L, Spong CY, Thom EA, Hauth JC, Leveno KJ, et al. Vitamins C and E to Prevent Complications of Pregnancy-Associated Hypertension. N Engl J Med [Internet]. New England Journal of Medicine (NEJM/MMS); 2010 Apr 8;362(14):1282–91. [http://dx.doi.org/10.1056/nejmoa0908056]

131. Petersen EE, Mitchell AA, Carey JC, Werler MM, Louik C, Rasmussen SA. Maternal exposure to statins and risk for birth defects: A case-series approach. Am J Med Genet [Internet]. Wiley-Blackwell; 2008 Oct 15;146A(20):2701–5. [http://dx.doi.org/10.1002/ajmg.a.32493]

132. Costantine MM, Cleary K. Pravastatin for the Prevention of Preeclampsia in High-risk Pregnant Women. Obstetric Anesthesia Digest [Internet]. Ovid Technologies (Wolters Kluwer Health); 2014;34(2):75–6. [http://dx.doi.org/10.1097/01.aoa.0000446288.30125.e2]

133. Bauer AJ, Banek CT, Needham K, Gillham H, Capoccia S, Regal JF, et al. Pravastatin Attenuates Hypertension, Oxidative Stress, and Angiogenic Imbalance in Rat Model of Placental Ischemia-Induced Hypertension. Hypertension [Internet]. Ovid Technologies (Wolters Kluwer Health); 2013 Mar 4;61(5):1103–10 [http://dx.doi.org/10.1161/hypertensionaha.111.00226]

134. Costantine MM, Tamayo E, Lu F, Bytautiene E, Longo M, Hankins GDV, et al. Using Pravastatin to Improve the Vascular Reactivity in a Mouse Model of Soluble Fms-Like Tyrosine Kinase-1–Induced Preeclampsia. Obstetrics & Gynecology [Internet]. Ovid Technologies (Wolters Kluwer Health); 2010;116(1):114–20. [http://dx.doi.org/10.1097/aog.0b013e3181e10ebd]

135. Pravastatin for the Prevention of Pre-eclampsia in High-Risk Women: A Phase I Pilot Study. Clinicaltrials.gov:NCT01717586.

136. Zheng J, Shan PF, Gu W. The efficacy of metformin in pregnant women with polycystic ovary syndrome: A meta-analysis of clinical trials. J Endocrinol Invest 2013: 36: 797–802.

137. Salvesen KÅ, Vanky E, Carlsen SM. Metformin treatment in pregnant women with polycystic ovary syndrome—is reduced complication rate mediated by changes in the uteroplacental circulation? Ultrasound Obstet Gynecol [Internet]. Wiley-Blackwell; 2007;29(4):433–7. [http://dx.doi.org/10.1002/uog.3965]

138. Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy: A risk factor for the development of severe preeclampsia. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 2001 Oct;185(4):781–5. [http://dx.doi.org/10.1067/mob.2001.117304]

139. Cotter A. Elevated plasma homocysteine in early pregnancy: a risk factor for the development of nonsevere preeclampsia. American Journal of Obstetrics and Gynecology [Internet]. Elsevier BV; 2003 Aug;189(2):391–4. [http://dx.doi.org/10.1067/s0002-9378(03)00669-0]

140. Salehi-Pourmehr H, Mohamad-Alizadeh S, Malakouti J, Farshbaf-Khalili A. Association of the folic acid consumption and its serum levels with pre-eclampsia in pregnant women. Iran J Nurs Midwifery Res 2012; 17: 461–466.

141. Li Z, Ye R, Zhang L, Li H, Liu J, Ren A. Folic Acid Supplementation During Early Pregnancy and the Risk of Gestational Hypertension and Preeclampsia. Hypertension [Internet]. Ovid Technologies (Wolters Kluwer Health); 2013 Feb 11;61(4):873–9 [http://dx.doi.org/10.1161/hypertensionaha.111.00230]

142. Effect of Folic Acid Supplementation in Pregnancy on Pre-eclampsia-Folic Acid Clinical Trial (FACT). Clinicaltrials.gov: NCT01355159

143. Howarth C, Gazis A, James D. Associations of Type 1 diabetes mellitus, maternal vascular disease and complications of pregnancy. Diabetic Med [Internet]. Wiley-Blackwell; 2007 Nov;24(11):1229–34. [http://dx.doi.org/10.1111/j.1464-5491.2007.02254.x]

144. Bhattacharya S, Campbell DM, Liston WA, Bhattacharya S. Effect of Body Mass Index on pregnancy outcomes in nulliparous women delivering singleton babies. BMC Public Health [Internet]. Springer Science + Business Media; 2007;7(1):168. [http://dx.doi.org/10.1186/1471-2458-7-168]

145. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet [Internet]. Elsevier BV; 2014 Aug;384(9945):766–81. [http://dx.doi.org/10.1016/s0140-6736(14)60460-8]

146. Panday M, Mantel G, Moodley J. Audit of severe acute morbidity in hypertensive pregnancies in a developing country. Journal of Obstetrics & Gynaecology [Internet]. Informa Healthcare; 2004 Jan;24(4):387–91. [http://dx.doi.org/10.1080/01443610410001685501]

147. Moodley J, Kalane G. A Review of the Management of Eclampsia: Practical Issues. Hypertens Pregnancy [Internet]. Informa UK Limited; 2006 Jan;25(2):47–62. [http://dx.doi.org/10.1080/10641950500543897]