ASSOCIATIONS BETWEEN HERPES SIMPLEX VIRUS TYPES 1 AND 2 (HSV-1 AND HSV-2), CYTOMEGALOVIRUS (CMV), EPSTEIN BARR VIRUS (EBV), HUMAN PAPILLOMA VIRUS (HPV), CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHOEAE INFECTIONS AND PREECLAMPSIA

by

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LIST OF PAPERS

This dissertation is based on three papers, referred to in the text by roman numerals (I-III):

I. Serological Associations between Herpes Virus Types 1 and 2 (HSV-1 and HSV-2), Cytomegalovirus (CMV), Epstein Barr Virus infections and Preeclampsia

II. Associations between maternal infections and Preeclampsia: A Systematic Review of Epidemiologic Studies

III. Associations between sexually transmitted infections and Preeclampsia: A Population-based Case Control Study

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ASSOCIATIONS BETWEEN HERPES SIMPLEX VIRUS TYPES 1 AND 2 (HSV-1 AND HSV-2), CYTOMEGALOVIRUS (CMV), EPSTEIN BARR VIRUS (EBV), HUMAN PAPILLOMA VIRUS (HPV), CHLAMYDIA TRACHOMATIS, AND GONORRHOEAE INFECTIONS AND PREECLAMPSIA

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Background: Atherosclerosis, endothelial dysfunction and inflammation are thought to be key pathophysiologic processes in preeclampsia. The basic thesis of this dissertation is that maternal infections may trigger upregulation of proinflammatory cytokines in women with preeclampsia resulting in vascular injury.

Objectives: We evaluated the evidence for a potential infectious disease etiology for preeclampsia in three papers.

Methods: For the first paper, we conducted a 1:3 matched case control study. In this study we measured immunoglobulin G (IgG) antibodies to HSV-1, HSV-2, CMV, and EBV in serum samples obtained from 50 cases with preeclampsia and 150 normotensive controls, matched on age, parity and race. For the second paper, we conducted a comprehensive review of published studies that explored the association between both bacterial and viral infections, and examined the strength of this association. For the third paper, we investigated the association between self-reported Genital Warts (HPV), Genital Herpes (HSV-2), Chlamydia (*C. trachomatis*), Gonorrhea (*N. gonorrhoeae*) infections, sociodemographic, and behavioral risk factors and the risk of preeclampsia in a representative national sample of 10,847 reproductive age women.

Results: We found that seroconversion for HSV 1 /2 or CMV was associated with a five-fold increased risk for developing preeclampsia (OR 5.4, 95% CI 1.0-29.0) after adjusting for education, income, smoking, years of cohabitation, medical insurance, and type of birth control. Pooling of relevant epidemiologic data, also revealed a two-fold increased risk of preeclampsia associated with bacterial and viral infections (OR 2.1, 95% CI 1.8-2.6). Additionally, population-based results suggest that Genital Warts, Genital Herpes, and *C. trachomatis* significantly increased the risk of preeclampsia (OR 3.0, 95% CI 1.0-8.8; OR 7.4, 95% CI 1.4-47.4; OR 5.2, 95% CI 1.3-20.2, respectively), after adjusting for socio-demographic, behavioral, and infection-related risk factors.

Public Health Relevance: Given the widespread prevalence of these infections, and the potential to prevent infection, our findings have important public health implications in the context of potential preventive strategies and identification of high-risk individuals.

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1. INTRODUCTION

During pregnancy, a woman's body undergoes a number of physiological adjustments in order to provide for the growth and development of her fetus, while at the same time maintaining homeostasis. Every organ system is affected, including her immune system. Concurrently, the fetus is an active participant in trans-placental exchange of resources by altering its own development as its regulatory processes matures. The physiological tug of war that ensues is complex, interrelated, and changes constantly throughout pregnancy. Any aberration to this system can adversely affect the progress and outcome of the pregnancy. Such an aberration is found in women who develop preeclampsia.

The American College of Obstetrics and Gynecology (ACOG) defines preeclampsia as a systolic blood pressure greater than 140 mmHg or a diastolic blood pressure greater than 90 mmHg accompanied by proteinuria of 300 mg/24 hr urine collection, or more than 2+ on a voided or 1+ on a cathetherized random urine specimen, in a previously normotensive woman. In the past, a major weakness in the preeclampsia literature has been the lack of a gold standard definition for preeclampsia. This is illustrated by a preeclampsia literature that is saturated with diverse terminologies to describe this disorder. For example, terms such as toxemia, pregnancy induced hypertension, preeclampsia, and preeclamptic toxemia, have all been used in the literature to describe hypertension in pregnancy.[32] Lack of universal agreement on preeclampsia diagnosis has significantly increased the likelihood of case misclassification, and may have led to

varying measures of strength of association between exposure and disease across studies, even among those studies evaluating identical parameters.

Furthermore, preeclampsia researchers have primarily focused on characterizing physiological processes in patients known to have preeclampsia, but unfortunately, it has been impossible to determine whether these processes cause preeclampsia or are the result of the disease itself. In addition, many early investigations were retrospective and thus were not able to establish the nature of the relationship between risk factor and disease. Nevertheless, many theories of probable causes have emerged over the years, but unfortunately, some were not grounded in sound biological principles. In order to prevent a disease, it is necessary to know what the etiology and pathogenesis of the disease is and whether there are methods and tools to help predict what population is at greater risk.

Preeclampsia is distinguished from other hypertensive disorders of pregnancy such as chronic hypertension and gestational hypertension, by the presence of protein in the urine. In the past, known risk factors associated with proteinuria, including diabetes, smoking and obesity, were not controlled for in some studies of preeclampsia pathogenesis. In addition, it is now recognized that preeclampsia is not a one-disease disorder, but rather a clinical syndrome. The possibility that several factors interact to cause this disorder further complicates efforts to find both its prevention and cure.

Atherosclerosis, inflammation and endothelial dysfunction have been suggested as central to the pathogenesis of preeclampsia. The observed inflammatory response in both diseases may be induced by a number of factors. For example, Syndrome X (adiposity, hyperinsulinemia, hyperglycemia, and elevated blood pressure) is now strongly implicated in both diseases, [33] and syndrome X involves inflammation.[45] [158] Pro-inflammatory mediators may also be induced by

infection. Because some infectious diseases are highly prevalent in the general population, pregnant women will continue to be at risk for acquiring new infections during pregnancy. For example, herpes virus infections such as HSV-1 and HSV-2, or CMV establish lifelong persistent asymptomatic infections, and if acquired during pregnancy can cause a number of adverse pregnancy outcomes such as, neonatal herpes, intrauterine growth restriction (IUGR), miscarriage, and preterm labor.

Endothelial dysfunction and excessive inflammation are common occurrences in preeclampsia, and we know that infection clearly triggers an inflammatory response in infected hosts. So, taken together, it is reasonable to suggest that for at least some of the pathways preceding onset of preeclampsia, that infectious agents may be contributing factors. Therefore, the basic thesis of this dissertation is that maternal infections may stimulate upregulation of proinflammatory cytokines in preeclamptic pregnancies, resulting in vascular injury. If this thesis is correct, then, the infectious disease hypothesis for preeclampsia, like that of atherosclerosis, could be pursued more intensely. But more appropriately, the knowledge gained from continued research may be of widespread usefulness in understanding the pathogenesis of preeclampsia, and may lead to development of preventive and therapeutic strategies in the future.

2. BACKGROUND

2.1. DIAGNOSIS AND CLASSIFICATION

An important consideration in classifying hypertensive disorders of pregnancy is the differentiation between pre-pregnancy hypertension and new onset hypertension. There are currently four different types of pregnancy-associated hypertensive disorders (Table 1): 1) pregnancy-associated hypertension; 2) pre-existing hypertension; 3) preeclampsia superimposed upon chronic hypertension; and 4) eclampsia.

Table 1: Classification of Hypertensive Disorders of Pregnancy

DISORDER	DEFINITION	INCIDENCE
Pregnancy-associated hypertension	New onset hypertension that occurs after 20 weeks of gestation and settles within 6 weeks of delivery, characterized by a rise in blood pressure to >140/90 mmHg	6-7% of pregnancies 2-8% of pregnancies Hypertension with proteinuria(>0.3 g/day)
Pre-existing hypertension	Chronic hypertension diagnosed before pregnancy or earlier than 20 weeks of gestation, and persisting after delivery, characterized by a rise in blood pressure to >140/90 mmHg	3-5% of pregnancies
Preeclampsia superimposed on chronic hypertension	As Above	15-25% of hypertensive pregnancies
Eclampsia	Generalized convulsion during hypertensive pregnancy, labor, or within 7 days of delivery	0.05% of pregnancies

Source: Data from Mortl MG and Schneider MC 2000 , Walker JJ.2000 (modified table).

Gestational hypertension is defined as a systolic blood pressure >140 mmHg or diastolic blood pressure >140 mmHg that develops before 20th week of gestation without the occurrence of proteinuria, and is generally considered benign.(66) This diagnosis is nonspecific and can include women who may eventually develop preeclampsia and women who manifest preeclampsia symptoms without proteinuria. Final diagnosis is made after delivery when it is determined whether the woman had preeclampsia.(203)

Preeclampsia is defined as a systolic blood pressure greater than 140 mmHg or diastolic blood pressure greater than 90 mmHg after 20th weeks gestation in a previously normotensive woman, and the occurrence of proteinuria in the last three or four weeks of pregnancy. Proteinuria is defined as urinary excretion of protein (≥ 0.3 g) in a 24-hr specimen.

Chronic hypertension is defined as systolic blood pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg, or both that occurred before pregnancy and is present before 20th week of gestation, or persists longer than 12 weeks postpartum.

Preeclampsia superimposed on chronic hypertension is diagnosed when a woman with chronic hypertension develops new onset proteinuria after 20th weeks of gestation.

Eclampsia occurs in a woman whose preeclampsia has advanced to a more severe stage and involves seizures that cannot be attributed to other causes.

The incidence of preeclampsia among chronic hypertensive and gestational hypertensive pregnancies are 3 to 5 percent and 6 percent respectively. (106) The incidence of preeclampsia appears to have increased in recent years. This is likely due to an increase in numbers of pregnancies among older women, and a rise in numbers of multiple births, both of which are associated with high risk of preeclampsia. For example, according to the National Center for Health Statistics, in 1998, birth rates among women ages 30-44 and number of births to women

45 and older reached the highest levels in 30 years. In addition, overall twin births increased almost 50%, and significantly larger increases were observed in women ages 45 to 49. [190]

In contrast, the incidence of eclampsia (0.05%) has declined considerably in the past several decades. Data from the United Kingdom [52] indicate a 20-fold decline in eclampsia incidence since 1922. Similarly, data from Australia, and New Zealand, [39] show declines in eclampsia incidence occurring between the years 1928 and 1933 (3.2/1000), and from 1956 to 1958 (0.8/1000). This decline is primarily an artificial one and reflects the practice of early delivery or cesarean sections required in most preeclamptic pregnancies. Early delivery and cesarean sections have made it significantly less likely that pregnancies complicated by preeclampsia will progress to eclampsia.

2.2. CHALLENGES IN PREECLAMPSIA RESEARCH

A major challenge confronting researchers and practitioners is the fundamental philosophy that forms the guiding force in their respective disciplines, that is, the conduct of research versus delivery of patient care. For example, preeclampsia diagnostic criteria have in recent years taken either a restrictive or an inclusive approach. Restrictive diagnostic criteria, consider both new onset hypertension after 20 weeks and the presence of proteinuria greater than 3 g/24 hours. The inclusive approach, on the other hand, takes a much broader approach in defining preeclampsia. Proponents of the inclusive concept argue that diagnosis should factor in the multi-systemic nature of the disease and monitor the whole spectrum of symptoms and organs affected. Although it may be justifiable for clinicians to adopt an inclusive approach in clinical practice, a more restrictive approach is likely to reduce misclassification bias that may be introduced with an inclusive classification system. Resolving the conflicts posed by the two extremes of the

diagnostic spectrum, especially in the context of misclassification bias, is a critical factor that may ultimately affect translation of research findings into clinical practice.

The lack of consistency in defining preeclampsia has led to major confusion and disagreement among investigators. Discrepancies in hypertension classification have invariably revolved around whether or not proteinuria was included in the diagnosis of preeclampsia. For example in a review by Chappell and colleagues, [30] 73 journal articles were evaluated for a preeclampsia definition. Of the 73 journal articles, 67 used a general definition of preeclampsia. Of these 67 articles, 47 included a definition of preeclampsia, but the definitions varied considerably. Clear definition of preeclampsia that is uniformly used may prevent inclusion of patients who do not have preeclampsia or patients whose diagnoses are unclear. But most importantly, a standardized preeclampsia classification system will make comparisons between studies possible.

Some studies used diastolic BP and systolic BP inconsistently in the diagnosis. With the exception of one study, which used a blood pressure threshold of greater than 145/85 mmHg, most used a threshold of 140 mmHg for SBP and 90 mmHg for DBP. Finally, proteinuria measurement varied from study to study as well. Twenty nine articles used a proteinuria measurement of 0.3 gr/24 hr, 4 articles used 0.5 gr/24 hr, and others used dipstick analysis alone.[30] Dipstick measurements of protein in the urine have not been found to correlate well with 24-hour urinary collection protein excretion values in pregnant women.[120][95] Such inconsistencies have resulted in considerable disparities in incidence numbers among studies.

2.3. EPIDEMIOLOGY: PREECLAMPSIA INCIDENCE

Preeclampsia occurs in 3-10% of all pregnancies world-wide and in approximately 2-8% in the United States. [44] [79] Incidence varies depending on institution, diagnostic criteria, and patient population. For example, in a summary of a national hospital discharge database, the rate of preeclampsia was 2.6% and 0.6% for eclampsia, [155] whereas the Maternal Fetal Medicine Network trial of low-dose aspirin in preeclampsia estimated a 6.3% incidence of preeclampsia.[169] Disparity in preeclampsia incidence often stems from the use of hospital discharge diagnoses. In a validation study conducted by Ales and Charleson,[4] 25% of preeclampsia diagnoses were incorrectly determined by ICD9 codes, and 53% of ICD9 coding missed true preeclampsia diagnoses. In the case of The Maternal Fetal Network trial, only primiparous women were included despite the fact that they are five times more likely to develop preeclampsia in comparison to multiparous women. [114]

Disparities in incidence figures can also be the result of inclusion of women with superimposed preeclampsia or women with underlying maternal disease such as, chronic hypertension and diabetes mellitus. Inclusion of a higher risk population may significantly increase incidence estimates. In fact, underlying maternal morbidity may account for 20% to 40% of preeclampsia. [32][110][171] Moreover, mothers with underlying maternal disease may be better able to navigate the healthcare system, and possibly may have been over-represented in maternal and perinatal morbidity statistics. [129]

As of February 2004, 281 patients (including primiparous and multiparous gravidas) enrolled in the Prenatal Exposures and Preeclampsia Prevention (PEPP) project at Pittsburgh's Magee-Womens Hospital had a discharge diagnosis of preeclampsia. The overall rate of preeclampsia is 6.9% (205/2955) among nulliparous and 2.6% (76/2955) among multiparous women delivering at Magee. Magee's total obstetrical population comes from two sources: 1)

Magee's prenatal clinics (longitudinal cohort, N= 2211), and 2) private physician practices (crosssectional cohort, N= 744). The incidence of preeclampsia among nulliparous patients in the longitudinal cohort is 1.4% (31/2211). In contrast, the incidence of preeclampsia among the cross sectional participants is 23.4% (174/744). These contrasting incidence numbers clearly demonstrate that preeclampsia rates can vary widely depending on which subgroup of the population is being studied.

In comparison to preeclampsia incidence figures generally reported in the literature (3-10%), the incidence at Magee is significantly lower (1.4%) among nulliparous patients. This is likely a reflection of Magee's strict diagnostic criteria or due to characteristics of the patient population. Patients who volunteer to participate in research studies at Magee may be different from the general population on important characteristics that may affect preeclampsia incidence rates.

2.4. EPIDEMIOLOGY: PREECLAMPSIA RISK FACTORS

Preeclampsia is a serious complication of pregnancy affecting primarily first pregnancies. Other risk factors include multiple gestation pregnancies, previous history of preeclampsia, maternal age < 20 and >35, race, family history, chronic hypertension, obesity, and diabetes. (196)(44)(168)(169)(3)

2.4.1. Primiparity

Primiparous women have consistently been found to be at significant risk for developing preeclampsia, whereas multiparous women with no previous history of the disease are rarely affected. For example, MacGillivray found a preeclampsia incidence of 5.6% among nulliparas and only a 0.3% incidence among multiparas in a Scotish cohort consisting of 5,878 women.(114) In another population study, Seidman and colleagues reported a 2.8% preeclampsia incidence in

a cohort of 5,591 nulliparous Israeli women. [164] At present, it is still not clear why primiparous women are more likely to develop preeclampsia. Because preeclampsia is a disease of placentation, it is possible that first pregnancy placentation may be distinctive enough to be a potentially relevant factor in the origin of this disease.

In summary, numerous studies have consistently implicated primiparity as a major risk factor for preeclampsia.

2.4.2. Multiple Gestation Pregnancies

Maternal conditions associated with excessive placental size, such as multiple gestation pregnancies, [26] hydatidiform moles, [135] and hydrops fetalis [163] have all been implicated with an elevated risk for preeclampsia. Zhang et al, in an analysis of pooled results from 6 studies of the relationship between multiple gestation pregnancies and the risk of preeclampsia, found that in comparison to singleton pregnancies, women with twin pregnancies were three times more likely to develop preeclampsia.[205] In a review of a population databank from Scotland, [27] the impact of parity on the risk of preeclampsia was evaluated. Among twin gestations, both primiparous (OR 3.41, 95% CI 2.9-4.1) and multiparous women (OR 7.29, 95% CI 6.0-8.9) were at increased risk of developing preeclampsia. Similarly, in a population-based cohort study (N=3407), Coonrod et al found that among twin gestations, the risk of preeclampsia was 4 times higher in nulliparous compared to multiparous women.[38] The same study suggested that advanced maternal age coupled with multi-fetal gestations significantly increased the risk of preeclampsia.

A recent analysis of prospective data from two trials of low-dose aspirin use found that women with twin pregnancies (N=684) were twice more likely to develop preeclampsia (RR 2.48, 95% CI 1.82-3.38), when compared to singleton gestation pregnancies (N=2946). This

finding tends to hold true even after adjustments for age, race, mean arterial pressure (MAP), and smoking. [169]

Thus, the epidemiologic evidence so far suggests that multiple gestation pregnancies are at significantly higher risk of preeclampsia compared to singleton pregnancies.

2.4.3. Previous History of Preeclampsia

Women who experienced preeclampsia in a previous pregnancy are at substantially increased risk of preeclampsia in subsequent pregnancies. For example, Lie and colleagues reported a 1.7% risk of preeclampsia among second pregnancies in a Norwegian population-based study of women with no prior history of preeclampsia. [108] If women had preeclampsia in the first pregnancy, risk was substantially increased (13.1%). Furthermore, the risk of preeclampsia in a subsequent pregnancy was higher with increasing severity of the disease, and particularly high if preeclampsia developed before 30 weeks' gestation [47] in the index pregnancy. In a prospective investigation conducted by Sibai and colleagues, out of 125 women with severe preeclampsia in the second trimester, 35% had normal blood pressure during a subsequent pregnancy and almost twice as many (65%) developed preeclampsia. Furthermore, of those who developed preeclampsia one third developed preeclampsia at ≤ 27 weeks, one third at 28 to 36 weeks, and another one third at ≥ 37 weeks. [166]

Visser et al found that the severity of preeclampsia was also related to levels of proteinuria. For example increases in proteinuria of 0.3-3 gr/24 hrs were associated with a preeclampsia recurrence risk of 12% and almost twice as high (22%) when proteinuria levels were ≥ 3 g/24 hrs in the index pregnancy. [192]

In summary, the relationship between previous history of preeclampsia and recurrence of the disease is well documented. Having had preeclampsia in a previous pregnancy increases the likelihood that a subsequent pregnancy will also be affected by preeclampsia.

2.4.4. Age

Young age (under 20) has frequently been reported as risk factor for preeclampsia.[155] This finding has not been replicated in all studies. It is possible that the observed age-associated risk is due to the fact that younger women are more likely to be primiparous, and therefore parity may be the underlying mechanism that places younger women at risk for preeclampsia. However, a nested case control study by Mittendorf et al, revealed no association between young maternal age (less than 19) and preeclampsia.[125] Furthermore, several population studies that adjusted for parity did not indicate that younger women were at a greater risk of preeclampsia.[76] [160]

There seems to be consistent evidence, however, that older women (over 35) are at increased risk of preeclampsia. [149] In the same study mentioned above, Mittendorf, et al found that women older than 34 were at increased risk of preeclampsia (OR 2.5, CI 1.8-3.5) in univariate analysis, but after adjusting for parity, no independent effect of advanced maternal age was observed. In a review of the literature on older maternal age and pregnancy outcome, Hansen JP found that in comparison to younger women, older women were two to four times more likely to develop preeclampsia.[73] Similarly, in a Finnish population study, Hartikainen A-L et al showed that women older than 35 were at higher risk (OR 2.5, 95% CI 1.5-4.1). [76]

Despite many reports linking advanced maternal age with increased risk of preeclampsia, it is difficult to determine with certainty whether older women are indeed at greater risk compared to younger women. Some studies have not adjusted for parity and those that have, found parity

and other factors such as multiple pregnancies confounded the relationship between age and preeclampsia. In addition, Chesley suggested that the association between older maternal age and preeclampsia is likely due to the tendency of older women to have essential hypertension. [32] It is possible that the observed association between advanced maternal age and preeclampsia may be due to the higher frequency of multiple pregnancies in older mothers, which places older mothers at greater risk.

2.4.5. Race

Many studies indicate that race is associated with increased risk of preeclampsia. [130] For example, Eskenazi et al,[55] in multivariate analyses of preeclampsia risk factors showed that black race was a significant risk factor for preeclampsia but only in nulliparous women (adjusted OR 12.3, 95% CI 1.6-100.8). In 1992, Savitz, in a study of pregnancy outcomes derived from North Carolina's 1988-1989 vital statistics data, found similar preeclampsia incidence in both black and white women. [159]

Interestingly, traditional preeclampsia risk factors vary across ethnic groups. For example, Knuist, et al (1998), in a prospective investigation of preeclampsia predictors found that unlike in white women, increase in diastolic blood pressure did not predict preeclampsia in black women, but increased maternal age was more likely to be a predictor among blacks. The observed race-associated relationship may be attributed to the tendency of black women to also suffer from chronic hypertension, and obesity. [94]

In summary, the available epidemiologic evidence suggesting black race as risk factor for preeclampsia is currently unclear. Many studies suggest that black women are twice more likely to develop preeclampsia compared to white women. However, it should be noted that many of

these studies did not consistently account for potential confounders including sociodemographic factors, obesity and chronic hypertension.

2.4.6. Family History

Several lines of evidence suggest that preeclampsia may be an inheritable disorder, but the exact inheritance pattern has not yet been found. [7]

For example, Chesley and colleagues reported a 26% incidence of preeclampsia in daughters of women with preeclampsia, but only 8% incidence in the daughter-in-law. [31] Similarly, data from a pregnancy cohort in Scotland that had been followed for several decades found that preeclampsia was more likely to occur in sisters (2.5- to 3.4-fold), mothers (4-fold), and mothers-in-law (4.4%) of women who have had preeclampsia, compared to mothers of controls (3.5%).[182]

These findings were replicated in a prospective investigation of 368 primigravid women. Eighteen women (18/368) reported having a mother and, or sister who developed preeclampsia. Of this eighteen, 27.8% (5/18) developed preeclampsia compared to only 8.3% (29/350) with no family history of preeclampsia (RR 3.4, 95% CI 1.5-7.6).[34] In addition, family history was associated with severity of preeclampsia. For example, among women who had a family history of preeclampsia, 22.2% (4/368) developed severe preeclampsia, compared to 5.1% (18/350) with no history of preeclampsia (RR 4.3, 95% CI 1.6-11.5). [34][47]

Similarly, Arngrimsson et al studied the genetic and familial predisposition to preeclampsia in a defined population in Iceland. In this study, inheritance patterns in four-generation families were followed through both sons and daughters. Authors were not able to differentiate between autosomal recessive and autosomal dominant inheritance. [7]

According to a population-based study of approximately 1.7 million births in Norway, [108] a man who has fathered a child in a previous preeclamptic pregnancy is twice as likely to father a preeclamptic pregnancy with a different woman. Interestingly, men whose mothers had preeclampsia while pregnant with them are twice as likely to father a child who is the product of a preeclamptic pregnancy. This suggests a genetic predisposition to preeclampsia that can be transmitted both maternally and paternally.

An additional approach employed in determining a potential genetic influence on preeclampsia has been the study of frequency of preeclampsia in different twin zygosity groups, but so far findings have been inconclusive. In two studies of monozygotic twins, none of 10 twin pairs were found to be concordant for preeclampsia. [185][184]

In summary, a familial predisposition to preeclampsia has been documented in many studies, suggesting that genetic factors aid in the development of this disorder. However, the study of a genetic predisposition to preeclampsia is hindered by the multi-systemic nature of the disease. For example, there is currently no single agreed upon preeclampsia phenotype. Furthermore, preeclampsia is a pregnancy specific disorder and therefore any potential preeclampsia allele is limited to pregnant women. Considering that delivery of the fetus is the only effective cure for this disorder, expression of a preeclampsia gene may be interrupted in the process. [198]

2.4.7. Chronic Hypertension

Eskanazi (1991), reported in a case control study that preeclampsia was twice more likely to occur in women with a previous history of hypertension.[55] In another study, Rey and Couturier,[147] found that women with chronic hypertension were ten times more likely to develop preeclampsia than normotensive women. Furthermore, perinatal deaths were more

common in women who had chronic hypertension with superimposed preeclampsia (101/1000) compared to controls (12/1000). [147] Page and Christianson prospectively followed a cohort of black (N= 2880) and white women (N=10,074) and divided them into four groups: 1) with chronic hypertension; 2) without chronic hypertension; 3) with superimposed preeclampsia; and 4) without superimposed preeclampsia. Perinatal deaths among women with chronic hypertension were 2-to 3-fold higher compared to normotensives without proteinuria and ten times higher among women with chronic hypertension and superimposed preeclampsia. [135]

In summary, women with chronic hypertension are at substantial risk of developing preeclampsia.

2.4.8. Body Mass Index (BMI)

Several epidemiologic studies indicate that increased maternal adiposity, as measured by high body mass index (BMI) significantly increases the risk of preeclampsia (three- to six-fold increase).[55] Sibai and colleagues, in a large multi-center trial studying the effect of calcium supplementation on the incidence of preeclampsia, found that women with BMI greater than 34 kg/m2 early in the second trimester had the highest incidence of preeclampsia (12.6%).[169] Nulliparous women with this level of BMI were at a 4.9 fold increased risk of preeclampsia, whereas multiparous women were at 5.1-fold increased risk.[181] This association remained significant after adjusting for race, prior history of preeclampsia and clinic service (OR 3.5, 95% CI 1.7-7.5).

In a case control study conducted by Mittendorf and colleagues [125], heavy women (BMI > 30) were at increased risk for preeclampsia (Adjusted OR 2.7, 95% CI 1.6-4.4). Additionally, in a population-based study of 96,801 primiparous women who delivered singleton births in Washington State between 1992-1996, both obese women (BMI ≥ 30) (OR 3.3, 95% CI 3.0-3.7)

and women who were overweight before pregnancy (BMI 25-29) were more likely to develop preeclampsia (OR 2.0, 95% CI 1.8-2.52). [51] In a prospective multicenter study of aspirin use for the prevention of preeclampsia, Sibai and colleagues demonstrated an independent dose-response relationship between relative weight and incidence of preeclampsia in primiparous women. [167]

In summary, evidence from several well-controlled studies indicates that obesity is a significant risk factor for preeclampsia.

2.4.9. Protein Intake

World War I and World War II brought an explosion of reports describing a potential relationship between protein intake and preeclampsia. It was widely speculated that the disease was the result of high intakes of protein, and that evidence for this could be found in the decreased incidence of preeclampsia attributed to food shortages during war years. Chesley questioned the reliability of these findings. He suggested that the observed decline in preeclampsia was more likely due to a decline in primiparous births during the war. Most males remaining at home during this time had large families, and had wives who had pregnancies as multiparas. [31] In addition, many studies did not control for the possible association between protein intake and maternal socio-demographic factors. For example, protein intakes are often associated with socioeconomic factors such as poverty, poor eating and health habits. Furthermore, the exact processes involved in dietary protein break down and synthesis and their potential influence on blood pressure regulation is currently not known.

In summary, due to poor study design and a lack of understanding of the biochemical mechanism underlying the effect of dietary protein metabolism on blood pressure regulation, no firm conclusions on the link between dietary protein and blood pressure can as yet be made. [134]

2.4.10. Diabetes

Diabetes is also a risk factor for preeclampsia. The exact mechanism driving this relationship is not known, but obesity and insulin resistance may be predisposing factors. For example, several lines of evidence suggest an association between obesity, type-2 diabetes, insulin resistance, and hypertension. [143][144][111][126][122] Among 140 nulliparous black women with known fasting glucose and insulin, Sowers et al found fasting insulin levels to be 1.8-fold higher in preeclamptics compared to normotensives.

Garner et al, in a prospective study of 334 pregnant diabetic women, reported a preeclampsia incidence of 10% among diabetics compared to 4% in controls. [66] Furthermore, the rate of preelampsia among diabetics was elevated with increasing severity and duration of diabetes, from 9% among diet-controlled gestational diabetes to 30% among women with White classes D, F, and R. [66][129]

In summary, women with pregestational diabetes are twice more likely to develop preeclampsia compared to women with gestational diabetes.

2.4.11. Cigarette Smoking

Smoking has consistently been associated with a reduced risk of preeclampsia, even after cessation of smoking. At present, the exact mechanism underlying this association is not known, but suggestions made in the past attribute this protective effect to decreased expansion of plasma volume among smokers, [114] hypotensive effects of toxic byproducts (thiocyanate) of smoking, and to vasoconstriction and platelet aggregation resulting from nicotine-induced inhibition of thromboxane production. [93][204]

Cnattingius et al, in a population-based study of 317,652 nulliparous women aged 15 to 34 years who delivered singleton pregnancies in Sweden from 1987 through 1993, found that

maternal smoking was associated with significantly reduced risks of mild and severe preeclampsia (RR=0.6 and 0.5, respectively). However, smoking at least 10 cigarettes per day was associated with increased rates of perinatal mortality (from 24 to 36 per 1000), abruption placentae (from 31 to 67 per 1000), and small for gestational age (from 28% to 68%). [37]

A dose response relationship was reported in a prospective study that used data from the Collaborative Perinatal Project. After controlling for prepregnancy body mass, age, socioeconomic status, and race, both past smoking and smoking during pregnancy was associated in a dose response pattern with reduced risks of gestational hypertension and preeclampsia. For example, among women who smoked ≥ 10 cigarettes/day, the relative risk of preeclampsia was 0.5 (95% CI 0.4-0.7) in comparison to nonsmokers. [206]

Similar findings were observed in another population-based study comprising the National Birth Registry of Sweden in 1993 and data collected from 1990 to 1994 at the Malmo University Hospital. Multivariate regression analysis for the University-based study showed that, in comparison to non-smokers, moderate smokers (1-9 cigarettes per day) had a lower incidence of preeclampsia (OR 0.4, 95% CI 0.2-0.6). This finding was replicated in the National series, in which preeclampsia incidence was also significantly lower for moderate smokers (OR 0.6, 95% 0.5-0.7). [109]

In summary, the evidence suggesting a reduced risk of preeclampsia associated with smoking has been consistent. This protective effect remains even after cessation of smoking. Further research is needed to reveal the mechanism for the smoking effect.

2.4.12. Prevention Studies

Preeclampsia prevention studies are hindered by the lack of knowledge of the precise pathohysiologic mechanisms underlying this disorder. Therefore, prevention strategies may not alter development of disease, but merely treat its symptoms. Another limitation is over-reliance on lowering of high blood pressure as a target of prevention. In preeclampsia, elevation of blood pressure tends to occur at a later stage in pregnancy in comparison to other serious early-onset symptoms. Therefore, elevation in blood pressure, by itself, may not be a sensitive marker for the underlying pathophysiology observed in preeclamptic pregnancies. In the past, because of over-reliance on second trimester blood pressure elevations as indicator of preeclampsia, a large number of studies have mistakenly classified gestational hypertension as preeclampsia.

Ideally, in order to predict who will develop preeclampsia, factors involved in the disease process should be identified prior to onset of disease, and should be biologically plausible. This increases the likelihood that treatment strategies will target risk factors that actually matter in the disease process. Moreover, preeclampsia affects but 3% of the population, which means that even if an effective treatment becomes available, the potential exists that a large number of the obstetrical population will be treated unnecessarily. Nevertheless, one could argue that preeclampsia sequelae are sufficiently serious to warrant a search for effective treatment strategies.

Numerous controlled studies targeted oral calcium supplementation as a potential preventive strategy. The rationale for using calcium is based on calcium's potential for lowering blood pressure. A few studies found significant reductions in preeclampsia incidence, but others reported no change. In a meta-analysis of randomized controlled trials, calcium supplementation was found to be highly effective in preventing preeclampsia (OR 0.38, 95% CI 0.22-0.65).[25]

This study showed a statistically significant 1.68 mmHg decrease in systolic blood pressure among hypertensive women supplemented with 1 g of calcium per day. These results were not replicated, however, in a subsequent large double-masked trial (RR 0.94, 95% CI 0.76-1.16) [107] and six other double-masked studies. Authors of the meta-analysis questioned the clinical significance of a 2 mmHg reduction in systolic blood pressure in the absence of a reduction in diastolic pressure, and went on to recommend calcium supplementation only for women who would benefit the most from supplementation, such as women with osteoporosis.

Aspirin has long been suggested as another approach to treating preeclampsia. A number of early clinical trials and meta-analyses have suggested that low dose aspirin is effective in preventing preeclampsia. The rationale for this suggestion is based on the observation that preeclampsia was found to be associated with disturbances in prostanoid and platelet function, [14][197][160][15][115][188][78][46][36] and that aspirin may be effective in decreasing platelet thromboxane synthesis while maintaining vascular wall prostacyclin synthesis. [46][35][137] For example, Sibai et al, in a randomized multicenter study of 3,135 nulliparous women, found that preeclampsia incidence was 26% lower in women who received 60 mg aspirin daily compared to placebo group (RR 0.7, 95% CI 0.6-1.0). [167] In a multicenter study conducted in England (Collaborative Low-dose Aspirin Study in Pregnancy (CLASP)) (N=6,927), the use of aspirin (60 mg daily) was associated with a reduction of 12% preeclampsia incidence. These findings were contradicted by several subsequent randomized trials, which suggested that low dose aspirin had little effect on preeclampsia incidence. [68][152]

The Jamaica Low-dose Aspirin Study Group, found no differences in preeclampsia incidence between primiparous women (N= 6,275) randomized to 60 mg daily of low-dose aspirin and those on placebo (OR 1.15, 95% CI 0.92-1.44) [68]. Similarly, Rotchell et al, in a

randomized trial of 3,647 women to 75 mg controlled-released aspirin or placebo, found no difference between treatment and placebo groups (2.2% in the treatment group and 2.5% in the placebo group developed preeclampsia.[152] One major criticism of these trials was their enrollment of low-risk women. Enrollment of a low risk population may have made it difficult to detect any effect of low-dose aspirin on preeclampsia incidence. In response to this criticism, the National Institute of Child Health and Development (NICHD) in 1998 sponsored a multicenter randomized trial of low-dose aspirin. [28][132] In this trial, high-risk preeclamptics including 471 women with pre-gestational insulin-treated diabetes mellitus, 774 women with chronic hypertension, 688 women with multifetal pregnancies, and 606 women with a history of preeclampsia during a previous pregnancy, were randomized to 60 mg low-dose aspirin daily or placebo. Results showed no beneficial effect of low-dose aspirin in any of the preeclampsia high-risk subgroups.

In summary, evidence from large clinical trials, so far, do not indicate that routine calcium or aspirin supplementation reduce the incidence of hypertension.

2.5. EPIDEMIOLOGY OF HERPES SIMPLEX VIRUSES

2.5.1. Herpes Simplex Types 1 and 2

HSV-1 and 2 are members of the alpha Herpesviridae family of viruses. HSV-1 infects the oropharynx, and is usually not transmitted by genital contact. [128][41][118] It is estimated that 90% of people worldwide are seropositive for HSV-1 by the fourth decade of life, especially those of lower socioeconomic groups. [40] For example, seroprevalence surveys of western populations in the post-World War II era found that 80 to 100 percent of middle-aged adults of lower socioeconomic status were seropositive for HSV-1, as compared to 30 to 50 percent of adults of

higher socioeconomic groups.[41] However, there has been an overall decline in HSV-1 seroprevalence in industrialized countries in recent years. For example, 40 to 63% of people are now found to be seropositive for HSV-1. [40] It is unclear, whether the declining rates are due to changes in hygiene or to a protective effect imparted by previous infection with HSV-2. In a prospective study by Langenberg et al, approximately two-thirds of new HSV-1 infections were symptomatic and genital infections due to HSV-1 were as common as oropharyngeal infections (0.5 cases per 100-person years).[105] In the United States, over 600,000 new cases of herpes infection are anticipated every year .[202]

In contrast to the HSV-1 declining rates, seroprevalence surveys indicate that HSV-2 infections have been increasing rapidly over the past several decades. US population-based surveys indicate that HSV-2 seroprevalence has increased from 16.4% [89] between 1976 and 1980 to 21.7% [60] between 1988 and 1994. Furthermore, HSV-2 prevalence varies considerably depending on the population studied. For example, among STD clinic patients, HSV-2 seroprevalence ranges from 8% to 83%, [128][42][77][44][41][124] among prostitutes from 75% to 96%, [128][41][59] and in blood donors from 5% to 18%.[42][60][44][41][59] It is difficult to determine whether the observed increase in HSV-2 infections is due to actual increases in incidence or to improvement in diagnosis and treatment. Also, assessment of the extent of HSV-2 infection is made difficult because HSV-2 is not a reportable disease in most states. Furthermore, most people are asymptomatic and thus are unaware that they are infected.

One can differentiate between the two viral types based on the region of the body where they establish latency. HSV-1 normally resides in the trigeminal ganglion in the vicinity of the ear. HSV-2 establishes latency in the sacral ganglion at the base of the spine. Both viral types, however, are capable of straying away from their usual location. Symptoms of infections are less

severe when the virus establishes latency away from its traditional site of residence. Severity of infection is also affected by the host immune system and duration of infection. A person is considered to have a primary HSV infection, if antibodies to HSV antigens were absent in an initial serum sample and were later found to be present in a subsequent sample. The symptoms of primary infections with HSV-1 and HSV-2 are similar, but a primary HSV-2 infection is usually more severe and reactivates with 16-fold greater frequency (Lafferty WE, et al 1987). Number of sexual partners, age of first sexual intercourse, and a history of other STDs are all consistent risk factors for HSV-2 infection.

Serological studies are critical to the documentation of HSV infection because as many as 75% of infected individuals acquire genital HSV-2 infection silently or have initial symptoms that are nonspecific. [20]

2.5.2. HSV Types 1 and 2 infection in pregnancy

Between 6-50% of women attending antenatal clinics are seropositive for HSV-2 (Table 2). Although, neither HSV-1 nor HSV-2 poses major health threats, a first episode of genital herpes close to labor increases the risk of neonatal herpes. This is primarily because of a lack of HSV antibodies in maternal blood stream. Mothers with recurrent HSV infection have ample antibodies in their blood stream to protect the fetus, however, discerning a primary HSV infection from a recurrent one requires careful consideration. For example, in a study of 29 pregnant women who were presumed to have primary HSV infection based on symptomatology alone, only 4 (14%) were found to actually have first episode disease as determined by type-specific HSV serologic assay. [81] In addition, severity of infection may also be influenced by pre-existing immunity to HSV infections. For example, studies have shown that prior HSV-1

infection may impart protection against HSV-2 acquisition. [24][118][177] Nonetheless, primary infection appears to have the greatest influence on adverse pregnancy outcomes.

Several culture and serology studies have suggested an increased risk of adverse pregnancy outcomes, including spontaneous abortion, preterm birth, and intrauterine growth restriction in pregnancies complicated by primary genital herpes infection [21][20][128][199][202]. For example, in a study conducted by Brown and colleagues at the University of Washington, 7,046 patients susceptible to HSV infection showed that neonatal herpes occurred significantly more often among women with new serologic evidence of HSV infection at the time of labor (4 of 9) compared to those whose primary episode had concluded prior to labor (0 of 94) ($p<0.001$). [20] The incidence of neonatal herpes is influenced by the socioeconomic status, age and past sexual activity of the population studied.

Table 2: HSV-2 Seroprevalence in Antenatal Clinic Attenders

CITY	NUMBER TESTED	% POSITIVE	TEST	REFERENCE
Tokyo	90	6	gG2 Immunodot	Hashido M, et al (1990)
Padua, Italy	NK	8.4	gG2 Immunodot	Nahmias AL, et al (1990)
Seville, Spain	NK	9.7	gG2 Immunodot	Nahmias AL, et al (1990)
Birmingham, Alabama (whites)	NK	11.4	gG2 Immunodot	Nahmias AL, et al (1990)
Taiwan	NK	13.5	gG2 Immunodot	Nahmias AL, et al (1990)
Sydney	229	14.5	gG2 ELISA	Cunningham AL, et al (1993)
Stockholm 1969	941	17	gG2 ELISA	Forsgren M, et al (1994)
Lyon	NK	17.3	gG2 Immunodot	Nahmias AL, et al (1990)
Rejkjavik, Iceland	NK	18.8	gG2 Immunodot	Nahmias AL, et al (1990)
Stockholm 1983	1759	32	gG2 ELISA	Forsgren M, et al (1994)
Stockholm 1989	1000	32	gG2 ELISA	Forsgren M, et al (1994)
Stanford, USA 1991	277	32	gG2 ELISA	Kulhanjian JA, et al (1992)
Atlanta (whites)	NK	34.9	gG2 Immunodot	Nahmias AL, et al (1990)
Sao Paulo 1988-1989 (low and middle class)	455	36	ELISA and Western blot	Weinberg A, et al (1993)
Seattle, USA 1990	201	37.8	Western blot	Brown ZA, et al (1995)
Sao Paulo 1988-1989 (very low income)	200	42	ELISA and Western blot	Weinberg A, et al (1993)
Atlanta (blacks)	NK	53.4	gG2 immunodot	Nahmias AL, et al (1990)
Pittsburgh, PA 2002	200	25	gG2 ELISA	Unpublished

NK= not known; ELISA = enzyme immunoassay; gG2 = glycoprotein G2

2.5.3. Cytomegalovirus (CMV)

CMV is a member of the beta herpes virus subfamily. CMV is common across geographic locations and socioeconomic groups (Table 3). In industrialized countries CMV infects approximately 50% of the adult population, and in underdeveloped countries an even greater proportion of the population (90%) are infected by age 2. [(49)] In the United States CMV infects between 50% and 85% of adults by age of 40. Common risk factors include: 1) non-white population; 2) low income; 3) breast feeding; 4) group care of children; 5) crowded living conditions and 6) sexual activity. Several sources, including estimates from large studies of blood donors, hospital workers and pregnant women, suggest an overall incidence of CMV

infection in adults at 1-2% per year. [176][11][10][70][179] Transmission of the virus generally requires close contact with infected persons excreting the virus in saliva, urine, or other bodily fluids. CMV can also be transmitted sexually, via breast milk and transplanted organs (CDC).

2.5.4. CMV infection in pregnancy

Cytomegalovirus (CMV) represents the leading cause of congenital viral infection in the US. CMV is one of the most serious causes of morbidity in newborns, and in many instances manifestation of illness does not become apparent until school age. For example, studies in both Sweden and the US indicate that a large number of infants with congenital CMV infection go on to develop sensorineural hearing loss. [75][84] It is estimated that in the US, the overall rate of congenital CMV infection is at 1% of live births, which amounts to about 40,000 new cases per year, [17] of whom approximately 10% are clinically apparent at birth. [64] Approximately 50% of infants with symptomatic and 15% of infants with asymptomatic congenital CMV infection will experience hearing loss. [64]

Throughout pregnancy, women can acquire either a primary or recurrent CMV infection. Primary infection can best be determined by seroconversion of IgG antibodies to CMV, ideally, with at least two serum samples during pregnancy. The presence of IgG and IgM antibodies to CMV in an initially seronegative serum sample is usually considered good evidence of a primary infection. However, the presence of antibodies may also be an indication of a prior infection. A recurrent infection is defined as the presence of IgG antibodies before conception. Primary infection can be transmitted by saliva, breast milk, cervical secretions at birth, sexual intercourse, and blood transfusion. In the US approximately 10% of women shed CMV at the time of delivery, rates as high as 40% have been reported in Taiwanese women. [165] Re-infection, however, usually does not have severe pathologic consequences. For instance, Adler et al [2]

showed that seropositive women were 90% resistant to infection by contact with CMV-shedding children, and along the same lines, Fowler et al [64] found that prior infection reduced maternal fetal transmission from 25% to 1% of pregnancies. Interestingly, several studies suggest that the rate of congenital infection is directly related to the prevalence of maternal CMV infection. For example, in populations with high CMV prevalence among women of childbearing age, the rate of congenital CMV infection tends to be high as well (Table 3). These findings, suggest that presence of antibodies to CMV does not necessarily prevent maternal transmission of the virus to her fetus, but does seem to prevent the fetus from developing serious disease. Primary infection is more likely to have serious health consequences for the fetus.

Table 3: Rates of Congenital CMV Infection In Various Populations In Relationship To Prevalence Of Maternal Seropositivity

LOCATION	PERCENTAGE MOTHERS SEROPOSITIVE	PERCENTAGE CONGENITAL CMV INFECTION	REFERENCE
Aarhus-Viborg, Denmark	52	0.40	Andersen HK, et al, 1979
Abidjan, Ivory Coast	100	1.40	Schopfer K, et al, 1978
Birmingham, Alabama, USA Low SES Middle SES	 77 36	 1.25 0.53	Stagno S, et al, 1986
Hamilton, Ontario, Canada	44	0.42	Larke RBP, et al, 1980
London, UK	56	0.30	Peckham CS, et al, 1983
São Paulo, Brazil Low SES Middle SES	 84 67	 0.98 0.46	Pannuti CS, et al, 1985
Seoul, South Korea	96	1.20	Sohn YM, et al, 1992

2.5.5. Epstein Barr Virus (EBV)

EBV is a member of the gamma herpes virus subfamily. EBV infection in humans usually occurs by exposure to saliva, mostly during childhood. Latent infection is established in lymphocytes. Unlike other herpes viruses, EBV causes no clinical manifestations in the vast majority of individuals. Once infected with EBV, a latent infection persists for life. In addition, the virus can be shed asymptomatically and thus easily spread from person to person through intimate contact, such as kissing, hence the expression "kissing disease." [139] In industrialized countries almost everyone has become seropositive by the age of 25 to 30 years, whereas seroconversion occurs earlier in developing countries. [57] For example, in the US, 50% of the population demonstrates EBV antibodies before the age of 5 years, 90 to 95% by adulthood. [57] EBV initially infects epithelial cells in the oropharynx. The B cells in nearby lymphoid tissue are then infected, and virus disseminates throughout the lymphoreticular system. [139] Like other herpes virus infections, EBV infection can reactivate. During this period of reactivation the virus is shed from the oropharynx, and occasionally from the cervix. [127][140][172][183] EBV has been recovered from genital mucosa of women with acute infectious mononucleosis, suggesting that the virus was disseminated from the oropharynx to distant mucosal sites or that infection was introduced through sexual contact. Consensus has yet to be reached on whether EBV infections can be transmitted sexually.

EBV infection can cause a benign lymphoproliferative disease known as mononucleosis, [121] but is not known to cause pregnancy-associated complications such as miscarriage or birth defects. This may be due to the rare occurrence of primary infections in pregnancy, since most people are already immune by childhood.

2.5.6. EBV infection in pregnancy

Only 1.3 to 4.2% of pregnant women in industrialized countries lack EBV antibodies, which means that primary infections rarely occur during pregnancy. For example, in various serological studies seroconversion ranged from 0.06% to 1.96% in pregnant women. [121][67][61][88]. EBV infections are not normally known to cause adverse pregnancy outcomes, but an early report by Icart and Didier showed correlation between presence of EBV antibodies during pregnancy and fetal abnormalities, but these findings were not replicated in a subsequent investigation. [61] In a more recent serological study designed to investigate mother-to-child EBV transmission, EBV antibodies were detected in 6% of neonates (5/83). Unfortunately, no follow up was conducted to determine if neonates developed abnormalities.

3. PATHOGENESIS OF PREECLAMPSIA

3.1. CYTOTROPHOBLAST INVASION IN PREGNANCIES COMPLICATED BY PREECLAMPSIA

Preeclampsia is a disorder of placentation. Implantation in human pregnancy differs from implantation in other mammals by the occurrence of a second physiologic invasion of the uterine wall. The first implantation occurs in the first 6 to 8 weeks of pregnancy followed by a much deeper cytotrophoblast invasion between the 11^{th} and 16^{th} week of gestation. The key pathological lesion in preeclampsia is the inability of cytotrophoblast to attain sufficient depth during the second invasion. It has been postulated that humans need this deeper physiological invasion to accommodate a larger brain. [116]

As can be seen in fig 1, preeclamptic cytotrophoblast invasion is shallow in comparison to normal pregnancy. Shallow invasion may result in poor vascular exchange between mother and fetus, frequently resulting in intrauterine growth restriction (IUGR) and perinatal mortality.[43] Furthermore, experimental studies have demonstrated the occurrence of incomplete modification of the uteroplacental spiral arteries by invading extravillous cytotrophoblast in the first and second trimesters of preeclamptic pregnancies.[194] Under normal circumstances, such as in normal pregnancy, spiral arteries are transformed from epithelial to endothelial phenotype. The invasion and modification of the maternal spiral arteries to create the uteroplacental arteries by extravillous cytotrophoblast with subsequent endothelial regeneration is a fundamental process in implantation. [91] This transformation does not occur in preeclampsia, causing vessels

to remain thick-walled and muscular. [207] As a result of defective invasion of trophoblastic cells, the maternal spiral arterioles are not transformed into the high volume, low resistance capacitance vessels capable of supplying the placenta with maximal blood flow. [54] The result is a hypoperfused placenta and an imbalance in maternal blood supply. The mother tries to compensate by elevating her blood pressure in an attempt to save her fetus by increasing its delivery of oxygen and nutrients. When she is no longer able to cope with fetal demands, the placenta signals for help by triggering a series of events that leads up to onset of preeclampsia. One such event is believed to be the release of cytotoxic factors that in turn can damage maternal endothelium, however the exact chronology of events is not known.

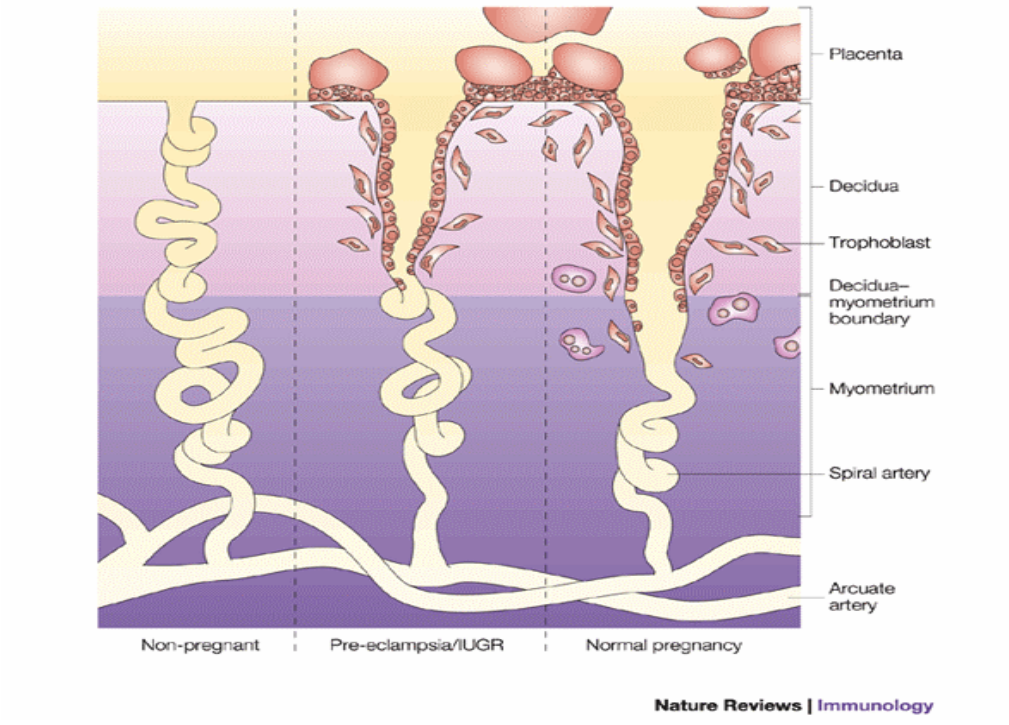

Figure 1: A Comparison Between Uninvaded Arteries (non-pregnant), Normal Pregnancy and Preeclampsia and IUGR

Source: Ashley Moffett-King. Nature Reviews Immunology 2002;2:656-663
Figure used with author's permission

3.2. UP-REGULATION OF INFLAMMATION

Preeclampsia is a multi-systemic disorder of human pregnancy complicating 2-4% of the obstetric population. Preeclampsia is characterized by new onset hypertension and proteinuria after 20th week of gestation in a previously normotensive woman, and is associated with preterm delivery, fetal growth restriction, and abruptio placentae. In the United States, from the years 1979 to 1986, preeclampsia-eclampsia was the second leading cause of maternal death and ranks between the second and third leading cause of maternal death in more recent years. [155] The search for a causative agent and potential unifying pathophysiological mechanism has been ongoing for decades, but what seems to be consistent is the occurrence of endothelial dysfunction in almost all aspects of the disease. In 1989, Roberts et al postulated that the clinical manifestations of preeclampsia, including hypertension, oxidative stress, and proteinuria could be explained by a generalized maternal endothelium dysfunction [150]. Redman, et al extended this hypothesis further by proposing that inflammation provides the oxidative insult to the endothelium seen in preeclampsia [145].

Endothelial cells line inside walls of blood vessels and are responsible for regulation of vascular tone and thrombosis. In preeclampsia serum, there is increased level of thromboxane-A2 and platelet aggregation in endothelial cells, which may affect vascular tone by inducing an imbalance of vasoconstriction and vasodilation, leading to disruption of endothelial cell function [131]. It is unclear, however, whether inflammation occurs before development of preeclampsia or is a consequence of the disease itself. Nevertheless, studies have tested the utility of white blood cell counts as indirect evidence of inflammation. Sacks et al found that normal third trimester pregnancy is characterized by activation of peripheral blood leukocytes, which is further increased in preeclampsia.[153] Similarly, Mellembakken et al found evidence of leukocyte

activation in venous blood from antecubital and uterine veins during cesarean sections in 30 women with preeclampsia [117].

Although studies have demonstrated evidence for an inflammatory response both in normal and preeclamptic pregnancies, in preeclampsia inflammation seems excessive. For example, a number of studies have found increased levels of pro-inflammatory cytokines, such as TNF-α, interleukin 6 (Table 4) and soluble phospholipase A2, as well as activated clotting and complement pathways, at higher levels in preeclamptics than in normotensive pregnancies.

Table 4: Studies Examining The Relationship Between Pro-inflammatory Cytokines and Pregnancy Outcome

REFERENCE/YEAR	NORMAL PREG CYTOKINE LEVELS (IN PG OR ML)			PREECLAMPSIA CYTOKINE LEVELS (IN PG OR ML)			TYPE OF STUDY
	IL-6	TNF-α	N	IL-6	TNF-α	N	
1. Rinehart et al/1999*	0.327	--	4	0.623	--	6	Cross sectional
2. Sanchez et al/2000*	--	694.8	179	--	920.1	125	Case control
3. Amory et al/2001	38,000	3,649	12	45,000	11,243	12	Case control
4. Benyo et al/2001	7.52	0.79	8	8.98	0.80	8	Cross sectional
5. Visser W et al/2002*	--	1.67	21	--	29	21	Case control

* Differences in cytokine levels between preeclamptic and control women were significant

3.3. LACK OF MATERNAL TOLERANCE TO PATERNAL/FETAL ANTIGENS

Under normal circumstances, cell surface antigens called Human Leukocyte Antigens (HLA) do not recognize self-antigens as foreign, and thus do not mount an adverse immune response. But when confronted with paternally derived fetal antigens, the potential exists for rejection. Mother and fetus, each have unique cell surface antigens (HLA). Despite the immediate danger of maternal immunologic recognition, the fetus manages to evade immune-mediated destruction. It

has been suggested, that the lack of HLA antigens on the syncytiotrophoblast and the presence of only the non-classic HLA-G antigen, [133] allows fetal trophoblast to avoid maternal immune recognition. Goldman-Wohl DS, et al found that the expression of HLA-G is reduced greatly in trophoblasts from spontaneous abortions and patients with preeclampsia, [69] in contrast to the high level of expression of HLA-G in trophoblasts from normal pregnancies. [29]

Several lines of evidence suggest that it is a lack of developed tolerance to paternal antigens that places a woman at risk for rejection of the pregnancy, manifested as preeclampsia. This concept is corroborated by epidemiological reports of higher risk of preeclampsia with excessive inflammation, the tendency for the disorder to occur more often among first pregnancies, with short duration of sexual cohabitation prior to conception, with change in sexual partner, and in women with a history of barrier contraceptive use. Primigravidity has consistently been demonstrated as the strongest risk factor for preeclampsia. A longer period of preconceptional sexual cohabitation with the father of the pregnancy is protective, [150] and a change of partner causes a woman's risk to revert to nearly the same level as for nulliparity. [151] Confirmation for this idea came from a Dutch study of 392 hypertensive multiparous patients, where it was demonstrated that multiparous preeclamptics changed partners more often (22-25%) compared to normotensive multiparous patients. A subsequent prospective study by Trupin et al showed that the incidence of preeclampsia between nulliparous (3.2%) and multiparous patients (3%) who changed partners was higher compared to that for nulliparous patients (1.9%) who did not change partners. [187] However, more recent work by Baso, et al and Skjaerven et al, demonstrated that it is not partner change but increased inter-pregnancy interval that elevates the risk for preeclampsia. [12][173] Basso, et al, extended this finding to suggest that subfertility may be the pathophysiology underlying the prolonged inter-pregnancy interval associated with

preeclampsia. Barrier contraceptive use has been linked to an elevation in risk for preeclampsia, but this has not been replicated in other, larger studies. [92][123]

3.4. MATERNAL INFECTIONS AS RISK FACTORS FOR PREECLAMPSIA

Intolerance to paternal antigens is not the only explanation for the immunologic response observed in preeclamptic pregnancies. An overall upregulation of immune mediators with resultant oxidative stress and endothelial dysfunction is triggered by infection. [112][145][153][154] Several epidemiological studies have linked both bacterial and viral infections to preeclampsia (Table 13). Sartelet et al, in a case control study, showed that malaria infection contracted during pregnancy was associated with preeclampsia (adjusted OR 3.3, 95% CI 1.1-9.5). [157]

Two further case control studies found that having urinary tract infections conferred significant 4-5 fold increased risks for preeclampsia. [86][125] These findings were replicated in a prospective investigation, which showed that asymptomatic bacteriuria was more common among preeclamptics (19%) compared to normal pregnancies (3-6%). [85] In another prospective study, Herrera JA et al, demonstrated a 64.7% reduction of preeclampsia in women treated for vaginal/cervical infections such as BV, *C. trachomatis*, *T. vaginalis* and group B streptococcus.[83] Similarly, in a Norwegian study, Trogstad LIS, et al showed an increased risk of developing preeclampsia among women who were seronegative for, and therefore at risk of acquiring, HSV-2, CMV and EBV. [186] In a recently published case control study conducted by Heine, et al, women with elevated titers of IgG to *C. pneumoniae* were found to have a three fold increased risk of preeclampsia (OR 3.1, 95% CI 1.2-7.9). [80]

4. RESEARCH STUDY

4.1. SPECIFIC AIMS

Preeclampsia is an unpredictable disease of human pregnancy and is responsible for considerable maternal and perinatal morbidity. The only effective cure is delivery of the fetus. There is evidence to suggest that endothelial injury and dysfunction are manifested early on in the disease process, and may be part of an excessive inflammatory response to pregnancy. We propose that maternal infections may be one trigger for such inflammatory activation. Infections may trigger the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 into maternal circulation. The resulting inflammation may stimulate recruitment of neutrophils and monocytes and activation of macrophages that may lead to induction of vascular injury.

The specific aims of this research are as follows:

Aim Study I: To determine the relationship between maternal infections with Herpes Simplex 1 and 2 (HSV-1 and HSV-2), Cytomegalovirus (CMV), and Epstein Barr (EBV) infections and the risk of preeclampsia.

Hypothesis 1: Women who lack IgG antibodies to HSV 1 / 2, CMV, or EBV in the beginning of pregnancy are at increased risk for developing preeclampsia, and women with elevated IgG antibodies to these viral infections early in pregnancy are less likely to develop preeclampsia.

Hypothesis 2: Women who acquire a primary HSV 1 / 2, CMV, or EBV infection during pregnancy are at increased risk of developing preeclampsia.

Aim Study II: To conduct a meta-analytic review of studies that explored the association between maternal infection and the risk of preeclampsia, and to determine the strength of this association.

Aim 1 Study III: To examine the relationship between Genital Warts (HPV), Genital Herpes (HSV-2), Chlamydia trachomatis, Gonorrhea and the risk of preeclampsia in a population-based cohort of reproductive age women.

Aim 2 Study III: To investigate multiple factors affecting infection status and their relation to preeclampsia.

Hypothesis 1: Women who have preeclampsia are more likely to report infections including Genital Warts, Genital Herpes, Chlamydia trachomatis and Gonorrhea in comparison to normal pregnancies.

Hypothesis 2: Behavioral, sociodemographic, and clinical factors may interact with infection to trigger preeclampsia.

4.2. METHODS (I)

4.2.1. Study design (I): Serological associations among HSV-1 and HSV-2, CMV, EBV infections and the risk of preeclampsia

Study I was a 1:3 matched case control study nested within the ongoing Pregnancy Evaluation Preeclampsia Prevention (PEPP) study at Pittsburgh's Magee-Womens Hospital. The ultimate goal of the PEPP study was to determine factors associated with preeclampsia and whether these factors could help improve prediction of the disease. Since the inception of PEPP in 1993 until

February 2002, a total of 2,892 pregnant women have been recruited. Blood samples were obtained from pregnant women at initiation of prenatal visits to the Outpatient Clinic at Magee-Womens Hospital. Magee-Womens hospital is the largest provider of obstetrical care in Allegheny County. Since Magee deliveries account for almost half of all live births in this county, this cohort was an excellent opportunity to study the relationship between maternal herpes virus infections and preeclampsia. In addition, PEPP's infrastructure tackles preeclampsia research in a coordinated manner, incorporating environmental, clinical and behavioral aspects of this maternal syndrome.

PEPP fulfilled our research needs as follows: 1) The continual expanding PEPP database with its extensive clinical, behavioral and epidemiologic data enabled the selection of the relevant nested case and control groups for the study, 2) PEPP throughout the years has built a repository of well documented interview, medical chart abstraction and laboratory outcomes data on risk factors for preeclampsia, and 3) PEPP employs standardized procedures to ensure adherence to study protocols and a balanced sample representative of the general population.

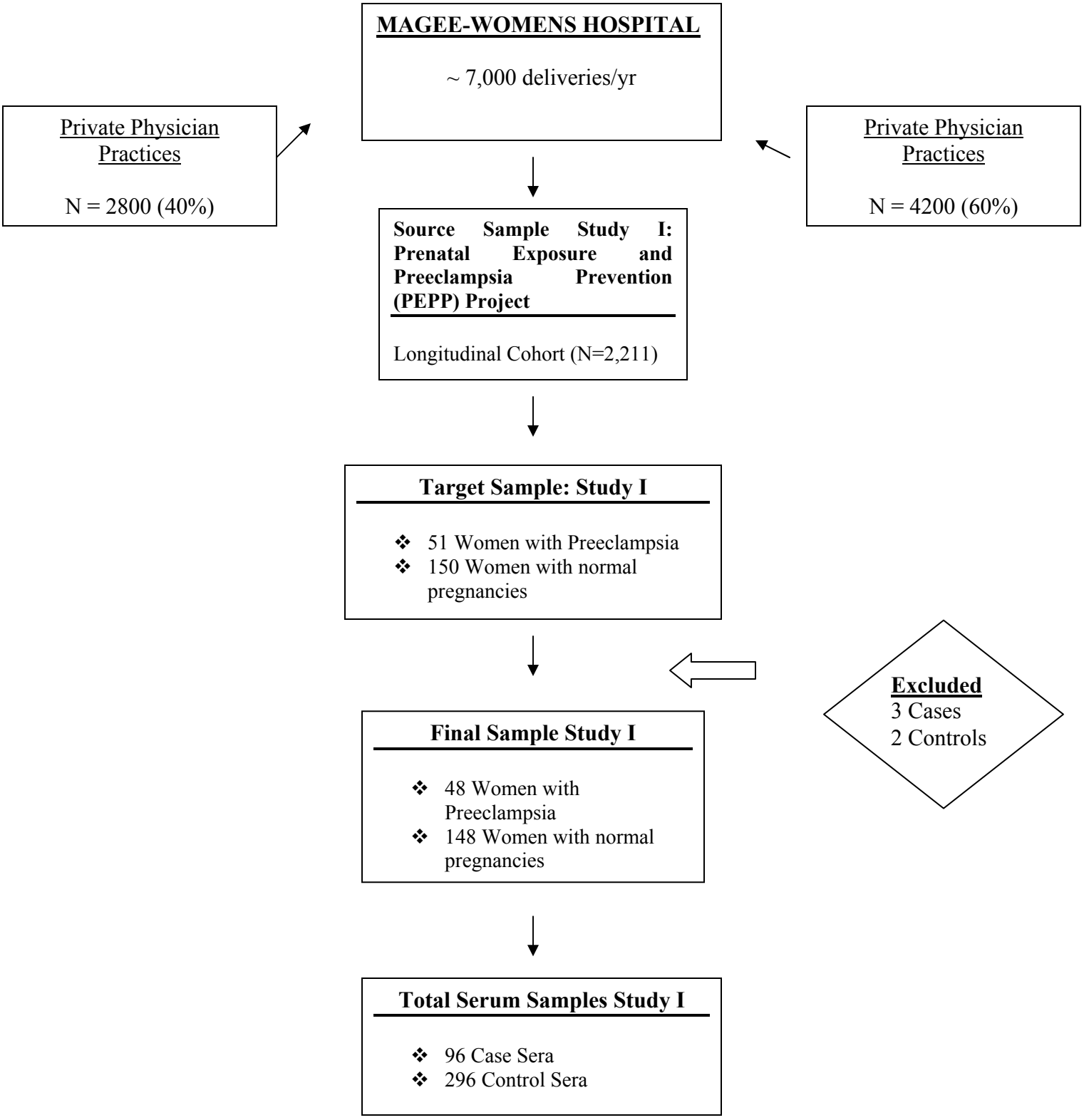

Figure 1: Study I Flow Diagram

4.2.2. Study Outcomes (I)

The main outcome in Study I was maternal HSV 1 / 2, CMV and EBV viral seroconversions.

4.2.3. Overall Eligibility Criteria (I)

A sample of healthy primigravida women between 16 and 44 years of age at 22 weeks or less of gestation were identified from PEPP's records and targeted for inclusion in the current study. Participants were originally identified at their first prenatal visit to the Outpatient Clinic at Magee-Womens Hospital. Consent was obtained from all eligible participants (Table 5) when they originally enrolled in the PEPP study, a brief baseline interview was conducted, and longitudinal blood samples taken as aliquots at the time they presented for prenatal care. For each preeclamptic case, three normotensive controls were selected, matched on age, parity and race. From an available cohort of 2,211 women registered for outpatient prenatal care, 50 preeclamptic cases and 150 normotensive controls were selected to represent a 1:3 case control design (Fig 2).

Controls were randomly selected from the same cohort. The earliest available serum sample obtained was chosen as early trimester sample and the latest available pre-delivery sample was chosen as convalescent sample. Due to lack of blood samples on 2 cases and 10 controls, a total of 96 cases and 280 control sera were retrieved from PEPP's bio-repository and shipped to the University of Washington Virology Laboratory in Seattle for determination of antibodies to HSV 1 / 2, CMV and EBV.

Table 5: Eligibility Criteria for Participants in the PEPP Study

CASES	CONTROLS
A) Elevated blood pressure (at least two readings fulfilling criteria) which returns to normal by 12 weeks postpartum a. ≥30 mmHg change in SBP or ≥15 mmHg in DBP from baseline* OR b. SBP ≥140 mmHg or DBP ≥90 mmHg if no baseline measurement B) Urinary protein a. Dipstick of random urine with ≥2+ on voided or ≥1+ on catheterized random specimen b. ≥0.5 g of protein in a 24 hour urine collection C) Hyperuricemia (≥ 1 S.D above the mean for gestational age)	A) Blood pressure a. < 30 mmHg change in SBP and < 15 mmHg in DBP from baseline and B) Urinary protein a. All dipsticks of random urines with <2+ on voided and < 1+ on catheterized random specimens b. < 0.3 g of protein in all 24 hour urine collections C) Hyperuricemia

* Baseline = average blood pressure prior to 20 weeks gestation.

4.2.4. Study Population (I)

The longitudinal cohort consisted of pregnant women who met all PEPP eligibility criteria but have not yet delivered. Furthermore, longitudinal patients had no history of diabetes or hypertension and met ACOG criteria for preeclampsia. The present study restricted sample selection to the longitudinal cohort, for several reasons: 1) because PEPP's case designation occurred after delivery, the potential for selection bias was significantly reduced. In addition, essential indicators of sexual behavior and other information on covariates were collected prior to determining cases and controls; 2) with the longitudinal cohort it was possible to capture women initiating prenatal care prior to 22 weeks gestation, thereby allowing viral seroconversion to be defined more precisely. In addition, a longer follow up allowed for collection of more complete clinical and specimen data.

Cases: Cases were selected from PEPP's longitudinal cohort, and entailed a diagnosis of gestational hypertension with proteinuria. Additional criteria for case selection required that final designation of preeclampsia be made 12 weeks postpartum, after blood pressure and proteinuria returned to baseline levels. Diagnosis of preeclampsia in both PEPP and the current study represents a modified version of the definition proposed by the American College of Obstetrics and Gynecology. The rationale for adopting a modified version was to minimize the likelihood of classifying transient hypertension during pregnancy as preeclampsia. Gestational hypertension was defined as an increase of 30 mmHg in systolic blood pressure or 15 mmHg in diastolic blood pressure compared with values obtained before 20 weeks of gestation or as having an absolute blood pressure ≥ 140/90 mmHg that developed after 20 weeks of gestation if first trimester blood pressures were not known. Proteinuria was defined as > 500 mg per 24-hour urine collection or ≥ 2+ (100 mg/dL) on a voided or ≥1+ (30 mg/dL) on a catheterized random urine specimen.

The following eligibility criteria were applied to cases: 1) primiparity, 2) single gestation, 3) no history of diabetes, 4) no history of cardiovascular disease, and 5) no history of hypertension. Multiparous women and women with twin pregnancies were excluded because the available epidemiologic evidence suggests fundamental differences in risk patterns associated with preeclampsia in these women. In particular, multiparous women who develop preeclampsia tend to have a history of prior preeclampsia and may be more likely to be at subclinical cardiovascular risk (J Roberts). Women with multiple gestations are excluded because their large placental size appears to place them at predictably higher risk for developing preeclampsia. Consequently, infection is less likely to be the cause of preeclampsia in such women. In addition, the substantially lower prevalence of preeclampsia in multiparous women and the substantially

smaller number of women with multiple gestations would make it difficult to achieve adequate sample size in these subgroups (J Roberts).

Controls: Controls consisted of pregnant women enrolled in the PEPP study who did not experience preeclampsia. Three times as many controls were selected from the same longitudinal cohort and met the same inclusion criteria as cases: 1) primiparity, 2) singleton gestation, 3) no history of diabetes, 4) no history of cardiovascular disease, and 5) no history of hypertension.

4.2.5. Data Collection (I)

Data Management: A master file was created containing sera from cases and controls extracted from PEPP's bio-specimen repository. This master file was divided into strata describing cases and controls by unique sample identification numbers and listed quantities available for the study. For each stratum, a serum aliquot was obtained from early and convalescent samples. After close scrutiny of records on this list, records missing the required 0.5 ml of maternal sera that was needed for the conduct of reliable viral serologies were excluded. Thus, the final study sample was comprised of 48 cases and 140 controls, with an overall total of 376 blood serum samples available for shipment. This excel file was then submitted to PEPP's laboratory repository where the sera was pulled and packaged for shipment. Sera was packaged according to this excel file in order to prevent mislabeling of vials. A second file, stripped of case control status and with the order of IDs rearranged, were included with the labeled and packaged sera and shipped to the virology laboratory at the University of Washington (Table 6). Results of serologic tests were recorded by laboratory personnel directly onto the second list (in an Excel spreadsheet). A third dataset was then created, by merging virology results to the baseline and clinical data file by sample record numbers.

Table 6: Example of blood serum master list containing the first two strata of the 1:3 case control study

STRATA	CC[1]	RECORD ID[2]	SAMPLE ID	SAMPLE DATE	SAMPLE TIME	AMOUNT
1	1	000112233	13579	12/23/99		
1	1	000112233	24687	8/15/00		
1	0	000456789	46810	7/25/99		
1	0	000456789	68103	2/18/00		
1	0	000678910	81012	1/20/00		
1	0	000678910	10125	10/18/00		
1	0	000345678	70012	4/15/00		
1	0	000345678	60034	8/12/00		
2	1	000891011	12146	2/18/01		
2	1	000891011	14168	10/20/01		
2	0	000910112	16182	1/17/01		
2	0	000910112	18204	10/12/01		
2	0	001011123	20226	4/10/01		
2	0	001011123	44246	12/05/01		
2	0	002456789	27289	3/10/01		
2	0	002456789	53435	5/06/01		

[1] *CC denotes case control status. For each stratum, a total of 2 (early and labor samples for the one case)case samples and 6 control samples (early and labor samples for the 3 controls) are listed*

[2] *Note that record numbers are identical for each pair, because each pair represents the same person, however, all sample ids are unique. Before samples are shipped to virology lab, the CC and RECORD ID columns will be deleted, and this modified list will be shipped packaged with the sera. Another copy of this modified list will be sent via e-mail to virology lab.*

Chart Reviews: An important data collection aspect in the present study was extraction of relevant information from PEPP's database to be used in the case control analysis. The first step involved selection of cases and controls from PEPP's longitudinal participant pool. Three controls were selected for each preeclampsia case and matched on age, parity and race. This was greatly facilitated by PEPP's in-house diagnostic group who reviews and determines final assignment of cases on an ongoing basis. Additional queries of prenatal charts were conducted to obtain baseline primary risk factors, demographic information, medical history and information on social habits (Table 7). Additional variables were created from existing ones, such as interval period between early and convalescent samples, and length of cohabitation. The cohabitation

variable was created by subtracting time of first sexual relations with partner from date of interview.

Table 7: Data Collected on Study Population

CATEGORY	DATA COLLECTED
1. Demographics	Age, education completed, marital status, current employment status
2. Behavioral	Length of cohabitation with father of pregnancy
3. Obstetric and gynecologic history	Pregnancies
4. Medical history	Diabetes, hypertension, STDs
5. Substance use	Tobacco use, alcohol use, drug use
6. Maternal anthropometric data	Weight, height, BMI
7. Medication use	Oral contraceptive use, other contraceptives
8. Laboratory data: viral serologies	IgG antibodies to HSV-1, HSV-2, CMV, EBV

Laboratory Data: Approximately 0.5 mL of blood sera from cases and controls were retrieved from -80ºC PEPP biospecimen storage at two time points (at 16-22 weeks gestation and at delivery), and shipped to the University of Washington virology laboratory for identification of serum antibodies to HSV-1, HSV-2, CMV and EBV.

4.2.6. Methodological issues related to measurement of infection status (I)

Detection of IgG Antibodies to HSV 1 / 2 Infections: Accurate assessment of maternal exposures to infectious agents is highly dependent on the assumption that laboratory assessment of serology has high sensitivity and specificity. In the past, seroepidemiologic studies were unable to accurately distinguish between HSV-1 and HSV-2 infections due to cross-reactive antibodies between the two types. But with the introduction of glycoprotein gG enzyme-linked immunosorbent assay (ELISA), it is now possible to reliably determine whether someone has been infected with HSV-1, HSV-2 or both. HSV glycoprotein ELISA's are now considered highly sensitive (>95%) and reproducible (coefficient of variation $\leq$ 5%). [82] The ELISA test

used in the present study (HerpeSelect 1 and 2), has been tested against the Western blot test (gold standard) in a group of sera from 241 prenatal patients [9] and found to have sensitivity and specificity of 96% and 95% for HerpeSelect-1. The sensitivity and specificity for HerpeSelect-2 were found to be 100% and 96%, respectively.

Timing of Seroconversion: The timing of blood collection can limit the interpretation of HSV, CMV or EBV serologic results. A basic assumption in determining a subject's serostatus is that change of infection status will occur only once, and hopefully this change will be captured in the time frame between collection of acute and convalescent samples. In some patients, antibody titers may fluctuate (rise and fall) significantly and in some instances fall to undetected levels within one month. Other patients may not develop significant antibodies at all. In these patients, misclassification of serostatus may be unavoidable. Preliminary data suggest that HSV-1 and HSV-2 antibodies can be detected a median of 2-3 weeks after the onset of first episodes of genital herpes.[9] Therefore, timing of blood specimen collection is of major importance, in determining seroconversion. It is possible that because of the shorter interval between acute and convalescent samples in preeclamptic subjects who deliver prematurely, as compared to normotensive subjects who do not, seroconversion may more likely be detected in normal sera. We addressed this potential problem by ensuring that time intervals between acute and convalescent sera were at least four weeks apart. In addition, we obtained gestational age adjusted risk measures of primary infection associated with preeclampsia. We also calculated time intervals between acute and convalescent samples to determine if sample intervals were significantly different for preeclamptics compared to normotensives.

4.2.7. Serologic methods for antibody testing (I)

HSV-1 and HSV-2: Maternal sera were tested using Enzyme-linked immunosorbent assay for the qualitative detection of human IgG class antibodies to HSV 1 / 2 (ELISA). The following procedures were followed for both HSV-1 and HSV-2: In the Focus Technologies HerpeSelect™ 1 ELISA IgG assay, the polystyrene microwells were coated with recombinant gG-1 antigen. Diluted serum samples and controls were incubated in the wells to allow specific antibody present in the samples to react with the antigen. Nonspecific reactants were removed by washing and peroxidase-conjugated anti-human IgG was added and reacted with specific IgG. Excess conjugate was removed by washing. Enzyme substrate and chromogen were added, and the color allowed to develop. After adding the Stop Reagent, the resultant color change was quantified by a spectrophotometric reading of optical density (OD). Sample OD readings were compared with reference cut-off OD reading to determine results. [82] Results were reported as positive, negative or equivocal. A positive result entailed an index level of > 1.10 and was presumptive for the presence of IgG antibodies to HSV 1 / 2. A negative result entailed an index value of <0.90 indicating no IgG antibodies to HSV 1 / 2 were detected. A result was equivocal if the index value was ≥ 0.90 but ≤ 1.10. As can be seen from Table 8 below, considering a 16% prevalence of HSV-1, and 25% for HSV-2 there is a high probability that a positive result with actually reflect actual disease (PPV).

Table 8: Predictive Values of ELISA IgG Testing for HSV-1 and HSV-2 According to Prevalence of Infection in Expectant Mothers [82]

PREVALENCE	HSV-1		HSV-2	
	PPV(%)	NPV(%)	PPV(%)	NPV(%)
50%	95.2	95.2	96.2	96.1
40%	93.0	96.7	94.5	97.4
30%	89.6	97.9	91.7	98.3
25%	87.0	98.3	89.5	98.7
20%	83.3	98.8	86.5	99.0
15%	77.9	99.1	81.9	99.3
10%	69.0	99.4	74.0	99.6
5%	51.3	99.7	57.4	99.8

Serologic Methods for Antibody Testing of CMV: Detection of antibodies to CMV infection was made with ABBOTT CMV Total AB EIA immunoassay. This assay is a solid phase enzyme immunoassay for the qualitative detection of antibody to CMV in human serum or plasma as an indication of past or current infection with CMV. The following procedures were followed: Polystyrene beads coated with heat inactivated CMV antigen were incubated with diluted serum. Any antibody to CMV that is present is bound to the antigen on the solid phase. After aspiration of the unbound material and washing of the beads, anti-human immunoglobulin (containing antibodies against IgA, IgG, IgM, heavy and light chains) conjugated with horseradish peroxidase (HRPO) is allowed to react with the antigen-antibody complex on the beads. Unbound enzyme conjugate is then aspirated and the beads are washed. Next o-Phenylenediamine (OPD) solution containing hydrogen peroxide is added to the beads and, after incubation, a yellow-orange color develops in proportion to the amount of antibody to CMV bound to the beads. The enzyme reaction is stopped by the addition of 1 N Sulfuric Acid. The absorbance of controls and specimens is determined using a spectrophotometer with the

wavelength set at 492 nm. Specimens giving absorbance values equal to or greater than the cutoff are considered reactive for antibody to CMV. [1]

Serologic Methods for Antibody Testing of EBV: Antibodies to Epstein Barr Virus (EBV) was assessed by the Sigma Diagnostic EBV-VCA IgG ELISA test system. This is an enzyme-linked immunosorbent assay (ELISA) designed for the qualitative detection of IgG class antibodies to Epstein Barr Virus viral capsid antigen (EBV-VCA) in human serum. The test procedure involves three incubation steps: 1) diluted test sera are incubated in multi-wells coated with EBV-VCA antigen, and antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components, 2) peroxidase conjugated goat anti-human IgG is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate, 3) the multi-wells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample. [170]

4.2.8. Data analysis strategy: Conditional logistic regression analysis (I)

Virology results were coded without knowing case control status, and baseline infection status and seroconversion evaluated in relation to preeclampsia in matched analyses. Odds ratios and 95% confidence intervals were estimated using conditional logistic regression with Stata statistical software (Stata Corporation, College Station, texas, version 7.0). A conditional analysis is appropriate in a 1:3 matched case control design, because it forms an exact likelihood function. Within each stratum, a likelihood function is formed based on an extensive listing of all

possible combinations of cases and controls, conditional on the total number of cases and controls in the stratum [161]. Only the discordant case control quartets regarding exposure were considered in analysis. Quartets where both cases and controls were positive or negative were excluded from analysis.

The *a priori* hypotheses were that lack of baseline IgG antibodies and viral seroconversion denoted risks for preeclampsia, and persistent elevations in viral IgG antibodies conferred protection against preeclampsia. Categorical variables were created for each virus indicating seroconversion or no seroconversion (coded yes=1/no=0) and summarized as frequencies by case control status. Cases and controls were compared according to the following variables: marital status, smoking before and during pregnancy, BMI, income, length of cohabitation with partner, medical insurance, gestational age, and non-barrier and barrier contraceptive methods. In addition, because time interval between acute and convalescent samples are typically shorter for preeclamptics compared to normotensives, frequency of seroconversion was adjusted for sample interval time and Mantel-Haenszel procedure used to test for differences among groups. Continuous variables were summarized by means and standard deviations, and statistically significant differences determined by the Student's t-test for variables that were normally distributed and the Wilcoxon test used for non-normally distributed continuous variables.

Presence or absence of IgG antibodies to herpes viruses was determined based on criteria established by Enzyme-linked Immunosorbent Assay (ELISA) manufacturers. An index level of greater than 1.10 was considered positive. Descriptive statistics for IgG prevalence were calculated for the overall study population and separately for preeclamptics (cases) and normotensives (controls). Cross tabulations were obtained for each herpes virus variable (1=positive, 0=negative) by case control status, summarized by frequencies. The nature and

relationship between continuous variables such as gestational age, smoking, sexual cohabitation, income, white blood cell count, platelet count and preeclampsia were assessed with scatter plots. Continuous variables will be summarized by means and standard deviations, and statistically significant differences determined by the Student's t-test for variables that were normally distributed and the Wilcoxon test used for non-normally distributed continuous variables. Odds ratios and 95% confidence intervals were the primary test for strength of each association with preeclampsia. The a priori hypothesis was that lack of baseline IgG antibodies denoted risk of preeclampsia.

Primary infection was defined as viral seroconversion. A participant, who was HSV, CMV or EBV seronegative at baseline (first trimester) and her subsequent serum sample (labor/delivery) became positive, was considered to have seroconverted. Categorical variables were created for each virus indicating seroconversion or no seroconversion (yes=1/no=0) and summarized as frequencies. Cases and controls were compared according to the following variables: marital status, smoking during pregnancy, BMI, income, length of cohabitation, medical insurance, gestational age, birth control methods. Because time interval between acute and convalescent sample is typically shorter for preeclamptics compared to normotensives, frequency of seroconversion was estimated by time interval and Mantel-Haenszel procedure used to test for differences among groups. Baseline status and seroconversion were evaluated in relation to preeclampsia in matched analyses.

Descriptive statistics for infection related variables were calculated for the study population as a whole and separately for cases and controls. In order to examine the impact of potential confounders on primary infections status, bivariate analyses using variables associated with case status and infection status were examined as stratification variables and summary

statistics calculated using the Mantel-Haenszel method. [161] Conditional logistic regression models produced odds ratios for baseline serologic positivity or serconversion associated with preeclampsia, adjusting for the effects of other relevant parameters. All parameters that were significant at the level of $p< 0.25$ were entered into a subsequent conditional regression analysis. Next, parameters significant at the level of $p < 0.10$ were identified. A final logistic regression model was then fitted using a level of 0.05 as entry criteria.

4.2.9. Sample size considerations (I)

In order to determine the number of subjects required for the study, we considered the following: 1) the prevalence of the disease in the general population, and 2) the prevalence (p_o) of the risk factor in the control population, and 3) establishment of the acceptable levels of Type I error and Type II error. Table 10 presents the number of subjects required to detect odds ratios of 1.5, 2.0, 2.5, 3.0 and 3.5, with respect to primary infection.

Table 9: Sample size needed to detect odds ratios of interest given various seroprevalence rates, case: control ratio of 1:3, two tailed α of 0.05 and β of 0.20. Numbers in the Table refer to the cumber of cases

ODDS RATIO	0.20	0.25	0.30	0.35	0.40	0.45
1.5	347	304	279	264	257	255
2.0	109	98	92	88	88	89
2.5	59	54	51	50	51	52
3.0	40	37	35	35	36	37
3.5	30	28	27	27	28	29

The sample size listed is the number of subjects needed in the exposed group. Triple this number of subjects is needed in the unexposed group

As can be seen in Table 9, assuming an overall prevalence of 30 to 50 percent exposure rate in controls and a 1 to 3 case control ratio, approximately 90 cases and 270 controls were needed to

detect greater than twofold increase in risk with an α risk of 0.05 and a β risk of 0.20 (80% statistical power).

4.2.10. Potential problems (I)

Confounding: We assessed the possibility that demographic, behavioral, and clinical parameters could impact the relationship between serology and preeclampsia. We were aware, however, that in any case control study unmeasured confounding may influence the observed associations.

Bias: Bias has been defined as "any systematic error in the design, conduct or analysis of a study that results in a mistaken estimate of an exposure's effect on the risk of disease." [161] Major types of biases in case control studies are, selection, misclassification and information biases. We accounted for selection bias by randomly selecting cases from a pregnancy cohort.

Misclassification bias occurs when patients are improperly assigned as cases or controls. This type of bias is a significant concern in preeclampsia studies due to a lack of gold standard in defining this disorder. Any misclassification would have the effect of biasing the observed association toward the null. Ideally, use of a rigorous case definition and juried case selection process may improve classification. We minimized the occurrence of this type of bias as follows: we expected that because preeclampsia is a relatively rare disease, controls were likely not have the disease.

Information bias was minimized because: 1) laboratory personnel were masked to case control status, and 2) women volunteered demographic and behavioral information prior to the occurrence of disease.

Data Validity: In order to ensure the reliability and validity of serologic measurements, sera were run in duplicate within the same assay. In addition, samples with different run dates

were retested pair wise on the same ELISA plate and borderline serology results were retested as well.

4.2.11. Preeclampsia Diagnosis (I)

Preeclampsia was diagnosed in accordance with guidelines proposed by the American College of Obstetrics and Gynecology (ACOG). Preeclampsia was defined as either a systolic blood pressure ≥ 140/90 mmHg or a diastolic level ≥ 90 mmHg on repeated measurements that developed after 20 weeks of gestation. Proteinuria was defined as > 300 mg per 24-hour urine collection or ≥ 2+ (100 mg/dL) on a voided or ≥ 1+ (30 mg/dL) on a catheterized random urine specimen. Final designation of preeclampsia was made 12 weeks postpartum, after blood pressure and proteinuria returned to baseline levels.

Covariates

Medical history and other behavioral risk factors were obtained by abstraction of prenatal charts. We created an additional variable, interval time by calculating time period between early and convalescent sample blood draws. Information on covariates included gestational age, oral and barrier contraceptive use, education, smoking, BMI, income, sexual cohabitation with partner, and medical insurance.

4.3. RESULTS (I)

Main characteristics of cases and controls are displayed in Tables 10 and 11. As can be seen, cases and controls did not differ significantly on gestational age, medical insurance, marital status, education, income, smoking, and contraceptive methods. However, a significant linear increase in preeclampsia was observed with increasing tertiles of BMI (p for trend = 0.03).

Table 12 shows virology results by case control status and trimester of pregnancy. Among the 48 cases, 23 (47.9%) lacked IgG antibodies to HSV 1 / 2, and 27 (56.3%) lacked IgG antibodies to CMV; among the 140 controls, 39 (27.9%) and 80 (57.1%) lacked antibodies to HSV 1 / 2 and CMV, respectively. Only 2 (4.2%) cases and 8 (5.7%) controls were EBV seronegative. We found that women who lacked antibodies to both HSV-1 and HSV-2 in early trimester were at significantly increased risk of developing preeclampsia (OR 2.9, 95% CI 1.3-6.4), whereas women who lacked antibodies to CMV (OR 0.9, 95% CI 0.4-1.9) and EBV (OR 0.7, 95% 0.2-3.6) were not. In contrast, persistent elevations in IgG antibodies to HSV 1 / 2 appeared to lower the risk of preeclampsia (OR 0.5, 95% CI 0.2-1.0), whereas elevations in IgG antibodies to CMV was not associated with preeclampsia (OR 1.1, 95% CI 0.5-2.4).

Five (10.4%) preeclamptics and four (2.9%) normotensives seroconverted for HSV 1 / 2 or CMV. Seroconversion for HSV 1 / 2 or CMV was associated with a five-fold increased risk for developing preeclampsia (OR 5.4, 95% CI 1.0-29.0). This association remained significant after adjustment for education, income, smoking, years of cohabitation with partner, medical insurance, and type of birth control. There was no significant difference in early trimester and convalescent mean sample interval times between cases and controls, thus sample interval times did not affect risk estimates. The majority of women were seropositive for EBV (95.8 % of cases and 94.3% of controls) in the beginning of pregnancy. Hence we did not have sufficient power to determine the association between EBV primary infection and the risk of preeclampsia.

Table 10: Obstetric and Socio-demographic Characteristics of Preeclamptic Cases and Normotensive Controls (Paper I)

Variable	Preeclampsia Cases (n=48)	Normotensive Controls(n=140)	Statistical Significance
Gestational age (wks), mean ± SD	14.1 ± 10.4	14.3 ± 11.1	ns
Gestational age (wks), mean ± SD at:			
1st blood collection	23.7 ± 14.3	21.1 ± 14.5	
2nd blood collection	24.3 ± 14.6	28.6 ± 14.7	
Sample interval time (wks), mean ± SD	7.0 ± 1.0	7.0 ± 1.0	ns
Medicare/Medicaid			
No	19 (39.6)	60 (42.9)	ns
Yes	29 (60.4)	80 (57.1)	
Marital Status			
Never Married	22 (45.8)	61 (43.6)	ns
Married	26 (54.2)	79 (56.4)	
Education			
< High School	29 (60.4)	69 (49.3)	ns
> High School	19 (39.6)	71 (50.7)	
Income			
< 10K	14 (29.2)	33 (23.6)	
10K-20K	5 (10.4)	25 (17.9)	ns
20K-35K	12 (25.0)	22 (15.7)	
35K-50K	11 (22.9)	38 (27.1)	
50K-75K	6 (12.5)	22 (15.7)	
BMI			
< 18	28(62.2)	105 (78.4)	
18-30	1(2.2)	2 (1.5)	p = 0.03*
> 30	16(35.6)	27 (20.1)	

Note: P values for gestational age were determined by use of t test, and for all other covariates by use of Chi-square test. * Test of trend determined with Mantel Haenszel Chi-square test

Table 11: Behavioral Characteristics and Contraceptive Method Use Among Preeclamptic Cases and Normotensive Controls (Paper I)

Variable	Preeclampsia Cases (n=48)	Normotensive Controls(n=140)	Statistical Significance
Non-Barrier Birth Control Methods			
No	10 (20.8)	25 (17.9)	ns
Yes	38 (79.2)	115(82.1)	
Barrier Birth Control Methods			
No	25(52.1)	66 (47.1)	ns
Yes	23 (47.9)	74 (52.9)	
Smoked Before pregnancy cigarettes/day			
1-9	5 (11.6)	19 (15.2)	ns
10-19	5 (11.6)	17 (13.6)	
> 20	33 (76.7)	89 (71.2)	
Smoked during pregnancy cigarettes/day			
1-9	6 (23.1)	24 (23.5)	ns
10-19	3 (11.5)	9 (8.8)	
> 20	17 (65.4)	69 (67.6)	

Table 12: Risk of Preeclampsia Associated With Herpes Virus Infections (Paper I)

Variable	Cases[(a)] (N = 48) n (%)	Controls[(a)] (N = 140) n (%)	OR[(e)]	95% CI
Primary Infection[(b)]				
Any (HSV 1 / 2, CMV)	5 (10.4)	4 (2.9)	5.4	(1.0-29.0)
Lack of IgG Antibodies[(c)]				
HSV 1 / 2	23 (47.9)	39 (27.9)	2.9	(1.3-6.4)
CMV	27 (56.3)	80 (57.1)	0.9	(0.4-1.9)
EBV	2 (4.2)	8 (5.7)	0.7	(0.2-3.6)
Elevated IgG Antibodies[(d)]				
HSV 1 / 2	19 (39.6)	78 (55.7)	0.5	(0.2-1.0)
CMV	21 (43.8)	60 (42.9)	1.1	(0.2-1.0)
EBV	46 (95.8)	132 (94.3)	1.4	(0.3-6.7)

(a) Cases and controls were matched for age, parity and race in all analyses
(b) Primary infection denotes participants who were seronegative at baseline and seropositive at delivery
(c) Lack of antibodies at baseline. Instances where both HSV-1 and HSV-2 were seronegative at baseline
(d) Patients with elevated IgG antibodies were seropositive (either HSV-1 or HSV-2) at baseline and remained seropositive at delivery
(e) Adjusted for smoking, BMI, Barrier (condom, spermicide, diaphragm, cervical cap, sponge) /Non-Barrier (oral contraceptive, Depo-Provera, withdrawal and no birth control method), gestational age, length of sexual cohabitation with partner, sample interval time

4.4. DISCUSSION (I)

To our knowledge, the present investigation is the first study to show a significant association between newly acquired HSV-1, HSV-2 or CMV infections and preeclampsia. However, because of the relative small sample size involved in this study, a larger sufficiently powered prospective cohort study, designed to examine the association between maternal infection and preeclampsia is needed to confirm our initial observations.

To date, eight epidemiologic studies have explored a potential association between maternal infections and preeclampsia. Sartelet et al, in a case control study, showed that malaria infection contracted during pregnancy was associated with preeclampsia (adjusted OR 3.3, 95% CI 1.1-9.5).[157] Two further case control studies found that having urinary tract infections conferred significant 4-5-fold increased risks for preeclampsia. [86][125] These findings were replicated in a prospective investigation which showed that asymptomatic bacteriuria was more common among preeclamptics (19%) compared to normal pregnancies (3-6%). [85] In another prospective study, Herrera JA et al, demonstrated a 64.7% reduction of preeclampsia in women treated for vaginal/cervical infections such as BV, C. *trachomatis*, T. *vaginalis*, G. *vaginalis* and Group B streptococcus [83].

In a Norwegian study, Trogstad LIS, et al showed an increased risk of developing preeclampsia among women who were seronegative for, and therefore at risk of acquiring, HSV-2 (OR 1.7, 95% CI 0.7-4.2), CMV (OR 1.6, 95% CI 0.8-3.2) and EBV (OR 3.5, 95% CI 1.1-10.6) infections.[186] Our results are consistent with Trogstad's findings of an increased risk of preeclampsia associated with being seronegative for HSV-2 and CMV in the beginning of pregnancy. Unlike Trogstad, however, we found no association between preeclampsia and EBV seronegativity (OR 0.7, 95% CI 0.2-3.6).

Another case control study conducted by Heine et al, women with elevated titers of IgG to C. *pneumoniae* were found to have a three-fold increased risk of preeclampsia (OR 3.1, 95% CI 1.2-7.9). [80] Similarly, in a recently published case control study, von Dadelszen et al, showed that women with early onset preeclampsia had higher levels of IgG to C. *pneumoniae*(0.354, 95% CI 0.067-0.659) as compared to normotensives (0.207, 95% CI 0.105-0.359). They also demonstrated higher IgG levels to CMV (79, 95% CI 49-179) among preeclamptics compared to women with normal pregnancies (49, 95% CI 45-70). [194] Unlike von Daedelszen and colleagues, we found no association between CMV positivity and preeclampsia (OR 1.1, 95% CI 0.2-1.0).

A few potential study limitations merits mentioning. Case control studies are prone to a number of biases, primarily, misclassification, selection and information biases. Misclassification bias occurs when patients are improperly assigned as cases and controls. This type of bias is a significant concern in preeclampsia studies due to a lack of consistency in defining this disorder. The current study population consisted of a well-defined longitudinal pregnancy cohort with rigorous preeclampsia diagnostic criteria. In this study, case designation occurred after delivery. Thus misclassification of cases was unlikely. Also, essential indicators of sexual behavior and other information on covariates were collected prior to determining cases, and laboratory personnel were masked to case control status. Further, we excluded multiparous gravidas and twin gestation pregnancies because the available epidemiologic evidence suggests that these women are more likely to develop preeclampsia. Consequently, infection is less likely to be the cause of preeclampsia in these women.

Although we adjusted for major preeclampsia confounding factors, we cannot discount the possibility that unmeasured infection-related risk factors such as number of sexual partners may have confounded the relationship between infection and preeclampsia.

Another potential source of bias was determination of infection status. Because the time interval between acute and convalescent samples may be shorter in preeclamptic subjects who may deliver prematurely, as compared to normotensives who do not, seroconversion may more likely be detected in normal sera. We addressed this problem by ensuring that time intervals between acute and convalescent sera were at least four weeks apart. HSV-1 and HSV-2 antibodies can be detected a median of 2-3 weeks after the onset of first episodes of genital herpes. [9] In addition, we obtained gestational age adjusted risk measures of primary infection associated with preeclampsia. We recognize, however, that in some patients levels of antibody titer may have varied and in some instances even declined to undetectable levels. In these patients, misclassification of serostatus is unavoidable.

Between 6-50% of women attending prenatal clinics are seropositive for HSV-2, whereas a larger proportion, are seropositive for CMV and EBV. [64] Although infected individuals mount an antibody response, they continue to harbor these viruses, and can have recurrences. Nonetheless, primary infection appears to have the greatest influence on adverse pregnancy outcomes. [18]

4.5. CONCLUSION (I)

Our data suggest that primary infection with HSV, and possibly CMV may be associated with preeclampsia. Primary infections with these herpes viruses may contribute to the inflammatory burden of preeclamptic pregnancies. However, whether *in utero* viral infections may influence preeclampsia risk is not known. One possible implication of our finding is that women who are seronegative at the onset of pregnancy may benefit from preventive strategies that may not only reduce the incidence of preeclampsia but might also lower maternal morbidity and perinatal mortality associated with this maternal syndrome.

4.6. METHODS (II)

4.6.1. Study design (II): Association between maternal infection and preeclampsia: A systematic review of epidemiologic studies

We searched MEDLINE, Cochrane, and Embase databases using a combination of search words: *preeclampsia, preeclampsia and infection, maternal infection and hypertensive disorders*. Inclusion criteria were: 1) study had to contain original data, 2) study design, 3) preeclampsia diagnosis, 4) techniques for detecting infection, and 5) adjustment for well-known preeclampsia confounders, including age, parity and multiple gestation pregnancies.

In addition to obtaining information on the methodology used in the original studies, we abstracted the following information: 1) name of author/authors and year of publication, 2) number of subjects, and 3) strength of association (odds ratios) and 95% confidence intervals (see Table 13). Exposure was defined as presence or absence of infection.

In the current meta-analysis, we required that studies utilize either the National High Blood Pressure Education Program (NHBPEP) criteria or the American College of Obstetrics and Gynecology (ACOG) criteria to define preeclampsia. Both define preeclampsia as a systolic

blood pressure greater than 140 mmHg or a diastolic blood pressure greater than 90 mmHg accompanied by proteinuria of 300 mg/24 hr urine collection, or more than 2+ on a voided or 1+ on a cathetherized random urine specimen, in a previously normotensive woman.

No institutional review board (IRB) approval was necessary, since only previously published data was used. All data was entered into Excel and later formatted as a STATA file in preparation for analysis.

4.6.2. Statistical Analysis (II)

The primary outcome was measure of association of bacterial or viral infections and the risk of preeclampsia compared to normotensive women. No adjustment for confounders was possible with the meta-analysis because only summary data was available. Individual study results were combined and heterogeneity between studies assessed with Q-statistics. [103] Based on a Q-statistic p-value greater than 0.10, which indicated absence of heterogeneity between studies, a fixed effects model was used to calculate pooled odds ratios. The fixed effects model assumes that risk estimates are identical when studies are combined and that between-study variations are due to random errors. [103]

We considered the potential for publication bias by checking whether risk estimate differences between studies distorted results of the meta-analysis. This was accomplished by visually inspecting a funnel plot for asymmetry. A funnel plot graphically depicts the logarithm of the study odds ratios against their standard errors.[53][180] All analyses were conducted with Stata™ 7.0 (Stata™ Stata Corporation, 4905 Lakeway Drive, College Station, Texas 77845 USA). Statistical significance was set at a probability level < 0.05.

4.7. RESULTS (II)

4.7.1. Review of studies (II)

Review of the literature yielded nine studies that have been conducted to date on the association between maternal infection and preeclampsia. Eight of these studies were published in peer-reviewed journals between 1986 and 2004. We excluded one published study because no control group was specified. [83] Of the remaining seven studies, one was retrospective, [86] three were case controlled,[153][125][80] and another three were prospective.[85][186][194] With the exception of two studies [157][186], the studies were conducted in the US. [85][86][125][80][194]

Four studies explored whether maternal exposure to bacterial infections (asymptomatic bacteriuria, urinary tract infection, malaria, and C. *pneumoniae*) [157][85][83][125] were associated with preeclampsia, and three examined this association with viral infections (HSV-2, CMV, and EBV).[186][80][194] We excluded one unpublished study, because exposure was defined differently from the studies included in the meta-analysis. Studies included in the meta-analysis used ACOG diagnostic criteria to define preeclampsia. The total combined population across the seven published studies was 1,382 preeclamptics and 4,555 normal pregnancies.

Characteristics of studies included in this review are detailed in Table 13. Sartelet et al, in a case control study, showed that malaria infection contracted during pregnancy was associated with preeclampsia (adjusted OR 3.3, 95% CI 1.1-9.5).[157] Two further case control studies found that having urinary tract infections conferred significant 4-5-fold increased risks for preeclampsia.[83][125] These findings were replicated in a prospective investigation which showed that asymptomatic bacteriuria was more common among preeclamptics (19%) compared to normal pregnancies (3-6%). [85]

In a Norwegian study, Trogstad LIS, et al showed an increased risk of developing preeclampsia among women who were seronegative for, and therefore at risk of acquiring, HSV-

2 (OR 1.7, 95% CI 0.7-4.2), CMV (OR 1.6, 95% CI 0.8-3.2) and EBV (OR 3.5, 95% CI 1.1-10.6) infections. [186]

Another case control study conducted by Heine et al, women with elevated titers of IgG to C. *pneumoniae* were found to have a three-fold increased risk of preeclampsia (OR 3.1, 95% CI 1.2-7.9).[80] Similarly, in a recently published case control study, von Dadelszen et al, showed that women with early onset preeclampsia had higher levels of IgG to C. *pneumoniae*(0.354, 95% CI 0.067-0.659) as compared to normotensives (0.207, 95% CI 0.105-0.359). They also demonstrated higher IgG levels to CMV (79, 95% CI 49-179) among preeclamptics compared to women with normal pregnancies (49, 95% CI 45-70). [194]

Table 13: Characteristics of Studies Included in the Meta-analysis

AUTHOR/YEAR	TYPE OF STUDY	PREECLAMPSIA DIAGNOSIS	INFECTIOUS AGENT STUDIED	TECHNIQUE USED TO DEFINE INFECTION	N (%)	OR (95% CI), % REDUCTION, INCIDENCE
Sartelet et al/1996	Case control	Maximum DBP of at least 90 mmHg hrs preceding delivery	Malaria	Histological observation of infected red blood cells or Malaria pigment in macrophages or Fibrinoid (placenta)	Ca: 17/32 (*53.1*) Co: 60/220 (*27.3*)	3.3 (1.1-9.5)
Hill et al/1986	Prospective	ACOG criteria	w/ asymptomatic bacteriuria w/o asymptomatic bacteriuria	Presence of more than 100,000 colonies of single bacterial species per 1 mL urine	Ca: 19/100(*19.0*) Co: 3/100 (*3.0*)	Preeclamptics: 19% incidence Normotensives: 3-6% incidence
Hsu and Witter/1995	Retrospective		UTI	Positive urine culture of > 100,000 bacteria	Ca: 214/785(*27.3*) Co: 127/785(*16.2*)	4.2 (1.05-5.09)
Mittendorf R et al/1996	Case control	ACOG criteria	UTI	Medical chart abstraction	Cases: 386 Controls: 2,355	5.3 (2.9-9.7)
Trogstad, et al/2001	Prospective	ACOG criteria	1)HSV-2 2)CMV 3)EBV 4)Toxoplasma *gondii*	1)HSV- IgG ELISA 2)CMV-IgG ELISA 3)EBV-antiEBV Recombinant EA IgG ELISA 4)Platelia Toxo-IgG	Ca: 6/33 (*18.2*), Co: 253/945(*26.8*) Ca: 19/33(*57.6*), Co: 650/945(*68.8*) Ca: 29/33(*87.9*), Co: 879/945(*93.0*) Ca: 3/33(*9.1*), Co: 91/945(*9.6*)	1.7 (0.7-4.2) 1.6 (0.8-3.2) 3.5 (1.1-10.6) 1.0 (0.3-3.5)
Heine RP, et al/2003	Case control	ACOG criteria	C. *pneumoniae*	Microimmunofluorescenc	Ca: 25/37(*67.6*) Co: 15/37(*40.5*)	3.1 (1.2-7.9)
Von Dadelszen, et al/200	Case control	National High Blood Pressure Education Program criteria	CMV+C. *pneumoniae*	CMV – IgG ELISA Fluorescent detection	Ca: 6/9 (66.7) Co: 46/113 (*40.7*)	2.9 (0.7-12.2) (Calculated in Meta-Analysis)

4.7.2. Meta Analysis (II)

Figure 4 shows a forest plot comparing odds of preeclampsia among women with either bacterial or viral infections during pregnancy. A forest plot illustrates the contribution of each study to the meta-analysis (its weight) and is represented by the area of a box whose center represents the size of effect estimated from that study (point estimate).[180] Pooled results revealed a statistically significant association of maternal infections with preeclampsia (OR 2.1, 95% CI 1.8-2.6) (Fig. 3), with no evidence of heterogeneity (Q = 7.5, p = 0.3), nor indication of publication bias (Fig. 4).

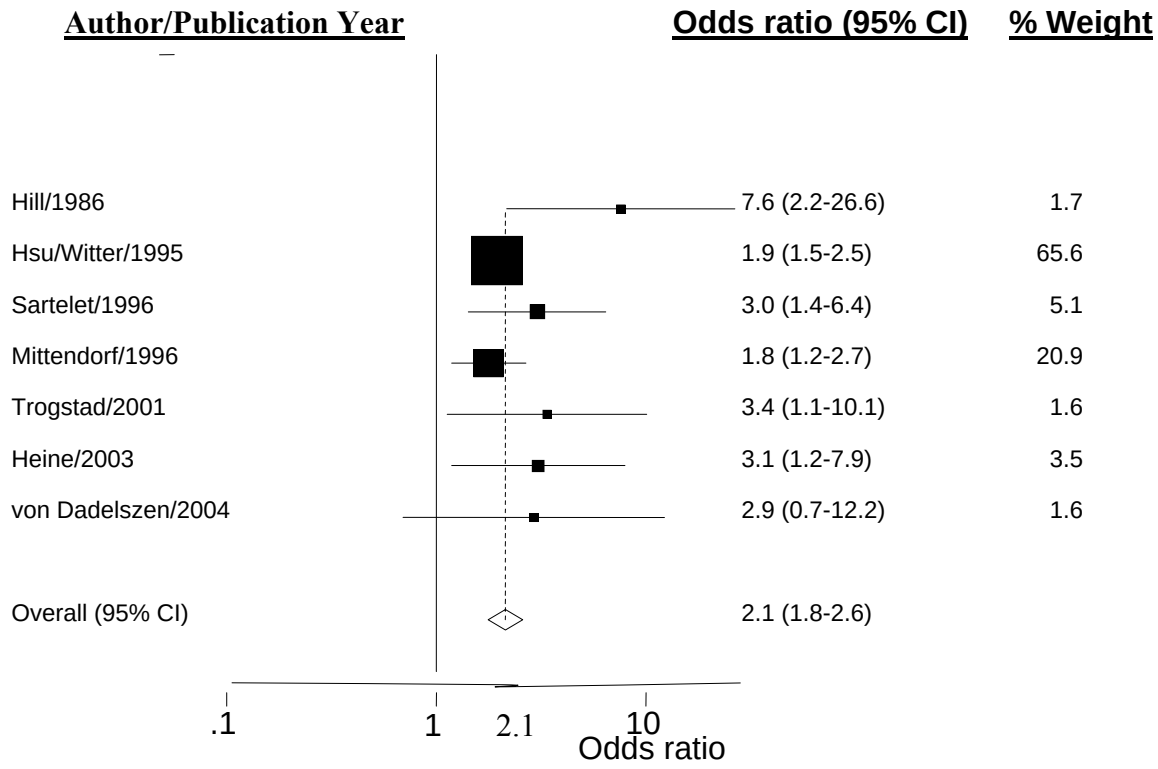

Figure 2: Association Between Bacterial and Viral Infections and Preeclampsia. Black squares and horizontal lines denote odds ratios and 95% confidence intervals for each study. The size of the black squares reflects the weight of each study. The Diamond shape denotes pooled odds ratio and 95% CI.

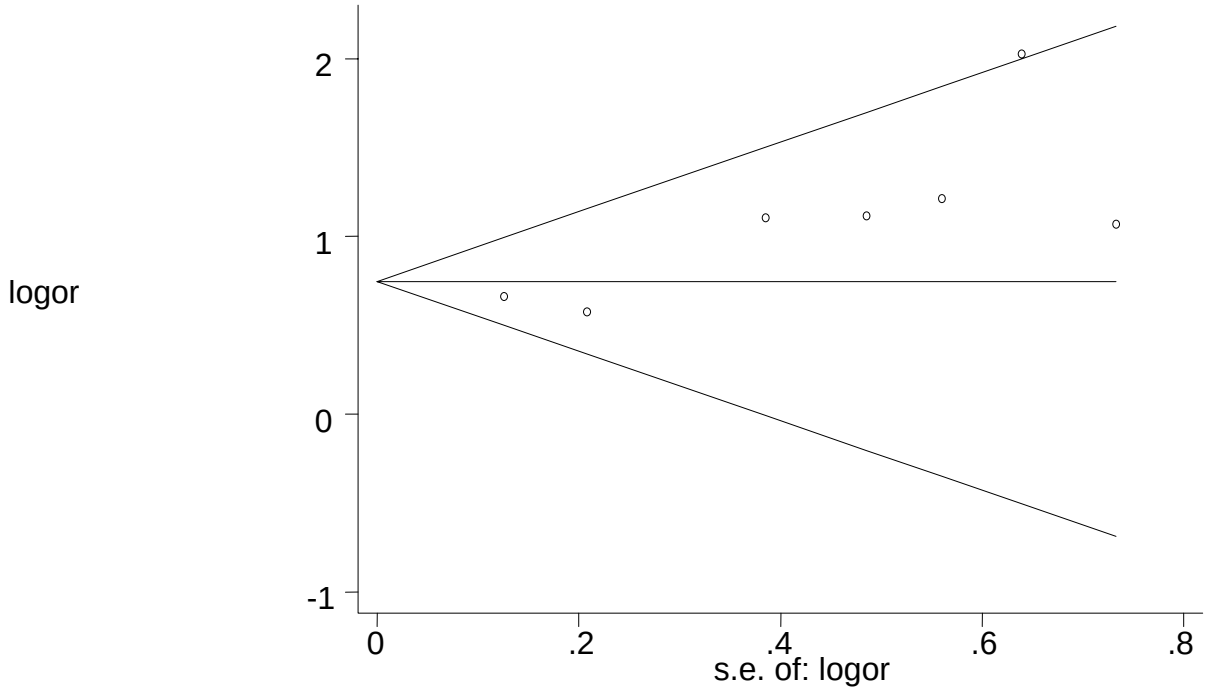

Figure 3: Funnel Plot (with pseudo 95% CI) to detect publication bias for studies exploring the association between maternal infection and preeclampsia. Odds ratios are presented on a logarithmic scale. Egger's test of publication bias: p=0.23

4.8. DISCUSSION (II)

Pooling of relevant epidemiologic data revealed a two-fold increased risk of preeclampsia associated with bacterial and viral infections. This finding is consistent with results obtained from individual studies. However, limitations of meta-analytic reviews should be considered when interpreting results. These limitations generally fall into two categories: 1) limitations carried over from when studies were originally conducted, and 2) those related to the conduct of the meta-analysis.

We addressed the first limitation, by defining *a priori*, inclusion and exclusion criteria. An important inclusion criterion required that a uniform preeclampsia diagnosis was implemented in all studies included in the meta-analysis. By doing so, we were reasonably certain that preeclampsia was defined consistently, which made it possible to make comparisons between studies. In the past, lack of consistency in defining preeclampsia often resulted in the erroneous inclusion of women with superimposed preeclampsia or women with underlying maternal disease such as, chronic hypertension and diabetes mellitus. Inclusion of a higher risk population can result in overestimation of preeclampsia incidence and can possibly inflate risk estimates.

Although there was no evidence of heterogeneity in this review, quality of meta-analytic results are nevertheless influenced by the quality of individual studies. If potential confounders were not adequately addressed when studies were originally carried out, combined results will inevitably reflect these limitations. This is of particular concern, since adjustment of confounders is not possible with aggregate data. However, given the consistency of risk estimates obtained across studies, it is unlikely that results from this review may be confounded by factors that were not controlled for originally.

The second limitation includes bias stemming from the conduct of a meta-analysis, such as publication bias. Publication bias is the tendency on the parts of investigators, reviewers, and editors to submit or accept manuscripts for publication based on the direction or strength of the study findings.[48] Typically this involves a higher acceptance rate of studies showing statistically significant results. In a meta-analysis, this type of bias can have the effect of skewing meta-analytic reviews toward a positive result. Inspection of the funnel plot in Fig.4 shows a symmetrical distribution of study results (shown in Fig. 4 as log odds ratio), we therefore, do not believe publication bias was a limitation in this review.

4.9. CONCLUSION (II)

Individual retrospective, case control and prospective studies have reported on the relationship between maternal infections and preeclampsia. Our combined results confirm these study findings. Preeclampsia is associated with an excessive intravascular, maternal inflammatory response. The cause of inflammation is not completely understood. However, infection clearly stimulates the immune system and may provide a ready explanation for preeclampsia-related inflammation.

4.10. METHODS (III)

4.10.1. NSFG Survey design (III)

Study III analyzed data collected in Cycle 5 of NSFG conducted between January and October 1995. The NSFG survey was designed and administered by the National Center for Health Statistics (NCHS), and represented a national probability sample of 10,847 civilian, non-institutionalized women between the ages of 15 and 44 years. The NSFG sample was drawn from 14,000 households interviewed in the 1993 National Health Interview Survey (NHIS). NSFG was conducted to produce national estimates of factors related to pregnancy and birth rates, including sexual activity, contraceptive use, infertility, and other health and health behavior related characteristics for the entire population of 60.2 million women 15-44 years of age [137]. In NSFG, the Primary Sampling Units, or PSU's, included all of the largest Metropolitan areas in the US. NSFG had 198 PSU's. Written informed consent was obtained from each participant before interviews were undertaken.

Of the 10,847 women who completed interviews, 6,483 were White, 2,466 were African American, 1,533 Hispanic and 365 were from other racial/ethnic groups. The final response rate for Cycle 5 of the NSFG was 81.9%. [141] Non-response rate (18.1%) was primarily due to an inability to locate sampled women. Missing data (don't know, refused, or not ascertained responses) were imputed, by matching reported data from a similar woman in the NSFG or the National Health Interview Survey (NHIS) to a woman with missing data. Less than one percent of the cases received an imputed value. [141]

NSFG data consists of two main data files: 1) Public Use File, and 2) the Omitted Items files. The Public Use File contains background information, pregnancy and a variety of socio-

demographic information, and the Omitted Items File consists of information on abortions, STDs, sexual risk taking behaviors, and other reproductive health measures.

4.10.2. Data collection (III)

Detailed and structured interviews were conducted in respondents' homes by trained interviewers with the aid of a computer assisted personal interviewing program (CAPI) installed on laptop computers. The CAPI program aided the interview process by selecting questions that were appropriate for each respondent, and incorporating skip patterns based on the intent and logic of questions. This program also assessed validity of responses, which helped to increase the quality of the data. On average, interviews took one hour and 43 minutes to complete. To ensure respondents' anonymity when answering sensitive questionnaire items, they were given the choice of using either an interviewer-administered or a self-administered interview mode. The self-administered mode involved the use of an audio computer-assisted self-interviewing process (Audio CASI). With Audio CASI, respondents heard the questions through headphones and self-entered answers into a laptop computer.

4.10.3. Study design (III)

The NSFG dataset contained records of 21,332 pregnancies reported by 10,847 respondents. Years in which pregnancies occurred ranged from 1964 to 1995. Of these records, case control designation was complete for only women who reported pregnancies between the years 1991 and 1995. This left 1,565 women, including 322 preeclampsia cases and 1,243 normotensive controls available for analysis. The prevalence of preeclampsia in the overall NSFG sample (Public Use File) was 1.5% (322/21,332). We then merged the Public Use File (containing socio-demographic and pregnancy data) with the Omitted Items File (containing STD and behavior-related data) by case identification number.

Out of the total 10,847 NSFG respondents, 434 women reported genital warts (4.0%), 215 genital herpes (2.0%), 490 Chlamydia trachomatis (4.5%) and 220 Gonorrhea (2.0%). However, because case control data were only available for the years 1991 through 1995, merging of STD data was limited to this time frame. The remaining years with available STD data were excluded from this analysis. The final sample consisted of 353 records with complete STD data for the period 1991 to 1995.

We also conducted secondary analysis on a subset of NSFG data. For this subset, we selected only primiparous respondents who reported single gestation pregnancies, no history of chronic hypertension, no history of gestational diabetes, and who were matched on age. Records missing complete STD information were excluded from this merged file as well. We were left with 340 records available for secondary analysis, which included 98 preeclamptics and 242 normotensives. This sub-sample provided approximately a 1:3 case control ratio.

The main outcomes in both analyses were preeclampsia and self-reported viral (genital warts, genital herpes) and bacterial (Gonorrhea and Chlamydia) infections.

Variable Definitions: Preeclampsia was defined by responses to the question: "During your pregnancy, did you have a medical problem that required medical attention beyond routine prenatal care, such as pregnancy-related high blood pressure, also known as preeclampsia?" This question was followed by a more detailed description of preeclampsia symptoms: "High blood pressure, which may be accompanied by proteinuria (protein buildup in urine) or edema (water retention and swelling), due to pregnancy or the influence of a recent pregnancy."

Bacterial and viral infections were ascertained from responses to the question: "Has a doctor or other medical provider ever told you that you had genital warts, genital herpes Gonorrhea, Chlamydia? Interviewers followed the question with a description of the infection.

For example for genital warts infection: "genital warts are usually painless warts on the vulva, cervix, or inside the vagina, caused by a virus (Human Papilloma) that is usually sexually transmitted." For genital herpes infection: "Genital Herpes is caused by a virus than can be sexually transmitted (Herpes Simplex Type 2). It should not be confused with herpes that causes cold sores or chicken pox (Herpes Simplex Type 1). In women, genital herpes causes sores on the cervix, vagina, and external genital area, and are sometimes painful and/or swollen." For Chlamydia: "Chlamydia is a sexually transmitted disease caused by a bacterial infection (*Chlamydia trachomatis*) and treated with antibiotic drugs. This infection often shows no symptoms in women." For Gonorrhea infection: "Gonorrhea, also known as 'GC' or 'Clap' is one of the most common sexually transmitted diseases and is caused by a bacterial infection (*Neisseria gonorrhoeae*) and treated with antibiotic drugs."

Socio-demographic Risk Factors: We created a three-category race variable (Non-Hispanic white, Non-Hispanic Black, and Hispanic) by combining the question related to ethnicity ("Are you of Hispanic or Spanish origin?), with the question designed to determine race ("Which of these groups: Alaskan Native or American Indian, Asian or Pacific Islander, Black, White would you say best describes your racial background?").

Marital status was categorized as: "currently married, not married but living with a partner or boyfriend, widowed, divorced, separated or never married." Level of education was ascertained from the question: "What is the highest grade or year of regular school you have ever attended. Possible responses were: Elementary/Junior High School (including 1st to 8th grade), High School (9th to 12th grade), and College and Graduate Professional School (1-7 years, or more)."

Behavioral Risk Factors: Aside from well-documented risk factors for preeclampsia including: 1) primiparity; 2) age; 3) low socioeconomic status; and 4) cigarette smoking, we obtained covariate information that may relate to the likelihood of infection. For example, we examined sexual behaviors, including age of sexual debut, number of sexual partners, frequency of sexual intercourse over the previous month, contraceptive use (barrier and non-barrier), length of cohabitation, and whether partner engaged in sexual relationships with other females or men. We also explored factors that may interact with sexual risk taking, such as IV drug use, sharing of needles, and having a partner who shared needles with others.

Statistical Analysis: We used STATA statistical software to merge, clean, and recode Public Use and Omitted Files variables. NSFG sample was not a simple random sample, in which all members of the population had an equal chance of being selected. To account for this, NSFG employed a sampling strategy that used weights designed to produce unbiased population estimates. The weights adjusted for: 1) the different sampling rates for Hispanic, black, and other women, 2) for non-response, and 3) for coverage rates. We therefore used STATA that accommodates unequal selection probabilities. With STATA we conducted both univariate and multivariate analyses of NSFG data and generated appropriate standard errors and p-values.

The *a priori* hypotheses for both primary and secondary analyses were that maternal viral infection denoted risk of preeclampsia, and sociodemographic, behavioral and clinical factors interacted with infection to trigger preeclampsia. Categorical variables were created for each bacterial and viral infection (coded yes=1/no=0) and summarized as frequencies by case control status. Continuous variables were summarized by means and standard deviations, and statistically significant differences determined by the Student's t-test for variables that were normally distributed and the Wilcoxon test used for non-normally distributed continuous

variables. For both primary and secondary analyses, variables that were missing 80% or more responses were excluded from logistic regression analyses (see Appendix A and B).

Univariate logistic regression analyses were used to explore whether genital warts, genital herpes, *C. trachomatis* and Gonorrhea infections, socio-demographic, and behavioral characteristics were associated with preeclampsia. Variables were entered one at a time, and individual log-likelihoods compared. Variables with p-values of 0.20 were considered important variables for inclusion in subsequent multivariate models.

Confounding was assessed, by determining whether univariate odds ratios were significantly altered (> 10%) upon addition of other relevant variables. Potential confounders included preeclampsia- and STD risk factors selected on the basis of previous literature, and biologic plausibility. Multicollinearity was accounted for by eliminating variables with redundant information, thereby reducing model variance.

Preeclampsia was considered as outcome variable in univariate and multivariate regression analyses. Bacterial (Gonorrhea and Chlamydia), viral (genital warts, genital herpes) infections, socio-demographic, known preeclampsia risk factors (age, race, parity, multiple gestation pregnancies, gestational diabetes, hypertension) and health behavior variables were included in the multiple logistic regression models and results expressed as adjusted odds ratios and 95% confidence intervals. We followed the same model building strategy for the secondary analysis, but did not make adjustments for known preeclampsia risk factors in multivariate analyses, since we had already matched on them at the design stage.

4.11. RESULTS (III)

4.11.1. Socio-demographic and health behavior characteristics (III)

As shown in Table 14, with the exception of income, cases and controls differed significantly on socio-demographic characteristics including region of residence, race, education, and marital status. Substance use (smoking) and abuse (IV drug use) were not significantly different. In general, the majority of sample respondents described themselves as non-Hispanic White (51.6% of cases and 63.1% of controls), while 30.9% of cases and 20.2% of controls were non-Hispanic Black. Hispanics accounted for 17.5% of cases and 16.7% of controls. The age of preeclampsia respondents ranged from 14.3 to 44.4 years (mean 26.5, SD 5.8) and 12.8 to 43.8 years for normotensives (mean 28.5, SD 6.3).

More than half of cases (59.8%) and controls (73.3%) were married, and the majority completed high school education. Of the single respondents, almost half cohabitated with a partner (41.8% of cases and 48.8% of controls), and half lived alone.

As expected, low birth weight babies were significantly more common among women who reported preeclampsia (18.2%) than normal pregnancies (7.8%, $p<0.001$). Prenatal care in the first six months of pregnancy was also commoner among women with preeclampsia (92.9% of cases versus 77.5% of controls, $p < 0.001$), and they were also, more often, recipients of Medicaid ($p <0.001$), and AFDC assistance ($p=0.04$). Very few women reported smoking during pregnancy.

Among barrier contraceptive users, cases (3.2%) used female condoms more often than controls (0.5%) ($p=0.07$), whereas no case control differences were observed in the use of male condoms (91.6% and 87.6%, respectively, $p=0.31$). Among non-barrier contraceptive users, women with preeclampsia used the rhythm method more often than controls (32.6% versus 19.4%, respectively, $p=0.02$).

Although not statistically significant, women with preeclampsia reported with greater frequency having had only one lifetime sexual partner compared to normotensive women (19.0% vs 9.3%). Very few women reported high-risk practices, including IV drug use (1.0% of cases versus 2.1% of controls), sharing of needles with others (0.3% of cases and 0.2% of controls), or sexual partners who had sex with men (0 % of cases and 2.2 % of controls). However, a slightly larger proportion of women suspected partners of having sexual intercourse with other women (17.8% of cases and 21.3% of controls). Of the preeclampsia cases, 16.8% reported being tested or treated for STDs in the previous 12 months, compared to 16.1% of controls. Additionally, one third of cases (30.5%), and controls (31.1%) reported being tested or treated for vaginal, UTI, or Pelvic infections in the previous 12 months. No case control differences were observed for frequencies of reported miscarriages or abortions.

In general, racial differences were seen for both socio-demographic, and behavior characteristics (Table 16). For example, Non-Hispanic blacks reported receiving Medicaid, AFDC and Food Stamp assistance significantly more often compared to Non-Hispanic Whites and Hispanics. Hispanics reported condom use significantly less often than Non-Hispanic whites and blacks ($p<0.001$). Non-Hispanic blacks, on the other hand, reported younger age of sexual debut ($p=0.02$), and larger numbers of sexual partners ($p=0.001$) with greater frequency compared to Hispanics and Non-Hispanic Whites.

Hispanics did not report any genital warts or gonorrhea infections. The prevalence of gonorrhea was significantly higher among Non-Hispanic Blacks (7.3% versus 0% for Hispanics and 1.0% for Non-Hispanic Whites, $p=0.02$).

4.11.2. Self-reported viral and bacterial infection (III)

Table 15 summarizes prevalence of maternal infection by case control status. Among the 102 preeclampsia cases with complete STD data, ten reported being told by a medical provider that they had genital warts (9.8%), seven respondents were told they had genital herpes (6.9%), eleven were told they had Chlamydia (10.8%), and only one reported Gonorrhea (1.0%). Among the 251 controls with complete STD data, eleven women reported Genital Warts infections (4.4%), six had Genital Herpes (2.4%), fourteen reported Chlamydia (5.6%), and six women reported Gonorrhea (2.4%). Both in the overall sample population and among women with preeclampsia, those with genital warts, genital herpes, *C. trachomatis*, or *N. Gonorrhoeae* infections, reported greater numbers of sexual partners, whereas those with no infections reported having had only one sexual partner.

Tables 17, 18, and 19 list results from logistic regression analyses, in which we examined the association between maternal infection, socio-demographic, and behavioral factors and the risk of preeclampsia. Our findings suggest that among women who reported genital warts, genital herpes, and Chlamydia trachomatis infections, the risk of preeclampsia was significantly greater (OR 2.5, 95% CI 1.2-5.4; OR 3.6, 95% CI 1.3-9.9; OR 1.9, 95% CI 1.1-3.1, respectively) in comparison to healthy pregnant women. These associations remained significant after adjusting for socio-demographic, behavioral, and infection-related risk factors (OR 3.0, 95% CI 1.1-8.5; OR 7.2, 95% CI 1.2-42.5; OR 5.2, 95% CI 1.3-20.2, respectively). Gonorrhea was not associated with preeclampsia neither in univariate or multivariate models.

We obtained similar findings in secondary analysis (Table 19). Genital warts, genital herpes and Chlamydia trachomatis significantly increased the risk of preeclampsia. In analysis set 1, Chlamydia (OR 1.1, 95% CI 0.4-3.0) was not associated with preeclampsia in univariate model.

However, after adjusting for socio-demographic and behavioral factors, Chlamydia was associated with 2.6-fold increased risk of preeclampsia (OR 2.6, 95% CI 0.5-13.2), but this association was not statistically significant. In contrast, Gonorrhea appeared to lower the risk of preeclampsia (OR 0.3, 95% CI 0.01-8.3).

Table 14: Percent Distribution of Self-reported Socio-demographic, and Behavioral Characteristics of Preeclampsia and Normotensive (Paper III)

Variable	**Preeclampsia Cases (%) (N=322)**	**Normotensive Controls (%) (N=1,243)**	**P***
Socio-demographic Characteristics			
Region			
Northeast	17.7	19.2	0.01
Midwest	23.3	25.6	
South	38.8	29.7	
West	20.2	25.5	
Race			
White, non-Hispanic	51.6	63.1	<0.001
Black, non-Hispanic	30.9	20.2	
Hispanic	17.5	16.7	
Marital Status			
Married or living w/ partner	59.6	73.3	<0.001
Single, or Living Alone	40.1	26.7	
Income			
> 70K	4.0	2.1	0.42
30K-70K	11.2	6.8	
< 30K	11.2	4.7	
Missing	73.6	86.4	
Education			
< High School	22.7	16.3	0.03
High School	73.9	79.5	
College/Graduate	3.4	4.2	

Table 14 (cont'd)

	Cases	Controls	p
Health Care Utilization and Food Assistance			
Medicare/Medicaid			
No	58.3	73.4	<0.001
Yes	41.7	26.6	
Foodstamp			
No	79.8	83.8	0.41
Yes	20.2	16.2	
AFDC			
No	24.5	14.2	0.04
Yes	4.7	1.2	
Missing	70.8	84.6	
Received Prenatal Care			
No	6.8	22.5	<0.001
Yes	92.9	77.5	
Missing	0.3	0.1	
Time Began Prenatal Care			
First Trimester	92.6	95.0	0.12
Second Trimester	7.4	5.0	
Trimester of Pregnancy	49.3	72.8	<0.001
First Trimester	0.0	3.1	
Second Trimester	50.7	24.1	
Third Trimester			
Obstetric Characteristics and Pregnancy Outcomes			
Treated for Miscarriage			
No	72.0	80.1	0.002
Yes	28.0	20.0	
Any Abortion			
No	94.7	94.2	0.86
Yes	5.3	5.8	
Low Birth Weight Baby			
No	81.9	92.2	0.001
Yes	18.2	7.8	
Baby's Gender			
Male	49.6	49.3	0.93
Female	50.4	50.7	

Table 14 (cont'd)

Non-Barrier Birth Control Methods	**Cases**	**Controls**	**p**
Oral Contraceptive			
No	16.8	19.7	0.56
Yes	83.2	80.3	
Depo-Provera			
No	85.3	90.7	0.17
Yes	14.7	9.3	
Withdrawal Method			
No	52.6	54.4	0.78
Yes	47.4	45.6	
Rhythm Method			
No	67.4	80.3	0.02
Yes	32.6	19.7	
Barrier Birth Control Methods			
Male Condom Use			
No	8.4	12.4	0.31
Yes	91.6	87.6	
Female Condom Use			
No	96.8	99.5	0.07
Yes	3.2	0.5	
Spermicide Use			
No	85.3	86.0	0.87
Yes	14.7	14.0	
Diaphragm Use			
No	86.3	92.2	0.11
Yes	13.7	7.8	
IUD			
No	99.0	98.5	0.73
Yes	1.0	1.5	

Table 14 (cont'd)

High Risk Behaviors	Cases	Controls	p
Frequency Intercourse			
≤ Once a month	15.0	15.8	0.27
2-3 a month	30.8	25.5	
Once a week	23.5	21.8	
2-3 times a week	29.6	29.1	
≥ 4 times a week	1.2	7.9	
Number of Sexual Partners			
≤ 1	19.0	9.3	<0.001
2-3	75.9	88.3	
4-5	2.6	0.7	
>5	2.6	1.7	
Age of First Sexual Intercourse			
≤ 15	20.0	26.3	0.50
15-20	62.1	57.4	
≥ 21	17.9	16.3	
IV Drug Use			
No	99.0	97.9	0.67
Yes	1.0	2.1	
Shared Needles			
No	0.0	100.0	0.20
Yes	100.0	0.0	
Partner Had Sex with Men			
No	100.0	97.8	0.31
Yes	0.0	2.2	
Partner Had Sex with Other Females			
No	82.2	78.7	0.53
Yes	17.8	21.3	
Tested or Treated for STD in Past 12 Months			
No	83.2	83.9	0.87
Yes	16.8	16.1	
Tested or Treated for Vaginal, UTI, or Pelvic Infection in Past 12 months			
No	69.5	68.9	0.92
Yes	30.5	31.1	

Table 14 (cont'd)	**Cases**	**Controls**	**p**
Chances of Being Infected With HIV High Medium Low None	 0.0 3.5 12.1 84.5	 0.9 0.4 22.2 75.9	 0.23
Substance Use			
Smoked During Pregnancy No Yes	 15.3 10.2	 22.3 19.8	 0.52

* Chi-square or Fisher's Exact test p-values

Table 15: Number and Percent Distribution of Maternal Infection by Case Control Status (Paper III)

Variable	**Preeclampsia Cases N=102* n (%)**	**Normotensive Controls N=251* n (%)**
Genital Warts No Yes	 92 (90.2) 10 (9.8)	 240 (95.6) 11 (4.4)
Genital Herpes No Yes	 95 (93.1) 7 (6.9)	 245 (97.6) 6 (2.4)
Chlamydia No Yes	 91 (89.2) 11 (10.8)	 237 (94.4) 14 (5.6)
Gonorrhea No Yes	 101 (99.0) 1 (1.0)	 245 (97.6) 6 (2.4)

* Sample size available to determine prevalence of infection across case control status.

Table 16: Frequencies and Percentages of Selected Maternal Characteristics by Race Groups (Paper III)

	Non-Hispanic White		Non-Hispanic Black		Hispanic	
	n(%)	N	n(%)	N	n(%)	N
Genital Warts	19 (8.1)	235	2 (2.9)	69	0 (0)	40
Genital Herpes	11 (4.7)	235	0 (0)	69	2 (5.0)	40
Chlamydia	18 (7.7)	235	4 (5.8)	69	3 (7.5)	40
Gonorrhea*	2 (0.9)	235	5 (7.3)	69	0 (0)	40
Male Condom Use**	177 (92.2)	192	52 (96.3)	54	20 (58.8)	34
Female Condom Use	1 (0.5)	192	2 (3.7)	54	1 (2.9)	34
Rhythm Method	50 (26.0)	192	8 (14.8)	54	9 (26.5)	34
Age first had sex*						
≤ 15 years	41 (21.7)	189	21 (38.9)	54	6 (17.7)	34
15-20 years	116 (61.4)	189	30 (55.6)	54	19 (55.9)	34
≥ 21 years	32 (16.9)	189	3 (5.6)	54	9 (26.5)	34

Table 16 (cont'd)

	Non-Hispanic White		Non-Hispanic Black		Hispanic	
	n(%)	N	n(%)	N	n(%)	N
# Sexual Partners in past 5 years**						
≤ 1	124 (13.8)	901	19 (5.7)	331	23 (9.1)	252
2-3	754 (83.7)	901	296 (89.4)	331	224 (88.9)	252
4-5	9 (1.0)	901	6 (1.8)	331	1 (0.4)	252
> 5	14 (1.6)	901	10 (3.0)	331	4 (1.6)	252
Age at pregnancy**						
< 19 years	33 (3.8)	879	26 (8.3)	312	10 (4.3)	235
19-34 years	684 (77.8)	879	246 (78.9)	312	187 (79.6)	235
> 34 years	162 (18.4)	879	40 (12.8)	312	38 (16.2)	235
Total Income						
< 30K	56 (32.6)	172	21 (47.7)	44	15 (48.4)	31
30K-70K	86 (50.0)	172	21 (47.7)	44	11 (35.5)	31
>70 K	30 (17.4)	172	2 (4.6)	44	5 (16.1)	31
Education**						
< High School	46 (4.9)	927	3 (0.9)	343	8 (3.1)	257
High School	109 (11.8)	927	82 (23.9)	343	83 (32.3)	257
≥ College	772 (83.3)	927	258 (75.2)	343	156 (64.6)	257
Medicaid**	180 (19.5)	924	188 (55.0)	342	88 (34.4)	256

Table 16 (cont'd)

	Non-Hispanic White		Non-Hispanic Black		Hispanic	
	n(%)	N	n(%)	N	n(%)	N
AFDC**	12 (6.3)	190	13 (24.5)	53	3 (8.8)	34
Food Stamp**	22 (11.6)	190	22 (41.5)	53	5 (14.7)	34
Get PNC	764 (82.4)	927	261 (76.5)	341	204 (79.4)	257
PNC 1st 6 mos	29 (3.8)	762	27 (10.3)	261	13 (6.4)	204
Parity*						
One	678 (73.1)	927	262 (76.4)	343	207 (80.5)	257
Two	249 (26.9)	927	81 (23.6)	343	50 (19.5)	257

* Chi-square or Fisher's Exact test p-values < 0.05

** p values < 0.001

Table 17: Analysis Set 1: Univariate Odds Ratios (OR) and 95% confidence intervals (CI) of Preeclampsia According to Self-reported Viral and Bacterial Infections, Socio-demographic, and Sexual Behavior Characteristics (Paper III)

	Univariate(b)		
	OR	**95% CI**	**p**
Genital Warts	2.5	1.2–5.4	0.02
Genital Herpes	3.6	1.3-9.9	0.01
Gonorrhea	0.7	0.1-3.3	0.62
Chlamydia	1.9	1.1-3.1	0.02
Race			
Non-Hispanic Black	1.7	1.3-2.2	<0.001
Hispanic	1.4	0.9-2.1	0.16
Education			
< High School	1.4	0.8-2.7	0.28
High School	0.9	0.5-1.8	0.90
Married	0.6	0.4-0.9	0.02
Age at Pregnancy			
19-34	2.5	1.3-4.9	0.01
> 34	3.5	1.4-8.8	0.01
First Trimester Pregnancy	3.5	2.8-4.4	<0.001
Sexual Partners			
2-3 partners	0.5	0.3-0.7	<0.001
4-5 partners	0.5	0.2-1.5	0.21
> 5 partners	0.6	0.4-0.7	<0.001
Age 1st Sex			
15-20 years	1.5	0.5-4.0	0.47
≥ 21 years	1.5	0.9-2.4	0.06
Partner Shared Needles	2.0	0.4-11.1	0.43
Smoking	0.4	0.3-0.7	0.001
Medicare/Medicaid	1.8	1.4-2.3	<0.001

Table 17 (cont'd)

	Univariate(b)		
	OR	**95% CI**	**p**
Prenatal Care (PNC)	4.2	2.5-7.3	<0.001
Began PNC in 2nd trimester	1.3	0.5-2.9	0.59
Gestational Diabetes	1.2	0.9-1.8	0.22
Pregnancy Order			
1st	2.7	1.6-4.5	<0.001
2nd	1.3	0.9-1.8	0.25
3rd	1.4	1.3-1.4	<0.001
4th	1.3	0.7-2.3	0.44
Treated for UTI	1.1	0.7-1.8	0.60

Referent category for race is Non-Hispanic White; for Education is High School; for Marital Status is married; for Partners in past 5 yrs is one partner; for Age 1st sex is less than 15; for Frequency Sex is ≤ 1 x/month. The reference category for viral and bacterial infection, Partner IV Drug Use, Smoking, Medicaid, Barrier and Non-barrier contraceptives is "No".

Table 18: <u>Analysis Set 1</u>: Multivariate Odds Ratios (OR) and 95% confidence intervals (CI) of Preeclampsia According to Self-reported Viral and Bacterial Infections, Socio-demographic, and Sexual Behavior Characteristics (Paper III)

Variable [(a)]	**Multivariate**[(b)]		
	OR	**95% CI**	**p**
Genital Warts	3.0	1.1–8.5	0.04
Genital Herpes	7.2	1.2-42.5	0.03
Gonorrhea	1.0	0.5-2.0	0.98
Chlamydia	5.2	1.3-20.2	0.02

Table 18 (cont'd)

Variable [a]	Multivariate[b]		
	OR	95% CI	p
Race			
Non-Hispanic Black	0.8	0.2-3.3	0.78
Hispanic	1.5	0.2-9.7	0.66
Income			
< 30K	3.1	1.1-9.1	0.04
30K-70K	1.3	0.5-3.6	0.65
Education			
< High School	3.0	0.3-27.5	0.34
High School	0.5	0.1-2.2	0.35
Not Married	1.4	1.0-2.1	0.07
Gestational Age	1.0	1.0-1.1	<0.001
All Sexual Partners			
2-3	0.9	0.5-1.8	0.76
4-5	1.2	0.1-20.5	0.88
> 5	0.6	0.2-1.9	0.40
Frequency Intercourse			
2-3 x/month	1.1	0.2-5.8	0.91
1 x/week	2.8	0.9-8.2	0.07
≥ 2x/week	2.1	1.2-3.7	0.01
Partner Had Sex w/ other females	2.6	0.8-7.9	0.11
Smoking	0.3	0.2-0.6	0.001
Medicare/Medicaid	3.2	2.5-4.0	<0.001
Food Stamp	0.4	0.3-0.7	<0.001

Table 18 (cont'd)

Variable [a]	Multivariate[b]		
	OR	95% CI	p
Male Condom	4.5	2.0-10.0	<0.001
Rhythm Method	3.5	2.2-5.8	<0.001
Tested or treated for UTI	0.6	0.2-1.4	0.21
Tested or treated for STD	0.6	0.3-1.5	0.26

[b] Adjusted for all other variables in the model, and additionally for age at pregnancy (three-year categories of <19 years to >34 years), Region (Northeast, Midwest, South, and West), Length of cohabitation, Gestational Diabetes, and High Blood Pressure

Table 19: Analysis Set 2: Univariate and Multivariate-adjusted Odds Ratios (OR) and 95% confidence intervals (CI) of Preeclampsia According to Viral and Bacterial Infections (Paper III)

	Univariate[a]		Multivariate[b]	
	OR (95% CI)	p	OR (95% CI)	p
Genital Warts	2.6 (1.1–6.6)	0.04	2.9 (1.1-7.5)	0.03
Genital Herpes	6.7 (1.2-38.2)	0.03	5.9 (1.1-32.8)	0.04
Gonorrhea	0.1 (0.0-1.3)	0.08	0.3 (0.0-8.3)	0.46
Chlamydia	1.1 (0.4-3.0)	0.84	2.6 (0.5-13.2)	0.25

[a] Cases and controls were matched for age, parity, and multiple gestation pregnancies. Cases and controls who reported hypertension, diabetes, and gestational diabetes were excluded from analysis

[b] Adjusted for Region (Northwest, Midwest, South, and West), prenatal care, gestational age, marital status, education, race, Medicaid, Food Stamp, AFDC, smoking, Barrier (condom, spermicide, diaphragm, cervical cap, sponge) /Non-Barrier (oral contraceptive, Depo-Provera, withdrawal and no birth control method) contraceptive use, cohabitation with partner, number of sexual partners in previous five years, frequency of intercourse (in previous month), IV drug use

4.12. DISCUSSION (III)

We observed significant associations between Genital Warts, Genital Herpes, and *C. trachomatis* and preeclampsia, which persisted after adjusting for both preeclampsia- and behavior-related risk factors. This study is, to our knowledge, the first study to explore the relationship between Genital Warts and Gonorrhea infections and the risk of preeclampsia. Previous epidemiologic studies have reported similar associations with respect to Herpes viruses (HSV-1, HSV-2, CMV, and EBV) and Chlamydia (*C. pneumoniae*), [80][83][86][125][157][186][194] but none have explored factors that could both affect infection status and interact with infection to trigger preeclampsia. Additionally, our results are consistent with previous studies that showed a protective effect of smoking (OR 0.3, 95% CI 0.2-0.6), which persisted after adjusting for socio-demographic, behavioral and infection-related risk factors.

A major strength of this study is that it is population-based, and therefore findings can be generalized to the general population. Furthermore, we were able to obtain detailed socio-demographic and STD-related characteristics to explore the association between maternal STD infection and preeclampsia and had sufficient power to detect significant associations.

A few study limitations should be considered when interpreting results. First, the present study relies on self-reports of sexually transmitted diseases and health risk behaviors, and is therefore prone to many forms of bias, including recall and social desirability bias. Ultimately, recall bias can result in both STD and health risk behaviors to be under or over-reported, either because respondents could not recall information or in the case of infection, were unaware of infection status. There is also the possibility for information to be withheld, if respondents perceived that their identities would not be kept anonymous or that their responses were not held confidential. Social desirability bias can occur if respondents perceived that investigators preferred certain attitudes or behaviors. [35]

NSFG used several techniques to maximize accuracy of responses and ensure privacy. NSFG interviewers assured respondents that both their identities and responses to questionnaire items would be kept confidential. Sensitive questionnaire items were left to the end, therefore allowing interviewers the time to establish rapport with respondents. Moreover, respondents were given the choice of a self-administered interview mode to answer more sensitive questions. They were asked at the end of interview, how likely it was that they would have given different answers had they chosen the interviewer-administered mode instead. Based on comparisons made with vital statistics and other external data, NSFG pregnancy and live birth self reports were found to be reliable. [141]

A second limitation is that case assignment was based on preeclampsia self-reports. Case misclassification has been a concern in preeclampsia studies due to lack of consistency in defining this disorder. Although a far more reliable method of determining preeclampsia incorporates well-defined diagnostic criteria, we found that preeclampsia prevalence in the NSFG sample (1.5%) was similar to that obtained from many studies utilizing clinical diagnoses. Preeclampsia occurs in 3-10% of all pregnancies worldwide and in approximately 2-4% in the United States.[44][79] Incidence varies depending on the patient population, institution and diagnostic criteria. For example, in a national hospital Discharge database, the rate of preeclampsia was 2.6%, [155] whereas the Maternal Fetal Medicine Network trial of low-dose aspirin in preeclampsia estimated a 6.3% incidence of preeclampsia. [169]

We believe it is unlikely that NSFG preeclampsia self-reports were widely inaccurate. Given the severity of preeclampsia, women may be more likely to remember whether they experienced a pregnancy complicated by preeclampsia. Nevertheless, it is still possible that some

preeclampsia cases were misclassified. This would most likely have the effect of attenuating associations.

4.13. CONCLUSION (III)

Both case control and prospective studies have reported on the relationship between maternal infections and preeclampsia. Preeclampsia is associated with an excessive intravascular maternal inflammatory response. The cause of inflammation is not completely understood. However, infection is known to stimulate the immune system and may provide a ready explanation for preeclampsia-related inflammation.

Clearly, firm conclusions cannot be drawn from self-reported STD data. Serology is a significantly more dependable indicator of infection. Ideally, pregnant women should be followed from the first through the third trimesters, with multiple blood draws available for STD testing. Further prospective studies are needed to understand better the temporal nature of STD infection and self-reported health risk behaviors. In addition data on medication use is helpful to determine the effect of treatment on infection and its relation to preeclampsia incidence.

5. PUBLIC HEALTH IMPLICATIONS

5.1. PAPERS I, II, III

Our findings suggest that maternal infections, particularly HSV-1, HSV-2, CMV, Genital warts (HPV) and Chlamydia trachomatis, significantly increase the risk of preeclampsia. Our findings have important public health significance. Given the widespread prevalence of these maternal infections, and the potential for their prevention, identification of subgroups of women with modifiable behaviors might ultimately contribute to prevention strategies.

Additionally, our research was the first to examine the relationship between primary herpes infections and preeclampsia. We found that seroconversion for HSV 1 / 2 or CMV during pregnancy, was associated with a five-fold increased risk of preeclampsia. One possible implication of this finding is that women who are seronegative at the onset of pregnancy may benefit from condom use.

Preeclampsia is associated with an excessive intravascular, maternal inflammatory response. The cause of inflammation is not completely understood. However, infection is known to stimulate the immune system and may provide a biologically plausible explanation for preeclampsia-related inflammation. However, whether *in utero* infections may influence preeclampsia risk is not known.

In addition, for most women, pregnancy represents a time in their lives when they are the most in contact with the health care system. For some women, pregnancy may be the only time they will see a medical provider on a regular basis. Obstetricians and primary care providers

have a unique opportunity to provide education and counseling to pregnant women at risk for STDs.

APPENDIX A: FREQUENCY DISTRIBUTION FOR VARIABLES IN PAPER I

Case control status

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = Normotensives	140	74.5	74.5	74.5
	1 = Preeclamptics	48	25.5	25.5	100.0
	Total	188	100.0	100.0	

Race

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = Black	56	29.8	29.8	29.8
	2 = White	132	70.2	70.2	100.0
	Total	188	100.0	100.0	

BMI category

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = < 18	133	70.7	74.3	74.3
	2 = 18 to 30	3	1.6	1.7	76.0
	3 = >30	43	22.9	24.0	100.0
	Total	179	95.2	100.0	
Missing	System	9	4.8		
Total		188	100.0		

Medicare/Medicaid

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	79	42.0	42.0	42.0
	1 = Yes	109	58.0	58.0	100.0
	Total	188	100.0	100.0	

APPENDIX A (cont'd)

Marital status

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = Never Married	83	44.1	44.1	44.1
	2 = Married	105	55.9	55.9	100.0
	Total	188	100.0	100.0	

Education

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = > HS	90	47.9	47.9	47.9
	2 = < HS	98	52.1	52.1	100.0
	Total	188	100.0	100.0	

Income Group

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = < 10K	47	25.0	25.0	25.0
	2 = 10-<20K	30	16.0	16.0	41.0
	3 = 20-50K	34	18.1	18.1	59.0
	4 = > 50K	49	26.1	26.1	85.1
	5 = Don't Know	28	14.9	14.9	100.0
	Total	188	100.0	100.0	

Smoking before pregnancy

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = Yes	24	12.8	14.3	14.3
	2 = No	22	11.7	13.1	27.4
	3 = Don't Know	122	64.9	72.6	100.0
	Total	168	89.4	100.0	
Missing	System	20	10.6		
Total		188	100.0		

APPENDIX A (cont'd)

Smoking during pregnancy

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = Yes	30	16.0	23.4	23.4
	2 = No	12	6.4	9.4	32.8
	3 = Don't Know	86	45.7	67.2	100.0
	Total	128	68.1	100.0	
Missing	System	60	31.9		
Total		188	100.0		

Time interval between blood draw

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = less than 6 months	12	6.4	6.4	6.4
	2 = greater or equal to 6 months	175	93.1	93.6	100.0
	Total	187	99.5	100.0	
Missing	System	1	.5		
Total		188	100.0		

Birth Control Pill

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	154	81.9	81.9	81.9
	1 = Yes	34	18.1	18.1	100.0
	Total	188	100.0	100.0	

Depoprovera

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	179	95.2	95.2	95.2
	1 = Yes	9	4.8	4.8	100.0
	Total	188	100.0	100.0	

No birth control

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	90	47.9	47.9	47.9
	1 = Yes	98	52.1	52.1	100.0
	Total	188	100.0	100.0	

Non barrier contraceptive

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	35	18.6	18.6	18.6
	1 = Yes	153	81.4	81.4	100.0
	Total	188	100.0	100.0	

Diaphragm

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	186	98.9	98.9	98.9
	1 = Yes	2	1.1	1.1	100.0
	Total	188	100.0	100.0	

APPENDIX A (cont'd)

Spermicide

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	169	89.9	89.9	89.9
	1 = Yes	19	10.1	10.1	100.0
	Total	188	100.0	100.0	

Condom

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	104	55.3	55.3	55.3
	1 = Yes	84	44.7	44.7	100.0
	Total	188	100.0	100.0	

Cervical cap

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	187	99.5	99.5	99.5
	1 = Yes	1	.5	.5	100.0
	Total	188	100.0	100.0	

Sponge

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	187	99.5	99.5	99.5
	1 = Yes	1	.5	.5	100.0
	Total	188	100.0	100.0	

APPENDIX A (cont'd)

Barrier contraceptive

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	91	48.4	48.4	48.4
	1 = Yes	97	51.6	51.6	100.0
	Total	188	100.0	100.0	

Any seroconversion

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	179	95.2	95.2	95.2
	1 = Yes	9	4.8	4.8	100.0
	Total	188	100.0	100.0	

HSV 1 / 2 seroconversion

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	181	96.3	96.3	96.3
	1 = Yes	7	3.7	3.7	100.0
	Total	188	100.0	100.0	

HSV-1 non primary infection

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	95	50.5	50.5	50.5
	1 = Yes	93	49.5	49.5	100.0
	Total	188	100.0	100.0	

APPENDIX A (cont'd)

HSV 1 / 2 non primary infection

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	91	48.4	48.4	48.4
	1 = Yes	97	51.6	51.6	100.0
	Total	188	100.0	100.0	

CMV non primary infection

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	107	56.9	56.9	56.9
	1 = Yes	81	43.1	43.1	100.0
	Total	188	100.0	100.0	

EBV non primary infection

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	10	5.3	5.3	5.3
	1 = Yes	178	94.7	94.7	100.0
	Total	188	100.0	100.0	

HSV-1 Negative

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	100	53.2	53.2	53.2
	1 = Yes	88	46.8	46.8	100.0
	Total	188	100.0	100.0	

APPENDIX A (cont'd)

HSV-2 negative

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	54	28.7	28.7	28.7
	1 = Yes	134	71.3	71.3	100.0
	Total	188	100.0	100.0	

HSV 1 / 2 negative

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	126	67.0	67.0	67.0
	1 = Yes	62	33.0	33.0	100.0
	Total	188	100.0	100.0	

HSV-2 non primary infection

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	145	77.1	77.1	77.1
	1 = Yes	43	22.9	22.9	100.0
	Total	188	100.0	100.0	

APPENDIX B: FREQUENCY DISTRIBUTION FOR VARIABLES IN PAPER III

Analysis Set 1

Preeclampsia

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	1,243	79.4	79.4	79.4
	1 = Yes	322	20.6	20.6	100.0
	Total	1,565	100.0	100.0	

Parity

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = Primipara	1,175	75.1	75.1	75.1
	1 = Multipara	390	24.9	24.9	100.0
Total		1,565	100.0	100.0	

Pregnancy Order

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = First	246	15.7	15.7	15.7
	2 = Second	223	14.3	14.3	30.0
	3 = Third	307	19.6	19.6	49.6
	4 = Fourth	399	25.5	25.5	75.1
	5 = ≥ Fifth	390	24.9	24.9	100.0
	Total	1,565	100.0	100.0	

Age at pregnancy

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = < 19 years	70	4.5	4.5	4.5
	2 = 19-34 years	1,139	72.8	72.8	77.3
	3 = > 34 years	252	16.1	16.1	93.3
Missing	System	104	6.7	6.7	100.0
Total		1,565	100.0	100.0	

Genital Warts

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	332	21.2	21.2	21.2
	1 = Yes	21	1.3	1.3	22.6
Missing	System	1,212	77.4	77.4	100.0
Total		1,565	100.0	100.0	

Gonnorhea

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	346	22.1	22.1	22.1
	1 = Yes	7	0.5	0.5	22.6
Missing	System	1,212	77.4	77.4	100.0
Total		1,565	100.0	100.0	

Chlamydia

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	328	21.0	21.0	21.0
	1 = Yes	25	1.6	1.6	22.6
Missing	System	1,212	77.4	77.4	100.0
Total		1,565	100.0	100.0	

HSV-2

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	340	21.7	21.2	21.2
	1 = Yes	13	0.8	0.8	22.0
Missing	System	1,212	77.4	77.4	100.0
Total		1,565	100.0	100.0	

Gestational Diabetes

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	1,415	90.4	90.4	90.4
	1 = Yes	147	9.4	9.4	99.8
	9 = Don't Know	1	0.1	0.1	99.9
Missing	System	2	0.1	0.1	100.0
Total		1,565	100.0	100.0	

Number of Sexual Partners

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = ≤ 1	171	10.9	10.9	10.9
	2 = 2-3	1,304	83.3	83.3	94.3
	3 = 4-5	16	1.0	1.0	95.3
	4 = > 5	29	1.9	1.9	97.1
Missing	System	45	2.9	2.9	100.0
	Total	1,565	100.0	100.0	

Frequency sexual intercourse

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = ≤ once/mo	38	2.4	2.4	2.4
	2 = 2-3 / mo	67	4.3	4.3	6.7
	3 = once/wk	55	3.5	3.5	10.2
	4 = > 5	72	4.6	4.6	14.8
Missing	System	1,333	85.2	85.2	100.0
	Total	1,565	100.0	100.0	

Smoking During Pregnancy

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	182	11.6	11.6	21.2
	1 = Yes	106	6.8	6.8	18.4
Missing	System	1,277	81.6	81.6	100.0
Total		1,565	100.0	100.0	

Race

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = White Non-Hispanic	927	59.2	59.2	59.2
	2 = Black Non-Hispanic	343	21.9	21.9	81.2
	3 = Hispanic	257	16.4	16.4	97.6
Missing	System	38	2.4	2.4	100.0
Total		1,565	100.0	100.0	

Marital Status

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = Single, or Living Alone	461	29.5	29.5	29.5
	1 = Married or Living w/Partner	1,103	70.5	70.5	99.9
Missing	System	1	0.1	0.1	100.0
Total		1,565	100.0	100.0	

Income

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = < 30K	94	6.0	6.0	6.0
	2 = 30K – 70K	121	7.7	7.7	13.7
	3 = > 70K	39	2.5	2.5	16.2
Missing	System	1,311	83.8	83.8	100.0
Total		1,565	100.0	100.0	

APPENDIX B Analysis Set 1 (cont'd)

Education

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = < High School	276	17.6	17.6	17.6
	2 = High School	1,226	78.3	78.3	96.0
	3 = College/Graduate	63	4.0	4.0	100.0
Total		1,565	100.0	100.0	

Medicare/Medicaid

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	1,096	70.0	70.0	70.0
	1 = Yes	464	29.7	29.7	99.7
Missing	System	5	0.3	0.1	100.0
Total		1,565	100.0	100.0	

Foodstamp

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	235	15.0	15.0	15.0
	1 = Yes	50	3.2	3.2	3.2
Missing	System	1,280	81.8	81.8	100.0
Total		1,565	100.0	100.0	

AFDC

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	255	16.3	16.3	16.3
	1 = Yes	30	1.9	1.9	18.2
Missing	System	1,280	81.8	81.8	100.0
Total		1,565	100.0	100.0	

Received Prenatal Care

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	301	19.2	19.2	19.2
	1 = Yes	1,262	80.6	80.6	99.9
Missing	System	2	0.1	0.1	100.0
Total		1,565	100.0	100.0	

Time Began Prenatal Care

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = First Trimester	1,190	76.0	76.0	76.0
	2 = Second Trimester	70	4.5	4.5	80.5
Missing	System	305	19.5	19.5	100.0
Total		1,565	100.0	100.0	

Any Abortion

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	269	17.2	17.2	17.2
	1 = Yes	16	1.0	1.0	18.2
Missing	System	1,280	81.8	81.8	100.0
Total		1,565	100.0	100.0	

Inject Drugs

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	280	17.9	17.9	17.9
	1 = Yes	5	0.3	0.3	18.2
Missing	System	1,280	81.8	81.8	100.0
Total		1,565	100.0	100.0	

Partner Use Drugs

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	264	16.9	16.9	16.9
	1 = Yes	9	0.6	0.5	17.4
Missing	System	1,292	82.6	82.6	100.0
Total		1,565	100.0	100.0	

Perception that partner had relationship with other females

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	218	13.9	13.9	17.9
	1 = Yes	55	3.5	3.5	18.2
Missing	System	1,292	82.6	82.6	100.0
Total		1,565	100.0	100.0	

Tested for AIDS

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	191	12.2	12.2	12.2
	1 = Yes	93	5.9	5.9	18.1
Missing	System	1,281	81.9	81.9	100.0
Total		1,565	100.0	100.0	

Tested for STDs in past 12 mos

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	241	15.4	15.4	15.4
	1 = Yes	47	3.0	3.0	18.4
Missing	System	1,277	81.6	81.6	100.0
Total		1,565	100.0	100.0	

Tested or treated for vaginal, UTI, or pelvic infection in past 12 mos

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	199	12.7	12.7	12.7
	1 = Yes	89	5.7	5.7	18.4
Missing	System	1,277	81.6	81.6	100.0
Total		1,565	100.0	100.0	

Condom use

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	32	2.0	2.0	2.0
	1 = Yes	256	16.4	16.4	18.4
Missing	System	1,277	81.6	81.6	100.0
Total		1,565	100.0	100.0	

Use rhythm method of birth control

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	219	14.0	14.0	14.0
	1 = Yes	69	4.4	4.4	18.4
Missing	System	1,277	81.6	81.6	100.0
Total		1,565	100.0	100.0	

Been told by MD had hypertension

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	260	16.6	16.6	12.7
	1 = Yes	28	1.8	1.8	18.4
Missing	System	1,277	81.6	81.6	100.0
Total		1,565	100.0	100.0	

APPENDIX B (cont'd)

Analysis Set 2(Adjusted for Primiparity, Gestational Diabetes, and Multiple Gestation

Preeclampsia

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	242	71.2	71.2	71.2
	1 = Yes	98	28.8	28.8	100.0
	Total	340	100.0	100.0	

Age at pregnancy

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = < 19 years	105	30.9	30.9	30.9
	2 = 19-34 years	203	59.7	59.7	90.6
	3 = > 34 years	13	3.8	3.8	94.4
Missing	System	19	5.6	5.6	100.0
Total		340	100.0	100.0	

Genital Warts

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	327	96.2	96.2	96.2
	1 = Yes	13	3.8	3.8	100.0
Total		340	100.0	100.0	

Gonnorhea

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	335	98.5	98.5	98.5
	1 = Yes	5	1.5	1.5	100.0
Total		340	100.0	100.0	

Chlamydia trachomatis

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	321	94.4	94.4	94.4
	1 = Yes	19	5.6	5.6	100.0
Total		340	100.0	100.0	

Genital Herpes

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	335	98.5	98.5	98.5
	1 = Yes	5	1.5	1.5	1.5
Total		340	100.0	100.0	

Number of Sexual Partners

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = ≤ 1	7	2.1	2.1	2.1
	2 = 2-3	183	53.8	53.8	55.9
	3 = 4-5	105	30.9	30.9	86.8
	4 = > 5	42	12.4	12.4	99.1
Missing	System	3	0.9	0.9	100.0
	Total	1,565	100.0	100.0	

Frequency of sexual intercourse

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = ≤ Once/mo	49	14.4	14.4	14.4
	2 = 2-3/mo	80	23.5	23.5	37.9
	3 = Once/wk	63	18.5	18.5	56.5
	4 = 2-3/wk	82	24.1	24.1	80.6
	5 = ≥ 4/wk	21	6.2	6.2	86.8
Missing	System	45	13.2	13.2	100.0
	Total	188	100.0	100.0	

Smoking During Pregnancy

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	213	62.7	62.7	62.7
	1 = Yes	127	37.3	37.3	100.0
Total		340	100.0	100.0	

Race

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = White Non-Hispanic	243	71.5	71.5	71.5
	2 = Black Non-Hispanic	77	22.7	22.7	94.1
	3 = Hispanic	20	5.9	5.9	100.0
Total		340	100.0	100.0	

Marital Status

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = Single, or Living Alone	170	50.0	50.0	50.0
	1 = Married or Living w/Partner	170	50.0	50.0	100.0
Total		340	100.0	100.0	

Income

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = < 30K	122	35.9	35.9	35.9
	2 = 30K – 70K	137	40.3	40.3	76.2
	3 = > 70K	81	23.8	23.8	100.0
Total		340	100.0	100.0	

Education

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	1 = < High School	183	53.8	53.8	53.8
	2 = High School	157	46.2	46.2	100.0
Total		340	100.0	100.0	

Medicare/Medicaid

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	231	67.9	67.9	67.9
	1 = Yes	108	31.8	31.8	99.7
Missing	System	1	0.3	0.3	100.0
Total		340	100.0	100.0	

Foodstamp

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	267	78.5	78.5	78.5
	1 = Yes	72	21.2	21.2	99.7
Missing	System	1	0.3	0.3	100.0
Total		340	100.0	100.0	

AFDC

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	289	85.0	85.0	85.0
	1 = Yes	50	14.7	14.7	99.7
Missing	System	1	0.3	0.3	100.0
Total		340	100.0	100.0	

Received Prenatal Care

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0 = No	66	19.4	19.4	19.4
	1 = Yes	274	80.6	80.6	100.0
Total		340	100.0	100.0	

Any Abortion

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	332	97.7	97.7	97.7
	1 = Yes	8	2.3	2.3	100.0
Total		340	100.0	100.0	

Inject Drugs

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	330	97.1	97.1	97.1
	1 = Yes	7	2.0	2.0	2.0
Missing	System	3	0.9	0.9	100.0
Total		340	100.0	100.0	

Perception that partner had relationship with other females

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	257	75.6	75.6	75.6
	1 = Yes	68	20.0	20.0	95.6
Missing	System	15	4.4	4.4	100.0
Total		340	100.0	100.0	

Tested for STDs in past 12 mos

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	284	83.5	83.5	83.5
	1 = Yes	56	16.5	16.5	100.0
Total		340	100.0	100.0	

Tested or treated for vaginal, UTI, or pelvic infection in past 12 mos

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	227	66.8	66.8	66.8
	1 = Yes	113	33.2	33.2	100.0
Total		340	100.0	100.0	

Condom use

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	37	10.9	10.9	10.9
	1 = Yes	303	89.1	89.1	100.0
Total		340	100.0	100.0	

Use rhythm method of birth control

		Frequency	Percent	Valid Percent	Cumulative Percent
Codes	0= No	262	77.1	77.1	77.1
	1 = Yes	78	22.9	22.9	100.0
Total		340	100.0	100.0	

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