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Maternal ABO Blood Phenotype and Factors Associated with Preeclampsia Subtype

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MATERNAL ABO BLOOD PHENOTYPE AND FACTORS ASSOCIATED WITH
PREECLAMPSIA SUBTYPE

by

Adriane Burgess

A Dissertation Submitted in
Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy
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ABSTRACT

MATERNAL ABO BLOOD PHENOTYPE AND FACTORS ASSOCIATED WITH PREECLAMPSIA SUBTYPE

by

Adriane Burgess

The University of Wisconsin-Milwaukee, 2017
Under the Supervision of Professor Teresa Johnson Ph.D., RN

Preeclampsia affects 3-8% of all pregnancies and is a global issue that significantly effects the short and long-term health of women and neonates. The pathophysiology of preeclampsia remains unclear and there seems to be two distinct subtypes, early and late onset. Each subtype may have a unique pathophysiology and set of risk factors. Preeclampsia is linked to long-term risk of cardiovascular disease in previously affected women. Subsequently, risk factors shared between preeclampsia and cardiovascular disease should be explored. The main aim of this study was to determine the strength of association between maternal ABO blood type and preeclampsia subtype. This hospital-based case control study was completed at one community hospital in the Mid Atlantic, United States. The study included 126 female subjects with early onset preeclampsia ($\leq 33\frac{6}{7}$ weeks gestation), 126 female subjects with late onset preeclampsia (≥ 34 weeks gestation) and 259 control subjects with no history of preeclampsia. Strict diagnostic criteria were used and preeclamptic subjects were classified by subtype based on gestational age at diagnosis. Data on ABO blood type, as well other physical and socio-demographic variables were extracted from the electronic health record. No significant association was noted between

preeclampsia subtype and non-O blood type ($p=.456$) and ABO blood phenotype trended towards significance ($p=.062$). After exclusion of subjects with comorbidities (CHTN, GDM and DM) from the sample ($n=403$), there was a significant association noted between ABO blood type and preeclampsia subtype ($p=.001$). A significant association was also noted between preeclamptic subjects with growth restriction and ABO blood type ($p= <.001$). Preeclamptic subjects with the B blood type had OR=3.44, 95% CI 1.58, 7.50 of having a growth-restricted fetus than did those with other blood types. Finally, when adjusting for race only, subjects with AB blood type had the following odds (OR=3.03, 95% CI 1.04, 8.80; OR=3.35, 95% CI 1.02, 11.03.) of developing preeclampsia and late onset preeclampsia respectively. When other clinical risk factors of preeclampsia are included in the model, AB blood type significantly predicts membership in the early onset preeclampsia subtype (OR=5.41, 95% CI, 1.19, 24.55) and was trend-level in the late onset group ($p=.053$). Preeclamptic women with B blood type had three times the odds of having a growth-restricted fetus, subsequently; they may require close ultrasound surveillance. AB blood type was significantly associated with three times increased odds of late onset preeclampsia. When included in a model with other common risk factors of preeclampsia, ABO blood type only accounted for a small amount of variability in the model. ABO blood type may not be a valuable addition to a preeclampsia-screening algorithm that already includes common clinical risk factors of preeclampsia. However, when controlling for other common clinical risk factors of preeclampsia, women with AB blood type had over 5 times the odds of developing early onset preeclampsia. Further research is necessary to examine if blood type regulates biomarkers that mediate the development of each preeclampsia subtype or in some way is associated with severe features of the disease.

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DEDICATION

To my parents,
my husband,
and especially Kate, Courtney, and Tyler

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CHAPTER 1

This chapter begins by introducing the problem of preeclampsia. Preeclampsia is linked to a variety of poor short and long-term outcomes for both mother and neonate. The disease is heterogeneous in nature and there are two distinct subtypes, early and late onset. Preeclampsia has been associated with increased risk of cardiovascular disease in previously effected women and shared risk factors should be investigated. Next, this chapter provides a pathophysiologic framework that describes the role of blood type in both disease processes. The purpose of this proposed dissertation study is given and research questions posed. Finally, contributions to the future of nursing science and practice are discussed.

Introduction

Incidence

Preeclampsia affects 3-8% of all pregnancies and is a global issue that significantly affects the short and long-term health of both women and neonates (Hutcheon, Liskonova & Joseph, 2011). Roughly, 1 in 12 pregnancies are affected by preeclampsia (Ilekis, Reddy & Roberts, 2007). Rates of preeclampsia have risen by 25% in the past two decades in the United States and the disease is linked to significant maternal morbidity and mortality as well as listed as a major contributor to prematurity (Ferrazzani et al., 2011; Wallis, Saftlas, Hsia, & Arish, 2008). Preeclampsia is a global issue, related to significant maternal and neonatal morbidity and mortality. Worldwide, hypertensive disorders of pregnancy are responsible for 76,000 maternal and 500,000 infant deaths each year (The Preeclampsia Foundation, 2016). Hypertensive disorders of pregnancy account for 16% of maternal deaths in developed countries and even

higher rates of maternal mortality related to these disorders are seen in Latin American and Caribbean countries (Khan, Wojdyla, Say, Gülmezoglu, & Van Look, 2006).

Maternal Complications

Hypertensive disorders of pregnancy account for 9.4% of maternal deaths in the United States (Creanga et al., 2015). In addition to the significant associated maternal mortality, preeclampsia is also linked to severe maternal morbidity, such as, eclampsia, acute renal failure, pulmonary edema, placental abruption, end organ damage and neurologic sequelae such as stroke (Sibai, Dekker & Kupferminc, 2005; Lisonkova & Joseph, 2013). These women, particularly those with early onset disease, are at higher risk for induction of labor and cesarean section (Alanis et al., 2008). The risk of serious complication increases with the severity of the disease (Hutcheon et al., 2011).

Infant Complications

Although preeclampsia is a maternal hypertensive disorder, with significant associated short and long-term maternal morbidity and mortality, there is also significant fetal and neonatal risk associated with the disease. Fifteen percent of pregnancies affected by preeclampsia result in spontaneous or medically indicated preterm birth (Alanis et al., 2008). There is a twofold risk of neonatal death in infants born to women diagnosed with preeclampsia, and significant associated neonatal morbidity, such as lower Apgar scores, seizures, and neonatal encephalopathy (Lisonkova, & Joseph, 2013). Fetal growth restriction (FGR) is commonly seen in infants of preeclamptic mothers, particularly those with early onset disease. Although rates of stillbirth are decreasing, women with preeclampsia continue to have far higher rates of stillbirth than unaffected pregnancies in both developed and developing countries (Hutcheon et al., 2011).

Definition and Diagnostic Criteria

Preeclampsia is defined by ACOG (2013) as the new onset of hypertension, with blood pressure greater than or equal to 140 mmHg systolic or greater than or equal to 90 mm Hg diastolic on two occasions at least 4 hours apart after 20 0/7 weeks gestation in a previously normotensive women, and proteinuria ($\geq 300\text{mg}$ in a 24-hour urine collection). In the absence of proteinuria the patient may have any one or more of the following in order to meet diagnostic criteria:

- platelets $< 100,000$ microliter
- serum creatinine > 1.1 or doubling of serum creatinine in the absence of renal disease
- elevated concentrations of blood liver transaminases to twice normal levels
- pulmonary edema
- new onset of cerebral or visual disturbances (ACOG, 2013, p. 4).

Non-proteinuric pre-eclampsia occurs in about 25% of all cases (Tranquilli, Brown, Zeeman, Decker & Sibai, 2013). Removal of proteinuria from the definitive diagnosis of preeclampsia proved important, as previously, many women with other signs and symptoms preeclampsia went undiagnosed solely due to lack of proteinuria. Unfortunately, while providers waited for women to become proteinuric in order to meet diagnostic criteria, the severity of the illness increased.

Severity. Due to the documented aggressive and dynamic nature of preeclampsia, there has been a change in nomenclature when documenting severity (ACOG, 2013). Preeclampsia is no longer documented as mild or severe, but rather “with severe features” and “without severe features” (ACOG, 2013, p. 8). Preeclampsia “with severe features” is documented when patients meet the basic criteria for diagnosis of preeclampsia and have any one or more of the below findings:

- systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg

- 110mmHg
- platelet count <100,000 per microliter
- impaired liver function as evidenced by severe persistent right upper quadrant or epigastric pain or elevated liver enzymes twice normal blood concentration
- progressive renal insufficiency as evidenced by worsening serum creatinine
- pulmonary edema
- cerebral or visual disturbances (ACOG, 2013).

Quantity of urinary protein, uric acid levels, FGR and edema are no longer diagnostic of increasing severity, however, may warrant closer surveillance of the pregnancy (ACOG, 2013).

Additionally, even in the absence of preeclampsia diagnosis, maternal report of individual symptoms such as new onset of cerebral or visual disturbance, severe epigastric or right upper quadrant pain, or an objective clinical finding of an elevation in blood pressure compared with an early pregnancy baseline blood pressure should result in close obstetrical evaluation (ACOG, 2013).

Classic Risk Factors

Clinical risk factors for preeclampsia include nulliparity, extremes in maternal age (<18 or >35 years), multiple gestation, obesity, African American race, utilization of assisted reproductive techniques, family history of preeclampsia, and history of previous preeclamptic pregnancy. Other preexisting diseases such as chronic hypertension, anti-phospholipid syndrome, lupus and diabetes have also been shown to increase risk of preeclampsia (Bartsch, Medcalf, Park, & Ray, 2016; Boyd, Tahir, Wohlfahrt & Melbye, 2013; Lisonkova & Joseph, 2013; O'Brien, Ray, & Chan, 2003; Paré et al., 2014). Women not affected by preeclampsia during their first pregnancy, have only a 1% chance of developing preeclampsia in a subsequent pregnancy if the pregnancy is with the same partner. However, if preeclampsia occurs in the first pregnancy, and the woman is pregnant again by the same partner, the chance of preeclampsia recurring is 7-15% (Hutcheon et al., 2011). Chance of recurrence increases even further in the

third pregnancy if both of the previous pregnancies were affected. Early gestational age at onset of preeclampsia also seems to increase risk of reoccurrence (Hutcheon et al., 2011).

Risk screening models. Due to the heterogeneous nature of disease, developing risk-screening models has proven difficult, as there are modifiable, non-modifiable, graded and interactional risk factors for preeclampsia (Osol & Bernstein, 2014). Additionally, over 200 biomarkers have also been explored alone and in combination, however, none have been revealed to be sensitive enough to result in prediction (Osol & Bernstein, 2014). Further research is necessary on the use of biochemical and biophysical markers already commonly collected during prenatal care for use in preeclampsia prediction.

Subtypes of Preeclampsia

Preeclampsia is heterogeneous in nature (Founds et al., 2011; Myatt et al., 2014). There are two distinct subtypes, early and late onset. It is important to note that these two subtypes are defined in relation to the timing of onset of the disease during pregnancy, not necessarily severity, and either subtype can have severe features (Kucukgoz Gulec et al., 2013). The American College of Obstetricians and Gynecologist guidelines for management of preeclampsia are based on the presence or absence of severe features. However, the subtypes seem to have differing clinical and laboratory findings of each subtype seem to differ (Kucukgoz Gulec et al., 2013). Subsequently, ACOG (2013) has called for more research into each preeclampsia subtype in order to determine if each subtype has a unique pathophysiology or risk factors and thus could require different management and/or treatment.

It has been difficult to synthesize research literature on preeclampsia subtypes as studies have used differing definitions of the early and late onset subtypes. In the literature, the definition of early onset preeclampsia varies, typically stated as onset of preeclampsia

symptomology occurring anywhere from prior to 28 0/7 weeks gestation to 37 0/7 weeks gestation (Tranquilli et al., 2013). Recently, the International Society for the Study of Hypertension in Pregnancy (ISSHP) set out to construct clear definitions of each preeclampsia subtype, in order to allow for improved synthesis of the research literature moving forward. Early onset preeclampsia is now defined by the ISSHP as occurrence of preeclampsia $\leq 33\ 6/7$ weeks gestation, while late onset preeclampsia occurs $\geq 34\ 0/7$ weeks (Lisonkova, & Joseph, 2013). Late onset preeclampsia is further subdivided to include preterm preeclampsia, which can occur anywhere between 34 1/7 weeks until 37 0/7 weeks gestation, and term preeclampsia, which occurs $\geq 37\ 1/7$ weeks gestation (Tranquilli et al., 2013).

Myatt et al. (2014) further support the importance of standardizing preeclampsia research design and provide guidelines for preeclampsia research. Myatt and colleagues (2014) also recognized variation in the definition of each preeclampsia subtype, noting that most research which explored preeclampsia subtypes do so in relation to timing of delivery, and utilized $<37\ 0/7$ weeks gestation and $>37\ 0/7$ weeks gestation as criteria for the early and late onset subtypes. Noting the significant increased risk of perinatal morbidity associated with preeclampsia cases that occur prior to 34 0/7 weeks gestation, Myatt and colleagues (2014) support the criteria outlined by the ISSHP and also recommend using $<34\ 0/7$ weeks gestation as criteria for early onset preeclampsia.

Early onset preeclampsia. Early onset preeclampsia is often more severe in nature than its late onset counterpart (Kucukgoz Gulec et al., 2013). It is suggested that early onset preeclampsia may have a significant genetic component and poor early placentation is seen more frequently in this subtype (Boyd et al., 2013; Steegers, Von Dadelszen, Duvekot, & Pijnenborg, 2010). Kucukgoz Gulec et al. (2013) observed increased rates of Hemolysis, Elevated Liver

Enzymes and Low Platelets (HELLP) syndrome, eclampsia, and admission to critical care units in women with early onset preeclampsia. These authors also reported that gestational age at admission seems to indicate worse neonatal outcomes. Early onset preeclampsia is also more often associated with abnormal uterine artery doppler velocimetry (Phillips, Janowiak, Badger & Bernstein, 2010) as well as FGR (Kucukgoz Gulec et al., 2013). Early onset preeclampsia may be unique in that there seems to be a significant uteroplacental component, as well as certain maternal pre-pregnancy characteristics that may predispose women to both the early onset subtype as well increased long-term cardiovascular risk (Phillips et al., 2010). “Early preeclampsia appears to be characterized by a state of high total vascular resistance and low cardiac output, whereas low total vascular resistance and high cardiac output mark term and near-term preeclampsia” (Phillips et al., 2010, p. 624). Although the ACOG Task Force (2013) did not construct preeclampsia treatment/management guidelines based on preeclampsia gestational age at onset, however, ACOG (2013) did make recommendations for initiation of late first trimester aspirin therapy based on prior pregnancy with early onset preeclampsia.

Late onset preeclampsia. Although early onset preeclampsia is often more severe in its presentation, late onset preeclampsia can also include “severe features” as described by ACOG (2013). The late onset subtype has been shown to have a higher incidence than early onset preeclampsia (Lisonkova, & Joseph, 2013). Boyd and colleagues (2013) suggest that environmental factors may contribute most to late onset preeclampsia.

Cardiovascular Disease and Preeclampsia

Previously it was thought that preeclampsia was completely cured by delivery of the pregnancy. However, more recently, research has begun to support that preeclampsia increases long-term risk of cardiovascular disease in women and infants who survive the pregnancy

disorder (Firoz & Melnik, 2011; Fraser et al., 2012; Geelhoed et al., 2010; Hermes et al., 2013; Herrera-Garcia, & Contag, 2014; Mongraw-Chaffin, Cirillo, & Cohn, 2010; van Rijn, et al., 2013). Women who were previously affected by early onset preeclampsia have been shown to have seven to nine times the increased risk of cardiovascular disease. Late onset preeclampsia has been shown to impart twice the risk of cardiovascular disease later in life in those who have been previously affected (Mongraw-Chaffin, Cirillo, & Cohn, 2010; van Rijn, et al., 2013).

Currently, no postpartum evidenced based cardiovascular risk reduction programs have been designed for women who have been diagnosed with either subset of preeclampsia, nor have screening strategies been created (Bushnell et al., 2014; Cusimano, Pudwell, Roddy, Cho, & Smith, 2014; Firoz & Melnik, 2011). Although traditional cardiovascular interventions may be used, there is no evidence to support their efficacy among women with preeclampsia and these interventions may differ by preeclampsia subtype (Celi et al., 2013; Firoz & Melnik, 2011; Veerbeek et al., 2015). Recent American Heart Association guidelines (2014) for the prevention of stroke in women reported that 18.2% of women with a history of preeclampsia had a cardiovascular event in the 10 years following the delivery of the effected pregnancy compared to 1.7% of women with uncomplicated pregnancies (Bushnell et al., 2014). With an understanding of the connection between cardiovascular disease (CVD) and preeclampsia, health care providers and women should view pregnancy as a unique opportunity, one that allows women and their health care providers an early glimpse into future health risk. Additionally, this provides women with an opportunity to begin to utilize preventative strategies proactively with an aim towards health promotion (Bushnell et al., 2014).

Blood Type as a Risk Factor

Due to the link between later life cardiovascular disease and preeclampsia, researchers should explore shared risk factors. Both cardiovascular disease and preeclampsia may be associated with blood type. Further examination of risk factors shared between the two disease processes is necessary, in the hope of establishing a clearer pathophysiologic basis for preeclampsia (Biswas et al., 2013; Bushnell et al., 2014). Several studies have revealed a correlation between ABO blood type and preeclampsia (Alpoim et al., 2011; Alpoim et al., 2012; Clark, Walker, Govan, Wu, & Greer., 2007; Hiltunen et al., 2009; Phaloprakarn & Tangjitgamol, 2013; Seyfizadeh et al., 2014; Than et al., 2011). No recent research has been done in the United States looking at the correlation between blood type and preeclampsia subtype.

Early onset preeclampsia has been linked to higher risk of cardiovascular disease than its late onset counterpart (van Rijn et al., 2013). It is proposed that the two subtypes have differing pathophysiology. Since ABO blood type has also been linked to cardiovascular disease, it is necessary to explore its role in the pathophysiology of both preeclampsia subtypes. With an understanding of the link between preeclampsia and cardiovascular disease, the pathophysiologic basis for preeclampsia may in fact be revealed through further investigation of biomarkers shared between preeclampsia and CVD (Biswas et al., 2013; Bushnell et al., 2014). Understanding non-manipulable risk factors of preeclampsia may help to find manipulable agents that can be used in treatment and management of preeclampsia (Shadish, Cook & Campbell, 2002).

Pathophysiologic Framework of the Study

Pathophysiology

There are four major human blood groups A, B, O and AB (Seyfizadeh et al., 2014). Current research supports that ABO blood phenotypes are now far more useful than in

hematology and transfusion medicine alone (Franchini & Lippi, 2015). Research indicates that blood phenotype may be linked to cancer particularly gastric and pancreatic cancers, cardiovascular disease as well as a variety of infectious disease (Biswas et al., 2013; Edgren et al., 2010; Franchini & Lippi, 2015). ABO antigens are expressed differently on the red cell surface as well as in a “variety of human cells and tissues, including epithelium, sensory neurons, platelets, and vascular endothelium” (Franchini & Lippi, 2015, p. 1). ABO blood phenotype affects hemostasis, due to varying levels of Von Willebrand factor (VWF) and Factor VIII by blood type (Franchini, Mengoli, Lippi, 2016). Non-O groups have approximately 25% higher levels of VWF than other types (Franchini & Lippi, 2015). Thusly, those with Non-O blood phenotypes are at increased risk for venous thrombosis, arterial thrombosis, myocardial infarction (MI) and ischemic stroke (Franchini & Lippi, 2015). The link between alteration in hemostasis seen in certain blood types as well as preeclampsia supports the biologic plausibility of this study.

Feminism

Traditional science holds significant power to improve outcomes, however, long held beliefs surrounding the biologic similarities of male and female bodies have hampered progress particularly related to cardiovascular research focused on women’s health. Although the basis of this study is highly pathophysiologic, feminism provides an excellent framework by which to examine the role preeclampsia plays in risk of future CVD. It is important for researchers in this area to have an understanding of how women’s oppression plays into their social reality (Yuill, 2012). A feminist framework allows for in-depth analysis of phenomena surrounding women bodies as well as their overall health, and provides a platform by which researchers can advocate

for the health of women and challenge a patriarchal dominated medical community (Routledge, 2007).

Purpose of the Proposed Study

This study will focus on identifying the strength of association between maternal blood type and preeclampsia subtype with an aim at improving identification and screening of those at increased risk for future cardiovascular disease. Additionally, the study will aim to identify risk factors and birth outcomes associated with both early and late onset preeclampsia.

Hypotheses and Research Questions

The research questions that will be addressed in this study include:

Research Question 1:

What are the maternal characteristics of women with preeclampsia and with each preeclampsia subtype?

Hypothesis 1: Individuals with early onset preeclampsia will have more traditional cardiovascular risk factors such as higher body mass index (BMI) and chronic hypertension and diabetes; additionally, more subjects in the early onset subtype will be of African American race and of lower socioeconomic class.

Research Question 2:

Is there an association between preeclampsia subtype and ABO blood phenotype?

Hypothesis 2: Early onset preeclampsia will be more highly correlated with non-O blood types than its late onset counterpart will. This directional hypothesis is based on several previous studies, which showed women with non-O blood types were at increased risk of preeclampsia due to elevated coagulation factors noted in these blood types as well as differences in inflammatory biomarkers (Alpoim et al., 2011; Hiltunen et al., 2009; Lee, Zhang et al., 2012; Phaloprakarn & Tangjitgamol, 2013). The main aim of these studies was not to assess this association by preeclampsia subtype, but rather preeclampsia as a homogenous group. Since

there is evidence to support two distinct subtypes of the disease, it is important to look at if blood type more significantly correlates with one subtype. Since cardiovascular risk is higher in the early onset subtype and non-O blood types correlate with increased cardiovascular risk (Chen et al., 2014; Etemadi et al., 2015; Gong et al., 2014a; Gong et al. 2014b; He et al., 2012; Karabuva, Carević, Radić, & Fabijanić, 2013; Lee, Zhang et al., 2012; Zakai et al., 2014), this author hypothesizes that non-O, blood types will also be more common in the early onset preeclampsia subtype.

Research Question 3:

What is the association between preeclampsia subtype and Rh type?

Hypothesis 3: Rh factor will not be associated with preeclampsia or preeclampsia subtype. In most previous studies which assessed the association between Rh factor and both preeclampsia and CVD no relationship was reported (Etemadi et al., 2015; He et al., 2012; López-Pulles et al., 2010; Shamsi et al., 2010). Although most studies did not find an association between Rh factor and preeclampsia, Sezik, Toyran and Yapar (2002) reported an association between O negative women and increased risk of HELLP syndrome. Subsequently, Rh data will be included in the study in order to determine if Rh factor is associated with either subtype of preeclampsia.

Research Question 4

What is the association between ABO blood type, platelet count at > 28 6/7 weeks gestation and preeclampsia development?

Hypothesis 4: ABO blood type will be associated with platelet count at $\geq 28\ 6/7$ weeks gestation in each preeclampsia subtype. Preeclampsia diagnostic criteria can include platelet count $< 100,000$ microliter in the absence of proteinuria, and platelet count $< 100,000$ microliter can also indicate increasing severity of the disease. ABO antigens are expressed on both the red

cell as well as on platelets (Franchini & Lippi, 2015). Since risk of preeclampsia is said to differ by ABO blood type, platelet count may also differ (Freitas, Alpoim, Komatsuzaki, Carvalho, & Dusse, 2013; Hiltunen et al., 2009; Lee, Zhang et al., 2012; Phaloprakarn & Tangjitgamol, 2013).

Research Question 5:

What is the association between ABO blood type and fetal growth restriction in preeclamptic women?

Hypothesis 5: ABO blood type will be associated with FGR in preeclamptic women.

Preeclampsia, particular early onset has been associated with increased risk of FGR (Kucukgoz Gulec et al., 2013). Hiltunen et al. (2009) discovered an association between AB blood type and growth restriction in preeclamptic women.

Research Question 6

What factors are significantly associated with preeclampsia and preeclampsia subtype?

Hypothesis 6: Non-O blood types will have increased odds of preeclampsia and preeclampsia early onset subtype. Traditional clinical and sociodemographic risk covariates such as increased BMI, nulliparity, lower socioeconomic status, Black race, extremes in maternal age will increase odds of the early onset preeclampsia subtype (Bartsch et al., 2016; Boyd et al., 2013; Lisonkova & Joseph, 2013; O'Brien, Ray, & Chan, 2003; Paré et al., 2014).

Study Setting

Due to accessibility, the electronic health record (EHR) from a large suburban community hospital in the Mid Atlantic United States that has roughly 3000 births per year was utilized to obtain the desired data. There are only two other small community hospitals with in the area; however, the majority of high-risk pregnancies are delivered at this hospital due to its NICU and high risk obstetrical facilities. Additionally, maternal fetal medicine specialists also

care for women not geographically close to this institution. Subsequently, women go on to deliver at this institution while following up with their high-risk care. This may result in a higher proportion of women with preeclampsia being seen at this institution.

Contributions to Nursing

Over the past 20 years, scientists have learned much about preeclampsia and the focus has moved away from blood pressure as the central etiology of the disease. Recent research has begun to focus on biomarkers that may provide insight into origins of the disease this however, has not resulted in earlier recognition or improved management (Myatt et al., 2014). In November of 2013, preeclampsia diagnostic criteria was updated and no longer requires proteinuria for diagnosis (ACOG, 2013). In turn, this may result in women that would previously not have been diagnosed with preeclampsia now being included in studies. Inclusion of this new population of women may in turn reveal new characteristics about women with preeclampsia. Many previous studies did not explore specific subtypes of preeclampsia; research aimed at exploring specific subtypes of preeclampsia may lead to an enhanced understanding of the pathophysiology of the disease (Myatt et al., 2014). A more clear understanding of the pathophysiology may improve prediction. Additionally, with the known association between cardiovascular disease and preeclampsia, a better understanding of risk factors that mediate the two diseases may lead to the creation of targeted therapies as well as cardiovascular preventive interventions specific to each subtype of preeclampsia.

Chapter Summary

Preeclampsia is heterogeneous in nature, each subtype is associated to a varying degree with poor long-term cardiovascular sequelae. Thusly, it is important to explore shared risk factors. If the primary hypothesis of this study is supported, non-O blood types will be

associated with an increased risk of early onset preeclampsia, which in turn may shed light on the pathophysiologic process that mediates its relationship to cardiovascular disease. This study may then provide new information on how to tailor cardio preventive therapies geared towards women who have had preeclampsia or aid in the design of tools aimed at preeclampsia prediction.

CHAPTER 2

Philosophical Framework and Literature Review

Introduction to the Chapter

The aim of this study is to 1) examine the association between ABO blood type, Rh factor and preeclampsia subtype and to 2) determine which other factors most significantly influence the development of each preeclampsia subtype. Additionally, epidemiological data will be collected in order to better describe each preeclampsia subtype. It is well established that women with preeclampsia have an increased risk of CVD (Mongraw-Chaffin, Cirillo, & Cohn, 2010; van Rijn, et al., 2013). Of the two subtypes, women with the early onset subtype have a significantly higher risk of cardiovascular disease than the late onset subtype (van Rijn, et al., 2013). It is hypothesized that due to the increased cardiac risk associated with non-O blood types, non –O blood types may also be associated with the development of early onset preeclampsia.

Chapter 2 begins by outlining the philosophical framework from which this study is grounded. Since preeclampsia is a uniquely female cardiac risk factor, feminism provides an excellent framework by which to address research gaps and pose questions. Feminist empiricism as well as the role feminism plays in cardiovascular research is described. Next, the proposed pathophysiology of preeclampsia will be described and non-classic biomarkers discussed. Finally, a review of the literature that assesses the current evidence on both the relationship between both ABO blood type and preeclampsia as well as cardiovascular disease and preeclampsia was completed in order to describe common themes.

Philosophical Framework

Cardiovascular disease (CVD) remains the leading cause of death in women in the United States, with heart attack the leading cause and stroke the third leading cause of mortality (Tindall & Vanderman-Winter, 2011). Although millions of dollars have been allocated towards research on cardiovascular disease, women have not seen the same improvement in outcomes, as have men. Rates of cardiovascular mortality have been declining in men since the early 1980s however, rates in women have been declining at a much slower rate, with CVD effecting 1 in 3 women (American Heart Association, 2015). Even within this gendered component of the disease, there continues to be racial disparities. CVD disproportionately affects African American and Hispanic women (Mosca, Hammond, Mochari-Greenberger, Towfighi, & Albert, 2013). In order to appropriately study this vexing problem, taking a gendered approach is necessary. A gendered approach requires studying both biologic as well as socially constructed differences between the lives of men and women (Davidson, 2012). Preeclampsia has significant implications for the long-term cardiovascular health of women, and is a cardiovascular risk factor unique to women. Thusly, more in depth study of preeclampsia and its relationship to cardiovascular disease needs to occur. Although blood type is not a uniquely female characteristic, it is important to assess how ABO blood type mediates preeclampsia risk as well as long-term cardiovascular disease risk in women, as this may improve prediction of both diseases.

Feminist empiricism. Feminist empiricism supports the basic principles of scientific inquiry, accepts that the world can be seen objectively and believes that knowledge is gained through experiences. The feminist empiricist also supports that research should be done by women on female participants in order to further maintain the feminist paradigm (Yuill, 2012).

There has been much debate as to what constitutes nursing knowledge, particularly how it relates to questions of basic science (Perry, 1994). Biologic research is indeed nursing research. For as nurses, we provide holistic care, and with this said, biologic phenomena cannot be separated from the holistic context (Perry, 1994). Perry et al. (1994) stated that “In order to prevent illness and promote health, it is necessary to generate knowledge about cellular processes that is less distorted and biased than that produced by a method entrenched in androcentrism” (p. 491). This quote speaks to the fact that the utilization of a feminist framework in biologic studies is paramount to the creation of new knowledge in this area.

Both women and men perpetuate androcentrism in the basic sciences (Perry, 1994). Historically, women and issues of women’s health have been excluded from basic science research as well as research agendas. A scientific community largely made up of men has designed these agendas. Subsequently, the health issues of women as well how women are effected by disease has not been studied as extensively due to allocation of funds towards androcentric research agendas (Perry, 1994). It is necessary to begin to focus basic science research on the health and wellness of women and continue to acknowledge the effect gender bias has had in cardiovascular research (McCormik & Bunting, 2002).

Feminist empiricism may be helpful in addressing research gaps specific to the biologic functioning of women by accounting for androcentric bias found in theories and methodologies often used in basic science (Perry, 1994). The researcher and the phenomena being studied are inextricably linked, thus objectivity in research is value laden (Perry, 1994; Potochnik, 2012). Social values shape ways of knowing in the scientific community, and thus scientific practice (Potochnik, 2012). This not only influences what studies are undertaken but also, hypotheses postulated, variables chosen, judgments made and answers found (Potochnik, 2012). Perry

(1994) suggests that increasing the number of women working within the scientific realm to determine and study biologic problems specific to women, has the potential to alter the research agenda and greatly improve outcomes in women.

Feminist Framework

A feminist framework can be used to gather both quantitative and qualitative data, as “it is the point of view of the researcher that makes research feminist in nature” (McCormick & Bunting, 2002, p.824). McCormick and Bunting (2002) listed eight criteria gathered from multiple authors, which define feminist research:

- “principle investigator was a woman,
- feminist methodology was used,
- the study had the potential to help the subjects as well as the researcher,
- the research focused on the experience of the woman,
- the investigator’s purpose was to study women within their role as women,
- the word “feminist” or “feminism” was used in the report,
- bibliographic references to feminist literature were made, and
- nonsexist language was used” (p. 824).

Several of the above criteria will be addressed as this author sets out to study the phenomena of preeclampsia and risk factors related to long-term cardiovascular disease.

Foundational to this study is the aspect that cardiovascular research, prevention strategies, and risk factor identification strategies do not adequately represent the needs and risk factors unique to women. When a feminist viewpoint is used to study cardiovascular disease in women, the potential for understanding the phenomena is enhanced. In addition, this author’s study aims to improve the health of women, valuing the experience of pregnancy as unique as well as sex specific.

Women’s Awareness of CVD

Clarke (1992) called for the use of a feminist methodology in health promotion research and asked researchers to consider gender and sex when designing and conceptualizing research

studies. For many years, CVD was socially constructed as a male illness, however, in the past decade, there has been a significant effort to increase women's awareness of their risk for this disease (Long, Taubenheim, Wayman, Temple, & Ruoff, 2008; Mosca, Hammond, Mochari-Greenberger, Towfighi, & Albert, 2013). Although awareness is improving, it continues to lag particularly in racial and ethnic minorities (Mosca, Mochari-Greenberger, Dolor, Newby, & Robb, 2010). Even with improvement in women's awareness of CVD risk, due to androcentric worldviews and gender bias in health research, how women are affected by, their risk factors for, and symptomology of CVD has been under studied (McCormick & Bunting, 2002).

Lack of research on how sex and gender specific factors affect cardiovascular disease and outcomes provide inadequate science for nurses to adequately educate and care for their female clients. Since health care providers are relatively unaware of the link between certain pregnancy complications and long-term cardiovascular risk, women remain under educated about their increased CVD risk if they had a pregnancy complicated by preeclampsia (Young, Hacker, & Rana, 2012). Subsequently, women are unable to make decisions to initiate lifestyle modifications aimed at prevention of CVD. Adams et al. (2014) called for improved communication and collaboration between obstetrical and primary care providers as well as increased patient education about the importance of pregnancy history to cardiovascular outcomes.

Feminist Research

In order to improve women's health research initiatives, in 1993, the NIH Revitalization Act required that women be included in clinical trials, the same year the FDA reversed its ruling that barred childbearing women from participating in clinical research (Mosca, Barrett-Connor, & Kass Wenger, 2011). Although these were great strides towards improvement and

identification of women's health issues, in 2003, the Food and Drug Administration Office of Women's Health (FDA OWH) reported that although women and men are equally participating in research, these results are not presented as gender specific (Mosca, Barrett-Connor, & Kass Wenger, 2011). It is integral to differentiate between the concepts of sex and gender (Hammarstrom et al., 2014). Examining biologic mechanisms unique to women allows researchers to begin to better understand their role in long-term health outcomes. A more clear understanding of uniquely female biologic concepts, such as preeclampsia and other pregnancy specific complications may be integral to improving health in women. There are biologic, social and environmental factors that must be considered when assessing risk and protective mechanisms that occur in the lives of men and women (Lorber & Moore, 2002). With this said, pregnancy is uniquely female, and the effect this process has on the long-term health of women must be considered.

Sex and Gender Specific Factors of Cardiovascular Disease

Yentl syndrome. In 1991, Dr. Bernadette Healy published an article in the *New England Journal of Medicine* describing Yentl Syndrome. Yentl syndrome is named after a woman in a short story who had to disguise herself as a man to receive equal treatment. Dr. Healy felt that women did not receive equal treatment for CVD until their symptomology presented like that of a man. She cited research showing that women were less likely to undergo coronary angiography, angioplasty and surgery when admitted to the hospital with similar diagnosis as their male counterparts; additionally women exhibited more debilitating angina then men and continued not to receive cardiac catheterization (Healy, 1991). It was not until after women had procedures such as cardiac catheterization and angioplasty or were determined definitively to have had a myocardial infarction that they received treatments at the same rate as men. At this

point, due to delayed treatment, major damage was often done. Dr. Healy described this inequitable treatment of women with cardiovascular symptomology as Yentl syndrome (Healy, 1991). Sex specific cardiovascular risk factors such as preeclampsia may further complicate identification of women at risk for CVD. Obstetrical risk factors for CVD such as preeclampsia, fetal growth restriction (FGR), and prematurity are not factored into cardiovascular risk models or acknowledged by health care providers as CVD risk factors further perpetuating Yentl syndrome as described by Dr. Healy over thirty years ago (Smith, Pudwell, Walker & Wen, 2012).

Biologic differences. Although there has been improvement in cardiovascular care in the past two decades, there continues to be more women than men living with, and dying from CVD and stroke, and women continue to have higher rates of hypertension than men (Mosca et al., 2011; McCormick & Bunting, 2002). Much of the early biomedical health research was conducted utilizing male participants, often generalizing these findings to women. Social issues notwithstanding, there are significant biologic as well as anatomical differences between the cardiovascular systems of men and women. Sex specific factors such as pregnancy, menopause, and hormones may have an effect on CVD (Craici, Wagner, & Garovic, 2008). These sex specific factors may influence onset and diagnosis of CVD, as well as its clinical course, efficacy of therapy, and outcomes (Craici et al., 2008). Biologic differences between men and women include; women have smaller coronary arteries, increased rates of mitral valve prolapse and left ventricular hypertrophy, lower hematocrit levels, and lower premenopausal blood pressures (McSweeney, Pettey, Souder, & Rhoads, 2011). These biologic dissimilarities can result in differences in CVD risk factors between the sexes (McSweeney et al., 2011).

Differences in symptoms. Women with coronary heart disease (CHD) present with different symptomology than do men and often present with silent myocardial ischemia. However, in contrast to men who often report crushing chest pain during episodes of cardiac ischemia, women frequently present with what health care providers often term ‘atypical’ chest pain. Women often report abdominal pain, persistent flu-like symptoms, shortness of breath as well as nausea and fatigue during periods of cardiac ischemia (McCormick & Bunting, 2002; Nicholson, 2007, p. 44). This atypical presentation often results in misdiagnosis and delay in treatment which may in turn effect long-term outcomes (McSweeney et al., 2011). However, researchers and clinicians should be cautious describing women’s symptoms as ‘atypical’ and work to redefine symptoms of heart disease to be inclusive.

Women as caregivers. In addition to sex specific factors, gender specific factors also play a role as to how women experience cardiovascular disease. Research has shown that women wait longer with symptoms before seeking cardiovascular care (Nicholson, 2007). A women’s delay in seeking care for CVD symptoms is often related to their role as wife, mother, and caregiver (Davidson et al., 2012). When considering timing of cardiovascular interventions after preeclampsia it is important to contemplate a women’s role as new mother. Women are often distracted caring for others, and put their own needs last; however, during the postpartum period they may be motivated to embrace lifestyle changes in order to promote the health and wellness of their family (Cusimano et al., 2014; Mosca et al., 2006; Seely et al., 2013). Women are often faced with role strain as they struggle to care for family, often balancing work outside the home. Having health care providers that honor mutuality in decision-making may improve a women’s willingness to access health care (Davidson et al., 2012). The socially constructed roles of women are important to consider when attempting to understand preeclampsia’s

relationship to long-term cardiovascular risk as well as when creating interventions to protect against or treat cardiovascular disease in women who have had preeclampsia.

Biomedical discourse. Historically, uniquely female biologic experiences have been characterized by medical establishments as being a flawed state or a deficiency, resulting in medicalization (Hyde, Nee, Howlett, Drennan, & Butler, 2010). This has occurred with menopause, which is more often considered a deficiency disorder by the medical community subsequently shaping the way women and health care providers view the biologic changes that accompany menopause (Hyde et al., 2010). Many times women feel guilty after a preeclamptic pregnancy due to poor infant outcomes that are often associated with this disease and may feel overwhelmed when learning of their risk of CVD at such a young age (Seely et al., 2013). Although preeclampsia may in fact be an independent risk factor of cardiovascular disease, it will be important to acknowledge that pregnancy is not a “flawed” state and not allow medical discourse to shape a women’s childbirth experience (McCormick & Bunting, 2002). Although it is important to recognize preeclampsia as a sex specific risk factor of CVD, nurses should use this an opportunity to empower women and advocate for improvements in their health and wellness as well as that of their family (McCormick & Bunting, 2002).

Health Care Provider Awareness of Preeclampsia and Cardiovascular Disease

As with CVD, in preeclampsia, when women are uneducated, they are often unaware of the signs and symptoms. Additionally, the physical complaints associated with preeclampsia are often non-descript. Because of this, health care providers often delay diagnosis and treatment (You, Wolf, Bailey, & Grobman, 2012). In obstetrical patients, health care providers need to be encouraged to listen carefully to women when they call into the office with reports of vague complaints such as they are “just not feeling right.” Health care providers in all settings tend to

minimize these reports, which are often missed opportunities for early evaluation and improved outcomes (Tsigas, 2006; Walsh, 2013). A common theme of women's experiences with both preeclampsia as well as cardiovascular disease is lack of recognition of symptomology by the medical community. Only 60% of primary care providers are aware of up to date American Heart Association guidelines for prevention of CVD in women (Ehrenthal et al., 2013), similarly, physician's awareness of preeclampsia as a risk factor for future cardiovascular disease is also low (Young, Hacker, & Rana, 2012). These gaps in recognition, response, as well as education may occur due to gender bias that has been perpetuated through medical and health care texts which are often outgrowths of "male-centered medical research" (McCormick & Bunting, 2002, p. 823). Nursing as a female dominated profession is ideally situated to bring voice to the gender specific disparities in the cardiovascular care of women (McCormick & Bunting, 2002).

Pathophysiology of Preeclampsia

In order to better understand possible shared risk factors between cardiovascular disease and preeclampsia an understanding of the hypothesized pathophysiology of preeclampsia is necessary. Preeclampsia has been studied in biologic, immunologic, genetic and pathophysiologic research (Charlton, Tooher, Rye, & Hennessy, 2014; Founds et al., 2011; Ileki, Reddy & Roberts, 2007). The disease seems to be resultant from a complex interplay of maternal and fetal factors (Hermes et al., 2013).

A common pathophysiologic theory of preeclampsia involves abnormal implantation of the placenta where trophoblasts insufficiently invade and remodel the uterine spiral arteries resulting in inadequate oxygen delivery to the fetus (Warrington, George, Palei, Spradley, & Granger, 2013). What is causative of this maladaptation remains unclear. It seems that the insufficient remodeling of the spiral arteries leads to ischemia, which in turn increases

angiogenic factors such as sFLT-1 and other inflammatory cytokines (Warrington et al., 2013). The release of these inflammatory factors into maternal circulation may result in fibrin disposition in the spiral arteries of the placenta, which can occlude blood to the fetus and result in FGR (Alpoim et al., 2011). There is also a decrease in proangiogenic factors and an increased production of reactive oxygen species. This in turn leads to maternal endothelial dysfunction (Warrington et al., 2013). Subsequently, due to the increased production of endothelin-1 and decreased availability of nitric oxide, extensive vascular abnormalities occur in end organs such as the brain and kidneys, resulting in hypertension, neurologic disturbances and proteinuria (Warrington et al., 2013).

Risk of CVD Based on Early Versus Late Onset Preeclampsia

Risk of CVD. Globally, one in three women dies of cardiovascular disease (CVD). CVD presents itself differently in women than in men, and often goes undiagnosed, making early identification of risk factors integral to the prevention of increased morbidity and mortality (Rich-Edwards, Fraser, Lawlor & Catov, 2014). Obstetric history is a unique risk marker in women that often goes unassessed (Spaan, Peeters, Spaanderman, & Brown, 2012). Typically early onset preeclampsia has been associated with increased severity of symptoms as well as worse maternal and neonatal outcomes than its late onset counterpart (Boyd et al., 2013; van Rijn et al., 2013). A large prospective study by Mongraw-Chaffin, Cirillo, & Cohn (2010) revealed that “cumulative cardiovascular disease death survival for women with early preeclampsia was 85.9% compared to 98.3% for women with late preeclampsia and 99.3% for women without preeclampsia” (p.1). Women with early onset disease have been shown to have a 7-8 fold increased risk of cardiovascular disease, whereas women with late onset disease have a twofold increase risk (van Rijn et al., 2013).

Shared risk factors. The link between pregnancy complications such as preeclampsia and future cardiovascular disease is not well understood. Research has revealed shared risk factors between cardiovascular disease and preeclampsia. Shared preeclampsia and CVD risk factors include increased prepregnancy lipids, triglycerides, BMI, as well as sub clinical elevation in blood pressure (Alsnes et al., Bushnell et al., 2014; 2014; Ray, Diamond, Singh & Bell, 2006; Rich-Edwards et al., 2014; Roberts, Bodnar, Patrick & Powers, 2011). Other underlying CVD risk factors such as metabolic syndrome may also play a role in the development of preeclampsia (Founds et al., 2011; Smith et al., 2009). Risk factors such as hypertension, diabetes and smoking seem to have more of an adverse effect on cardiovascular health in women and young people than in men and the elderly (Spaan et al., 2012). In young women, favorable cardiovascular profiles resulted in an 80% reduction in cardiovascular mortality when compared to young women who had two or more cardiovascular risk factors (Spaan et al., 2012). This points to the extreme importance of both primordial and primary prevention of cardiovascular disease in this group of women. With an understanding that women with a history of preeclampsia are at higher risk of CVD later in life, research aimed at exploring novel risk factors shared between preeclampsia and CVD may be helpful. Research may be helpful in revealing if pregnancy acts a stress test to expose women already at risk of CVD or rather if preeclampsia provides added physiologic stress that is in fact causal (Osol & Bernstein, 2014). Additional research aimed at revealing risk factors of preeclampsia may help to establish the relationship between pregnancy complications and chronic disease risk. This in turn will help clinicians alter the health trajectories of women who have experienced pregnancies complicated by preeclampsia. (Rich-Edwards et al., 2014).

van Rijn (2013) and colleagues suggest that women with early onset preeclampsia had two or more independent cardiovascular disease risk factors in addition to their preeclampsia diagnosis. These authors also observed higher BMI's in women with early onset preeclampsia, however elevated BMI could only partially explain the increased cardiovascular risk associated with early onset preeclampsia. In a recent study by Veerbeek et al. (2015), postpartum modifiable cardiovascular risk factors did differ by preeclampsia subtype: early, late, and pregnancy induced hypertension. The early onset group had an overall less favorable CVD profile postpartum. Specifically, there were significant differences in postpartum glucose and lipid levels in the early onset group. Interestingly, both early onset and PIH groups had higher rates of hypertension postpartum than the late onset group (Veerbeek et al., 2015).

Postpartum Pathophysiologic Effects of Preeclampsia

Hermes et al. (2013) stated that at 2.5 years postpartum, women with a history of gestational hypertension or late onset preeclampsia exhibit more cardiovascular risk factors and have higher risk of hypertension than women who had a normotensive pregnancy. These cardiovascular risk factors included "higher waist circumferences; higher BMI; higher systolic and diastolic blood pressures; higher prevalence of metabolic syndrome; higher levels of biochemical risk factors, including glucose, HbA1c, insulin, insulin resistance (HOMA), total cholesterol, triglycerides, and hs-CRP; and lower levels of high density lipoprotein (HDL) cholesterol" (Hermes et al., 2013, p. 474.e5).

Endothelial dysfunction. Endothelial dysfunction has been proposed to be a key factor in the pathophysiology of preeclampsia and may be the link between preeclampsia and long-term cardiovascular risk (Sandvik et al., 2013). Soluble fms-like tyrosine kinase (sFlt-1), and placental growth factor are antiangiogenic factors produced by the placenta, and may cause the

endothelial dysfunction that results from preeclampsia. Women with a history of preeclampsia have been shown to have markers of endothelial dysfunction one year after the index pregnancy. These markers of endothelial dysfunction were shown not to normalize until 11 years after the index pregnancy (Oslund et al., 2013). Lao (2014) suggests that in preeclampsia there is a decrease in endothelium-dependent dilatation. Vascular alterations, such as increases in the thickness of the carotid intima-media, result in alterations in blood pressure such as “higher brachial diastolic pressure, central systolic pressure, mean arterial pressure (MAP), and peripheral vascular resistance (PVR)” (Lao, 2014, p.250). Aykas et al. (2015) also found that alterations in the vascular structure and function associated with preeclampsia persisted after the index pregnancy. Melchiorre, Sharma, & Thilaganathan, (2014) reported that major adverse cardiovascular events occur at much higher rates in pregnancies affected by preeclampsia and risk of stroke and myocardial infarction remains significant for \geq three years postpartum. Although Oslund (2013) found normalization in endothelial dysfunction, women with a history of preeclampsia remained at higher risk for dysrhythmias, heart failure as well as cardiac death.

Cardiac changes. In addition to vascular changes, cardiac changes have also been shown to occur in patients with preeclampsia. In late onset preeclampsia, women had left ventricular remodeling as well as myocardial damage, whereas women with early preeclampsia seem to have more severe cardiac impairment, and in both groups, severe dysfunction and left ventricular hypertrophy could still be found at 1 year postpartum although higher rates were noted in the early onset group (Lao, 2014). In women who delivered preterm (<34) with preeclampsia, risk of cardiac death was even greater. In these women with early preeclampsia, one in seven would die from a cardiac event within three decades of delivery (Mongraw-Chattin et al., 2010).

Shared Pathway CVD and Preeclampsia

In order to bring light to the disparities in the cardiovascular care of women, gender specific cardiovascular risk factors must be explored. Although much research supports the link between preeclampsia and future CVD, the mechanism which connects the two is not clear. Scantlebury and Hayes (2014) propose several different pathways by which preeclampsia may increase risk of CVD

- 1) Increased risk of metabolic syndrome
- 2) Persistent endothelial dysfunction
- 3) Common underlying risk factors
- 4) Altered myocardial structure and function
- 5) Renal disease
- 6) Other non-classic risk factors

Non-classic biomarkers

A variety of biomarkers have been explored with an aim at improving prediction of preeclampsia. Visser et al. (2014) completed a metanalysis and systematic review of the literature looking at novel biomarkers that may predict cardiovascular risk. Due to the suggested common pathophysiology between cardiovascular disease and preeclampsia, Visser et al. (2014) hoped to better elucidate if these biomarkers could aid in preeclampsia prediction. The biomarkers which these authors reviewed included:

“inflammation (intercellular adhesion molecule [ICAM], vascular cell adhesion molecule [VCAM], interleukin-6 [IL-6], interleukin-10 [IL-10], and E-selectin), thrombosis (homocysteine, von Willebrand factor [VWF], fibrinogen, fibronectin, endothelin, D-dimer, plasminogen activator inhibitor-1 [PAI-1], tissue plasminogen activator [tPA]), and angiogenesis (vascular endothelial growth factor [VEGF], soluble Fms-like tyrosine kinase-1 [sFLT-1], and tumor necrosis factor alpha ([TNF-a])” (Visser et al., 2014, p. 373e2).

The authors broke these biomarkers into categories, those that effected inflammation, thrombosis, and angiogenesis (Visser et al., 2014). Levels of some of the specific biomarkers listed above are unique based on blood type. Although these authors did not find significant

differences in thrombotic biomarkers such as von Willebrand factor and fibrinogen in their review, previous studies have revealed a shared pathophysiology between thrombosis and preeclampsia and elevated levels of these markers in preeclamptic women. This shared pathway could explain the restriction of blood flow and reduced placental perfusion noted in preeclampsia (Visser et al., 2014). It has been shown that levels of VWF as well as other biomarkers vary based on blood type (Franchini & Lippi, 2015). Thusly, it is biologically plausible that ABO blood type may mediate the development of preeclampsia (Alpoim et al., 2013). Additionally, since preeclampsia influences risk of future cardiovascular disease and ABO blood type has been implicated in the development of both disease processes, this relationship must be further explored.

Review of the Literature: Preeclampsia and ABO Blood Type

Introduction

Preeclampsia affects 3-8% of all pregnancies (Hutcheon et al., 2011). It is a global issue and is related to significant maternal and neonatal morbidity and mortality. Rates of preeclampsia have risen by 25% in the past two decades in the United States and it is listed as a major contributor to prematurity (Ferrazzani et al., 2011; Wallis et al., 2008). Mothers diagnosed with preeclampsia have increased risk of poor short and long-term outcomes (Bushnell et al., 2014). Preeclampsia has been called the disease of theories and the pathophysiology has yet to be elucidated (Founds et al., 2011). It is hypothesized that different subtypes of the disease may exist.

Preeclampsia is defined by ACOG (2013) as the new onset of hypertension ≥ 140 mm Hg systolic or ≥ 90 mmHg diastolic after 20 weeks gestation, and proteinuria (≥ 300 mg in a 24-hour urine collection) or in the absence of proteinuria, platelets $< 100,000$ microliter, serum creatinine > 1.1 or doubling of serum creatinine in the absence of renal disease, and elevated

concentrations of blood liver transaminases to twice normal levels. When preeclampsia is diagnosed prior to 34 weeks in pregnancy it is defined as early onset preeclampsia, when occurring on or after 34 weeks gestation it is defined as late onset preeclampsia (Lisonkova, & Joseph, 2013). Typically early onset preeclampsia has been associated with increased severity of symptoms and more severe maternal and neonatal outcomes than late onset preeclampsia (Boyd et al., 2013; van Rijn et al., 2013). ACOG (2013) recently called for more research into the subtypes of preeclampsia in order to better determine if each subtype has a unique pathophysiology or set of risk factors thus requiring tailored therapies and interventions.

Preeclampsia and Cardiovascular Disease

Previously it was thought that preeclampsia was in fact cured by the imminent delivery of the baby. However, more recently, research has begun to support that preeclampsia is linked to long-term risk of cardiovascular disease in affected women (Firoz & Melnik, 2011; Fraser et al., 2012; Hermes et al., 2013; Mongraw-Chaffin, Cirillo, & Cohn, 2010; van Rijn, et al., 2013). The risk seems to be further increased in women with early onset preeclampsia. Women who were previously affected by early onset preeclampsia have been shown to have a 7-9 fold increased risk of cardiovascular disease. Women with late onset preeclampsia has been shown to impart a twofold increased risk of cardiovascular disease later in life in those who have been previously affected (van Rijn, et al., 2013).

Currently, no evidenced based cardiovascular risk reduction programs have been designed for women with preeclampsia, nor have screening strategies been created (ACOG, 2013; Firoz & Melnik, 2011). Although traditional cardiovascular interventions may be used, there is no evidence to support their efficacy in women with preeclampsia (Firoz & Melnik, 2011). More research is needed to identify variables, which may increase a woman's risk for

preeclampsia, so prediction, prevention and treatment of preeclampsia can be improved.

Additionally, we continue to look for what mechanisms connect hypertensive disorders of pregnancy to chronic cardiovascular disease later in life in women with a history of preeclampsia so that cardiovascular interventions can be tailored to this population (Scantlebury, & Hayes, 2014). The following review of literature will explore the link between maternal ABO blood type and preeclampsia.

Blood Type, Preeclampsia and CVD

In the early twentieth century scientist, Karl Landsteiner discovered the ABO blood phenotype, which was the first genetic polymorphism detected in humans (Etemadi et al., 2015; Franchini & Liumbruno, 2013). There are four ABO blood phenotypes, A, B, AB, and O. Today ABO blood type is used in far more than in transfusion medicine alone (Franchini & Lippi, 2015). ABO blood type can be the cause of illness such as the case in hemolytic disease of the fetus and the newborn, or in case of hemolytic reactions that occur after transfusion or transplantation (Franchini, & Liumbruno, 2013). ABO blood type has been shown to increase the incidence of some disease processes, such as cardiovascular disease, cancer, venous thrombus and resistance against certain pathogens (Hiltunen et al., 2009; Phaloprakarn & Tangjitgamol, 2013; Seyfizadeh et al., 2014; Than et al., 2011). With the known link between cardiovascular disease and preeclampsia blood type may be a biologically plausible moderator.

There has also been literature to suggest a correlation between maternal blood type and preeclampsia. However, no current literature explores the correlation between ABO type blood and preeclampsia subtype as currently defined by ACOG (2013). Pike & Dickins (1954) first looked into the relationship between a woman's blood type and preeclampsia and found that women with type O blood may be at increased risk for developing preeclampsia. More recently,

several other authors continue to support the relationship between blood type and preeclampsia, however, these authors have pointed to women with non-O blood types as being at increased risk of preeclampsia. Phaloprakarn & Tangjitgamol (2013) as well as other authors hypothesized that the correlation between non-O blood phenotypes such as A, B and AB and preeclampsia may be related to the fact that VWF is higher in non-O blood phenotypes, increasing the risk of thrombus (Alpoim et al., 2012; Alpoim et al., 2011). More research is necessary in order to better elucidate if different subsets of preeclampsia require different treatment and follow up and if different racial and ethnic populations are at increased risk of preeclampsia related to blood type distribution. The purpose of this author's dissertation research will be to explore the association between maternal blood type and preeclampsia subtype with an aim at improving identification and screening of those at increased risk for future cardiovascular disease as well as improving prediction, .

Methods Used to Conduct This Review

Search for Evidence

In order to identify literature relevant to preeclampsia and ABO blood phenotype CINAHL, The Cochrane Collaboration and PubMed databases were searched. These databases were chosen due to their inclusion of academic nursing, medical and allied health journals, books and other scholarly texts. The following keywords were used in the review *ABO blood group, Rh factor, preeclampsia, and gestational hypertensive disorders*.

Inclusion and Exclusion Criteria

Inclusion criteria include (a) research conducted on pregnant human subjects, (b) in English (c) published between January 2000 and January 2016 (d) in academic, peer reviewed journals. This time frame was used for the search due to the limited number of results returned

using a five year search window. Dissertations, letters to the editor, and other unpublished works were not included in the search, nor were solitary case studies. The initial search yielded 11 results, and the abstracts were reviewed to assess if each article met the aim of the review. One additional article was found when searching *gestational hypertensive disorders* and *ABO blood group*. The reference pages of selected articles were then reviewed to identify if there were other articles that may not have been revealed in the original search. One additional article was identified. Prior to completing the dissertation, the literature was searched in order to ensure the review was up to date, one additional article was added to the review. Subsequently, fourteen articles were included in the review.

Compilation of Evidence

Table of Evidence

The research literature was reviewed, synthesized and leveled using The U.S Preventive Task Force Levels of Evidence (Appendix A). A table of evidence was created (Appendix B). The table includes information on title, author name as well as level of evidence, aim of the research, sample size, results and strengths and limitations of each study. All fourteen articles are quantitative in nature and are one of the following designs, three systematic review and meta-analysis, five case-control studies, five cohort studies, and one cross-sectional design. These designs were appropriate for this review, experimental designs, such as randomized control trials do not lend themselves to this area of study.

Critical Appraisal of the Evidence

Synthesizing the Evidence

Maternal blood type. Hiltunen et al. (2009) reported that non-O blood group was not associated with preeclampsia; however, AB blood group was associated with higher rates of subgroups of preeclampsia such as early and severe as well as preeclampsia with FGR. These authors reported that O blood type was protective of pre-eclampsia with FGR ($P=0.03$) and in their study came close to significance in the early pre-eclampsia group ($P=0.08$) (Hiltunen et al., 2009). Lee, Zhang et al. (2012) stated that women with AB blood type had a higher incidence of gestational hypertensive disorders, while blood group O had the lowest odds of both preeclampsia and severe preeclampsia. Than et al. (2011) agreed, and reported that maternal blood groups may be helpful in early assessment of those pregnancies at risk of IUGR. Conversely, Alpoim et al. (2011) found that in their population, O and non-O blood types did not differ in frequency between preeclamptic and normotensive pregnancies. In a meta-analysis, Clark & Wu (2008) reported similar results to Alpoim et al. (2011). In another meta-analysis, Alpoim et al. (2013) suggest that maternal type AB blood increased risk for preeclampsia. While Phaloprakarn & Tangjitgamol, (2013) stated a 1.7 fold increased risk of preeclampsia for patients with A and AB blood types. Hentschke and colleagues (2014) also reported no difference in blood group distribution between preeclampsia and normotensive women. There is varied evidence on the correlation between maternal blood type and preeclampsia. More research is needed to clarify the association, as each study had differing population characteristics and were done on patients of varying ethnicities.

ABO blood type and ethnicity. Genetic variants of ABO blood types have been shown to be predictive of risk of hypertension in certain cultures in non-pregnant clients (Gasso et al., 2012). Distribution of blood group varies based on ethnicity (Hiltunen et al., 2009). There are a

limited number of studies found in the literature exploring the link between maternal blood type and preeclampsia diagnosis; this makes it difficult to ascertain the link between blood type and preeclampsia. The research reviewed was completed using various ethnic groups including the Thai, Hungarian, Turkish, Scottish, Finnish, Swedish, Iranian, Ecuadorian and Brazilian populations (Alpoim et al., 2012; Clark et al., 2007; Hiltunen et al., 2009; Lee, Zhang et al., 2012; Phaloprakarn & Tangjitgamol, 2013; Seyfizadeh et al., 2014). Phaloprakarn & Tangjitgamol (2013) found that in the Thai population women with A or AB blood types had higher rates of preeclampsia. Type O blood type is the most common blood type found in both the Thai population as well as the United States, whereas A is more common in both Japan and other European countries (Phaloprakarn & Tangjitgamol, 2013). None of the studies reviewed used American participants, which is an identified gap in the literature.

Von Willebrand Factor and FVIII. Differing ABO blood groups are associated with varying plasma concentrations of Von Willebrand Factor (VWF) and FVIII (Than et al., 2011). Plasma levels of VWF increase by blood type in the following order O>A>B>AB (Alpoim et al., 2013). “Mediators of endothelial cell dysfunction, including relative nitric oxide deficiency, fibronectin, von Willebrand factor, cell adhesion molecules, and cytokines, are upregulated in preeclampsia” (Bilhartz et al., 2011, p.696). VWF is a marker of hypercoagulability and may mediate platelet adhesion to the vessel wall particularly at sites of vascular injury (He et al., 2012). Since preeclampsia has been associated with fibrin disposition in the placental microvasculature it may be important to examine the role VWF plays in the development of the disease (Alpoim et al., 2011). ADAMTS13 is shown to remove VWF from circulation, in a deficiency of this substrate, VWF levels may rise, increasing the risk of thrombosis in the small vessels (Alpoim et al., 2011). Alpoim et al. (2013) showed that subjects with AB blood type had

higher levels of VWF as well as FVIII resulting in increased risk for thrombus formation.

However, when comparing O and non-O blood types, Alpoim et al. (2011) did not find differing levels of ADAMTS13 levels. Finally, after correction for FVIII levels, Witsenburg and colleagues (2005) did not find any difference between ABO blood types in terms of risk of pregnancy complications such as hypertensive disorders of pregnancy and IUGR.

Platelets. Pregnancy is a hypercoagulable state; it is hypothesized that in preeclampsia there is a deviation in the communication between the inflammation and coagulation cascades (Han et al., 2014; Rich-Edwards et al., 2014). Preeclampsia diagnostic criteria list platelet count as $>100,000$ per microliter in combination with blood pressure parameters as diagnostic of preeclampsia (ACOG, 2013) and in high risk women aspirin therapy is recommended for preeclampsia prevention (LeFevre, 2014). Platelets are formed from megakaryocytes in the bone marrow and referent levels indicate a balance in homeostasis and thrombosis. The imbalance that results between coagulation and anticoagulation factors in preeclampsia patients can result in poor placental and maternal end organ perfusion due to microthrombosis (Han et al., 2014). In addition to increasing coagulation, the increased platelet activation that occurs in preeclampsia may also mediate inflammation (Gonçalves Freitas et al., 2014).

Blood type antigens A and B are expressed on platelets (Lee, Zhang et al., 2012). Alpoim et al. (2011) postulated that the increased levels of Ultra Large VWF (ULVWF) seen in preeclamptic patients might be related to occlusion of the renal and placental arterioles that often occurs in this disease process. Interestingly, platelets do not adhere to smaller forms of VWF in the circulation after ULVWF binds to ADAMTS13. This may result in platelets blocking the microvascular of the placenta (Alpoim et al., 2011). Hiltunen et al. (2009) hypothesized that the effect blood type has on preeclampsia could be mediated by prothrombotic mechanisms.

PP13. Than and colleagues (2011) hypothesized that ABO blood type may influence gene and environment interactions due to how it functions at what they deem the “cross-roads” of the immune and coagulation systems (p. e21564). PP13 is a galectin, which functions at this crossroads and may be available in varying amounts in different blood groups (Than et al., 2011). Upon exploration, Than et al. (2011) reported that PP13 is an early marker for preeclampsia. PP13 is produced by the placenta however, is released into maternal circulation and works to bind other galactosides found on the ABO blood group antigen (Than et al., 2011). It is thought that in severe preeclampsia, PP13 may be more readily shed. This in turn may result in a maternal immune response, which is a suggested pathophysiologic basis for preeclampsia (Lee, Zhang et al., 2012). In their study, Than et al. (2011) state that women with B blood type had the highest serum levels of PP13 in all trimesters of pregnancy however, in this blood type, red blood cells had the weakest ability to bind to PP13 (Than et al., 2011). In contrast, AB blood type had the lowest serum levels of PP13 in the first trimester suggesting that PP13 more readily binds to certain ABO antigens altering serum-circulating levels. Than et al. (2011) stated that the proportion of individuals with AB and B blood type were low in their study so they could not adequately evaluate their association with risk of preeclampsia however, this study reveals that ABO antigens may play a unique role in preeclampsia risk by regulating the serum availability of other biomarkers (Than et al., 2011).

Factor V Leiden. Both Factor V Leiden (FVL) and blood group are common heritable influences, which may affect thrombotic risk (Alpoim et al., 2012; Clark et al., 2007; Hiltunen et al., 2009). Other research has shown that FVL may be influenced by the inheritance of a non-O blood type (Hiltunen et al., 2009). FVL has been associated with preeclampsia in previous studies (Clark et al., 2007; Hiltunen et al., 2009). Hiltunen et al. (2009) report an increased risk

for preeclampsia in those with FVL and FVL or FII G20210A combined, although this was not a statistically significant increased risk. In a study by Clark et al. (2007), there was no influence of either FVL or non-O blood type on risk of preeclampsia. Subsequently, based off their study, Clark et al. (2007) do not recommend routine screening for FVL to assess for risk of thrombotic events such as preeclampsia.

Interaction between blood type and other factors. Alpoim et al. (2013) and Than et al. (2011) both report that how various blood types interact with different biologic and hemostatic factors may be what increases risk of thrombotic disease in these patients. Phaloprakarn & Tangjitgamol (2013) suggest that tumor necrosis factor-alpha and intracellular adhesion molecule 1 tend to be more readily up regulated by the A allele, possibly increasing risk of preeclampsia in both the A and AB blood type. Seyfizadeh et al. (2014) reported that serum creatinine levels were significantly higher in AB blood type women in their sample. Since elevated creatinine is a known risk factor for preeclampsia, it is possible that blood type has an association with certain preeclampsia risk factors. Additionally, Lee, Zhang et al. (2012) propose that each ABO blood type may have differing rates of occurrence of certain cardiovascular risk factors such as hypocholesteremia, insulin resistance and endothelial damage that subsequently increase risk of preeclampsia.

Rh factor. Lee, Zhang et al. (2012) discovered that Rh positive women also have a slightly increased risk of developing preeclampsia than their Rh negative counterparts (Lee, Zhang et al., 2012). Sezik and colleagues (2002) reported in their study, that subjects with O negative blood had a higher risk of HELLP syndrome than other blood types. This risk was not reported in women with type O blood independent of Rh type (Sezik et al., 2002). These authors postulated that the Rh antigen or Rh incompatibility might be important factors in the

development of this most severe form of preeclampsia. They also hypothesized that the increased antibody load in O negative individuals could play a role in the increased incidence of HELLP syndrome seen in this population (Sezik et al., 2002).

Than et al. (2011) did not report any difference in the bioavailability of PP13 based on Rh type; no difference was still reported even when assessing levels of PP13 by ethnicity.

López-Pulles et al. (2010) also found no difference in rates of preeclampsia between Rh negative and Rh-positive women in their study.

Summary of Research Conclusions

Implications on Clinical Practice

Identification of risk factors specific to preeclampsia may allow clinicians to better understand the pathophysiology of the disease, and create an effective risk factor algorithm to identify women at risk for preeclampsia prior to conception (Alpoim et al., 2012). Globally, preeclampsia is associated with increased morbidity and mortality. Identifying biomarkers that promote early detection as well improve scientists understanding of the pathophysiology of preeclampsia is necessary in order to improve outcomes (Alpoim et al., 2011). Alpoim et al. (2011) suggests that monitoring FVIII, VWF and ADAMTS13 may be relevant. Phaloprakarn & Tangjitgamol (2013) suggest that tumor necrosis factor-alpha and intracellular adhesion molecule 1 tend to be more readily up regulated by the A allele. These inflammatory markers have also been associated with cardiovascular disease, further pointing to blood type as a mediator between the two disease processes.

As research continues to advance the science surrounding preeclampsia and with work toward prophylaxis, the idea of a specific screening algorithm is alluring (Jørgensen, Hedley, Gjerris, & Christiansen, 2014). With this said, it is important to create an algorithm that has a

high detection rate and low false positive rate (Jørgensen, et al., 2014). Research on biochemical markers such as ABO blood type should continue, in order to reach scientific consensus as to whether or not blood group would be a valuable addition to a screening algorithm. A screening algorithm could allow for early recognition of patients at risk for preeclampsia, providing practitioners with the opportunity to utilize prophylaxis or increase antepartal monitoring in order to improve pregnancy outcomes. Finally, even if ABO Blood type is not significantly different between preeclampsia subtypes, collection of data on various biomarkers by preeclampsia subtype may help to clarify or refute potential etiologic pathways (Myatt et al., 2014).

Limitations of the Review

Overall, there were a limited number of studies that have examined the association between blood type and preeclampsia. Many of the studies reviewed were retrospective in nature which may not allow for identification of potential confounders (Polit & Beck, 2012). Additionally, many of the studies had relatively small sample sizes and were completed on diverse ethnic populations. Since blood type varies by ethnicity, results are difficult to compare (Hiltunen et al, 2008; Lee, Zhang et al, 2012; Than et al., 2011). In 2013, there was a change in preeclampsia diagnostic criteria (ACOG, 2013). Due to the differing diagnostic criteria utilized as well as definitions of subtypes utilized, it is difficult to compare studies. Finally, none of the studies included information on neonatal and paternal blood type. Due to a postulated immunologic pathophysiology of preeclampsia, an interaction between fetal and/or paternal antigens and maternal ABO blood phenotype could also play a role in preeclampsia development.

Review of Literature: Cardiovascular Disease and ABO Blood Type

Introduction

Cardiovascular disease is a complex disease with a variety of risk factors (Lee, Lin et al., 2012). Cardiovascular disease is a vexing problem in the United States and around the globe. Thirty seven percent of deaths of women in the United States are said to be related to cardiovascular disease (Freibert, Mannino, Bush & Crofford, 2011). Scientists and health care providers continue to look for risk factors, which may improve early identification and treatment of those at risk. Since ABO blood type has been linked to increased susceptibility to certain disease processes, such as preeclampsia and has been associated with varying levels of clotting factors that increase risk of thrombus formation and cardiovascular risk it is important to explore further explore this association (Jassim, 2012).

Having a non-O blood type has been associated with nearly 6% of total deaths and as many as 9% of cardiovascular deaths (Etemadi et al., 2015; Franchini, & Liumbruno, 2013). Lee, Lin et al. (2012) postulated that since ABO blood type is inherited and premature coronary artery disease is often linked to inherited risk factors, ABO blood groups could mediate this risk. It is well established that ABO blood type increases risk of venous thrombus as ABO antigen expression determines levels of VWF and FVIII (Franchini & Lippi, 2015). Individuals with O blood types have 25% increased rates of VWF and FVIII, which is often protective of venous thrombus (Franchini & Lippi, 2015). Many studies suggest that the affect ABO blood type has on both hemostasis and plasma lipid levels influence other cardiovascular outcomes such as myocardial infarction and coronary heart disease (Franchini & Lippi, 2015). Accordingly, the following review of literature was completed in order to explore research on the relationship between ABO blood type and poor cardiovascular outcomes.

Methods Used to Conduct This Review

Search for Evidence

In order to identify literature relevant to cardiovascular disease and ABO blood phenotype CINAHL and PubMed databases were searched. These databases were chosen due to their inclusion of academic nursing, medical and allied health journals, books and other scholarly texts. The following keywords were used in the review, *ABO blood group and cardiovascular disease*.

Inclusion and Exclusion Criteria

Inclusion criteria were research conducted (a) on human subjects (b) age 19 and older, (c) in English (d) published in the past five years, (e) in academic, peer-reviewed journals. Dissertations and other unpublished works were not included in the search, nor were solitary case studies. The initial search yielded 75 results, and the abstracts were reviewed to assess if each article met the aim of the review. All articles included in the review used stroke, cardiovascular disease or atherosclerosis as the main outcome. Fourteen met the inclusion criteria for this literature review.

Compilation of Evidence

Table of Evidence

The research literature was reviewed, synthesized and leveled using The U.S Preventive Task Force Levels of Evidence (Appendix A). A table of evidence was created (Appendix C). The table includes information on title, author name as well as level of evidence, aim of the research, sample size, results and strengths and limitations of each study. All fourteen articles are quantitative in nature and are one of the following designs; one meta-analysis, six case-control studies, five cohort studies, and two cross-sectional design. These designs were

appropriate for this review, experimental designs, such as randomized control trials do not lend themselves to this area of study, as you cannot ethically manipulate blood type.

Critical Appraisal of the Evidence

Synthesizing the Evidence

ABO blood type. Gong et al. (2014b) found that having type A blood was significantly associated with cardiovascular disease even after adjustment for common cardiovascular risk factors. Additionally, type A blood was associated with more severe coronary atherosclerosis than other blood types (Gong et al., 2014b). Lee, Lin et al. (2012) reported in their study that participants with type A blood had significantly higher rates of coronary artery disease (CAD) as well as CAD that was more severe than in those with other blood types. This study also revealed type A blood to be an independent risk factor for both men and women with early onset CAD (Lee, Lin et al., 2012).

Etemadi et al. (2015) reported that those with non-O blood types had increased risk of total mortality, with most mortality being attributable to cardiovascular disease. In their metanalysis, He et al. (2012) noted a statistically significant difference in rates of CAD between blood groups, reporting that non-O subjects were at a significantly higher risk of developing coronary heart disease than those with an O blood type. Chen et al. (2014) also reported an association between non-O blood type and significant CAD although when analyzed by sex, the association was significant in men but not in women in their sample. Gong et al. (2014b) discovered that non-O blood types were an independent risk factor for CAD and MI.

Of all blood types, AB had the highest risk of CHD, followed by blood groups B and A (He et al., 2012). This risk remained, even after adjusting for other cardiovascular risk factors and when excluding diabetic patients (He et al., 2012). Karabuva, Carević, Radić, and Fabijanić

(2013) described that coronary artery disease occurred more often in males less than age 50 with type AB blood. A study by Zakai and colleagues (2014) discovered similarly, stating that AB blood type is a risk factor for stroke even after adjustment for age and race. On the contrary, in their study population of Bengali East Indians, Biswas et al. (2013) discovered that participants with AB blood type had decreased rates of coronary heart disease, compared with those with type O blood. In the review, only one study, Sode, Allin, Dahl, Gynzelberg, and Nordestgaard (2013) did not find an association between myocardial infarction and blood type.

Rh factor. Only two studies in the review included Rh factor as a variable. When studying Turkish participants, Etemadi et al. (2015) found no association between Rh factor and CVD, even when assessing for interaction between Rh factor and ABO blood type, no interaction effect was noted. In their meta-analysis He et al. (2012) also did not find an association between Rh status and poor cardiovascular outcome.

ABO as a mediator of CVD. Several studies performed mediation analysis in order to assess the role ABO blood type plays in mediating risk of CVD (Gong et al., 2014a; He et al., 2012). He et al. (2012) noted an interaction effect between BMI and ABO blood type. In obese or overweight non-O blood type women, blood type had a stronger relationship to CHD risk than in those women with BMI <25. This interaction was not seen in men (He et al., 2012). Gong et al. (2014a) explored CRP as an inflammatory mediator to CAD and MI. Gasso et al. (2014) found that ABO blood type might predict ACE plasma activity, which in turn could mediate CVD risk. The relationship between ACE plasma activity and blood type could prove relevant when designing risk models used to predict hypertension or when determining efficacy of ACE inhibitor pharmacotherapy (Gasso et al., 2014). Finally, since ABO antigens are also expressed on platelets, which play a role in both coagulation and inflammation, platelets may be an

important mediator of association between blood type and cardiovascular risk (Karabuva, Carević, Radić, & Fabijanić, 2013). Sode et al. (2013) reported that reduced activated partial thromboplastin time was seen in non-O versus O blood type, which further supports the role of coagulatory factors as mediator of cardiovascular risk.

Cardiovascular risk factors. Research has explored the link between ABO blood type and traditional cardiovascular risk factors. As described above He et al. (2012) revealed that BMI mediated CHD risk. In a study by Gong and colleagues (2014b), participants with blood group O had less traditional cardiovascular risk factors than those with blood type A and more severe coronary lesions. Conversely, Jassim (2012) found in their study that blood group O had higher levels of glucose, total cholesterol and higher blood pressures. Biswas et al. (2013) reported that just over 28% of participants with O blood type had a family history of cardiovascular disease.

Karabuva, Carević, Radić, and Fabijanić (2013) found that in the participants they studied with coronary artery disease, those with type B blood had lower HDL levels but did not find any difference in self-reported CAD risk factors among all blood types. Finally, Zakai et al. (2014) reported that participants with blood type AB had higher rates of diabetes than all other blood types; however, this association was non-significant when adjusted for race.

Cardiovascular pathogenesis. Each of the studies explored different cardiovascular end outcomes. Some explored acute cardiovascular events such as MI, while other studies looked at CAD as a chronic inflammatory process. Karabuva, Carević, Radić, and Fabijanić (2013) explored coronary artery disease as a chronic inflammatory process and discovered there to be no relationship between ABO blood type and magnitude of atherosclerotic lesions. These authors also reported no significant difference in distribution of ABO blood types among patients with

CAD versus healthy controls nor was there any difference in distribution of cardiovascular risk among blood types (Karabuva, Carević, Radić, & Fabijanić, 2013). Due to the known increased levels of VWF by blood type and subsequent risk of venous thromboembolism, Sode et al., (2013) also explored the interaction between ABO blood type, FVL and prothrombin mutations. Sode et al. (2013) discovered that there was not a consistent association between ABO blood type and MI. Other studies in this review have suggested a link between more acute cardiovascular events and blood type, particularly cardiac events that occur at a younger age and in women (Lee, Lin et al., 2012).

Genetic factors. In a recent Genome Wide Association Studies (GWAS) Reilly et al. (2011) set out to determine what genetic factors predispose participants to plaque rupture or myocardial infarction in participants with coronary atherosclerosis versus coronary disease without plaque rupture or MI. ABO was identified as a locus for MI in patients who had identified angiographic CAD (Reilly et al., 2011). Reilly and colleagues (2011) reported that “ABO GWAS signal for myocardial infarction in patients with angiographic CAD is mediated by the glycotransferase-deficient isoform that encodes the ABO blood group O phenotype” (p. 7). Subsequently, the higher risk of thrombus at time of MI in non-O blood types, even in those with less extensive atherosclerotic plaques, seems to be related to an interaction between non-O ABO glycotransferase activity and coronary thrombus rather than atherosclerosis (Reilly et al., 2011). Thus, in patients with angiographic CAD, blood type O is protective of MI. With an understanding of the genetic loci that exist for CAD, Wauters et al. (2013) took the work of Reilly and colleagues (2011) further and explored genetic variants of CAD. With an understanding that the development of CAD is heritable and complex, there may be genetic factors that overlap and subsequently mediate different pathologic cardiovascular outcomes. .

Inflammation. ABO antigens are expressed on endothelial cells and platelets, and play a role in motility, adhesion and proliferation of these cells (Karabuva, Carević, Radić, & Fabijanić, 2013). Subsequently, ABO antigens play a role in the inflammatory process. Gong et al. (2014a) report a higher prevalence of systemic inflammation in non-O blood types. Recent GWAS have linked the ABO locus to “circulating levels of soluble intercellular adhesion molecule-1, soluble P-selectin, soluble E-selectin, and angiotensin-converting enzyme” (Karabuva, Carević, Radić, & Fabijanić, 2013, p. 351). Interleukin-6 (IL-6) and tumor necrosis factor (TNF) have also been found to be mediators between blood type and cardiovascular disease (Gong et al., 2014a). Gong et al. (2014a) discussed the association between the ABO gene and C-reactive protein which is a marker of inflammation often elevated in CAD and MI. C-reactive protein was found to be higher in non-O blood types and mediated the risk of CAD and MI, in these blood types (Gong et al., 2014a).

Cholesterol. Genetic studies have revealed that ABO blood types are inherited from chromosome 9 locus 9p34 (Gong et al., 2014b). This chromosomal locus controls cholesterol balance and non-O blood types have increased cholesterol absorption (Chen et al., 2014; Gong et al., 2014b; Lee et al., 2012). Other GWAS studies have shown differing levels of plasma lipids based off blood type, and a recent study estimated that 10% of CHD risk in non-O blood types is mediated by low-density lipoprotein cholesterol levels (Chen et al., 2014; Etemadi et al., 2015). Thusly, ABO blood types influence on cardiovascular disease may be mediated at least in part by cholesterol (Chen et al., 2014). Chen et al (2014) found that those with O blood types had lower levels of total cholesterol and LDL cholesterol than all other blood types, with mean levels among other blood types to be roughly the same.

More specifically, studies by Etemadi et al (2015), and Lee, Lin et al. (2012) all reported that those with type A blood had higher total cholesterol and LDL. Etemadi et al (2015) reported that those with type B blood had lower lipid levels. However, in their study, overall, blood glucose was the only significant variable and non-O blood groups had higher blood glucose levels (Etemadi et al., 2015). These authors did not believe higher plasma lipid levels in type A individuals can alone explain the increased mortality in non-O persons because, lipids levels in AB and B type individuals were actually lower than those with O type blood (Etemadi et al., 2015). Lee, Lin et al. (2012) found similarly, and reported that the “association of blood group A and CAD/MI was independent of atherogenic lipid profiles” (p. 1819). In a study by Biswas and colleagues (2013), AB blood type had the highest levels of HDL with lowest HDL levels in individuals with O blood type.

Conversely, when analyzing LDL levels, highest levels were found in participants with O blood type with lowest levels in those with AB blood type (Biswas et al., 2013). Although these findings are contradictory to all other studies in the review this may be related to the ethnicity and genetics of the population they studied.

Summary of Research Conclusions

Implications on Clinical Practice

The majority of the studies reviewed found subjects with non-O blood types to be at higher risk of cardiovascular disease than those with O blood types. Several studies found subjects with AB blood types in particular to be at higher risk of cardiovascular events than all other blood types (Chen et al., 2014; Etemadi et al., 2015; Gong et al., 2014a; Gong et al. 2014b; He et al., 2012; Karabuva, Carević, Radić, & Fabijanić, 2013; Lee, Lin et al., 2012; Zakai et al., 2014). Etemadi et al. (2015) reported, total mortality is increased in non-O blood types and

much of this was attributable to cardiovascular risk. The association between non-O blood type and cardiovascular risk may be related to the 25-30% increase in VWF and FVIII in these individuals. The increase in these blood proteins in non-O individuals may result in increased platelet adhesion and subsequent plaque formation. It may be important for health care providers to consider blood type when screening individuals for heart disease risk. In addition to earlier identification, an understanding of how biomarkers differ by blood type may allow for the design of more individualized therapies for the prevention and treatment of CVD.

Limitations

It is well established that frequencies of blood type vary in different ethnicities/populations. The studies in the review were completed on individuals from Chinese (4), Iranian (1), Iraqi (1), Taiwanese (1), European backgrounds (UK, Belgian, Danish) (2), Croatian (1), Spanish (1) Indian (1), as well as individuals from the United States (2). Since studies did not include individuals from all ethnicities, findings from the review may not be generalizable. Additionally, the small sample size of some of the studies and lack of representation of more rare blood types in certain populations may also make it difficult to generalize the findings.

Cardiovascular endpoints were not well defined in all studies. Studies reviewed a variety of cardiovascular outcomes including MI, stroke and both acute and chronic CAD. Differences in the associations found between blood types in some of the studies could be related to the pathophysiology of the cardiac outcome being studied. Interestingly only one study focused on individuals with early onset cardiovascular disease (Lee, Lin et al., 2012). Early onset CVD may have a different pathogenesis than atherosclerotic lesions that accumulate with age and subsequently may not stem from the same pathologic background. It may be possible that ABO

blood type plays a more significant role in modulating the outcome in acute coronary events versus coronary events that are considered chronic.

Although A, B, AB and O are the most common blood groups; there are ABO subgroups as well. Subgroups are determined when there are noted to be decreasing amounts of antigens on the RBC's as well as in secretors in the saliva (Franchini & Liumbruno, 2013). Subgroups are most common on the A allele, with the two most common subgroups being A1 and A2. Approximately 80% of blood type A or AB are classified as being part of A1 subgroup (Franchini & Liumbruno, 2013). The other 20% are classified as A2 (Franchini & Liumbruno, 2013). Since in most studies individuals with type A or AB blood were shown to be at highest risk of cardiovascular sequelae, further refining research to examine the subgroups of the A allele may be helpful in identifying those who are at highest risk for the development of cardiovascular disease.

Conclusion

Certain ABO blood types have been shown to convey higher risk of certain disease processes. ABO blood type may mediate the development of certain cardiovascular pathology. Most studies in the review implicate non-O blood types with the development of CVD, with individuals with blood type A and AB at most cardiovascular risk. The authors of these studies hypothesize that the 25-30% increase in VWF and FVIII in non-O blood types may contribute to the increased cardiovascular risk due to the increase in platelet adhesion and plaque formation. Other authors explored the link between ABO blood type, inflammatory markers and cardiovascular risk. Interestingly, both markers of coagulation and inflammation have been explored in the preeclampsia literature and seem to play a role in the pathogenesis of this disease as well. Cardiovascular disease continues to be the leading cause of death in women and even

with new treatments, rates of CVD in women is not decreasing at the same rate as it is in men. With that said it is important to consider uniquely female risk factors for CVD. Exploration of risk factors shared between preeclampsia and CVD, such as ABO blood type, may allow for earlier identification of women at risk of both outcomes and help to more clearly define the pathophysiology of preeclampsia.

Preeclampsia, ABO and CVD

In both reviews of the literature, there were similar themes noted. Although results varied, in both reviews, non-O blood type was most frequently cited as increasing risk of both CVD and preeclampsia. With individuals with blood type AB being at highest risk of both outcomes. However, most of the studies in both reviews did not find an association between Rh factor with either CVD or preeclampsia. Since ABO antigens are expressed on platelets, platelets were implicated in both reviews as mediators in the risk of development of both disease processes. In the literature review exploring ABO blood type and CVD, increased cholesterol absorption as well as increased blood glucose levels were seen in non-O individuals as well as increased rates of other traditional cardiovascular risk factors such as increased BMI (Etemadi et al., 2015, He et al., 2012; Gong et al., 2012b). These risk factors have also been proposed as preeclampsia risk factors (Lee et al., 2012; Bodnar, Ness, Markovic, & Roberts, 2005).

Finally, inflammatory mediators were implicated in increasing risk of both CVD and preeclampsia. Traditional cardiovascular risk factors, such as cholesterol absorption and BMI, as well as coagulation factors also seem to vary based on blood type. These factors have also been implicated in the development of both CVD and preeclampsia. Subsequently it is possible that blood type mediates this relationship between the two disease processes due to its role in

modulating cardiovascular risk factors as well as levels of inflammatory and coagulation factors that may increase risk for both disease processes.

Conclusion

ABO is expressed on a variety of different tissues, blood components and bodily fluids (Seyfizadeh et al., 2014). ABO blood phenotype has been linked to increases in inflammation and coagulation due to differing levels of inflammatory markers and blood clotting proteins that have been implicated in both preeclampsia and cardiovascular disease (Gong et al., 2014a; Franchini & Lippi, 2015; Visser et al., 2014). Since preeclampsia leads to increased risk of cardiovascular disease and has been linked to many similar pathologic mechanisms it may be important to consider if ABO blood type mediates increased risk of preeclampsia. Research on the role of ABO blood type in the development of preeclampsia has yielded inconsistent results. However, several of the studies reviewed revealed an association between non-O blood types and preeclampsia diagnosis, specifically, AB blood type (Hiltunen et al., 2008; Lee et al., 2012; Phaloprakarn & Tangjitgamol, 2013).

Chapter Summary

Preeclampsia continues to threaten the health of women. Implications of the disease are far reaching and also include poor neonatal outcomes and increased cardiovascular risk later in life. With an understanding that preeclampsia is a cardiovascular risk factor unique to women, the importance of the use of a feminist theoretical framework was discussed. The pathophysiology of preeclampsia is not clear; however, it has been proposed that there are different subtypes of the disease. The role that blood type plays in the development of both cardiovascular disease and preeclampsia was explored, and commonalities were discovered in the research on the association between blood type and both disease processes. With an

understanding that each preeclampsia subtype may have a distinct pathophysiology, further research is necessary to explore the role blood type plays in the development of preeclampsia subtype.

Chapter 3

Methods

Chapter Introduction

Recently, much literature has been published on the relationship between pregnancy complications and risk of chronic disease. Upon further review of the literature, recent evidence has revealed an association between preeclampsia and future cardiovascular disease. The pathophysiology of preeclampsia has yet to be elucidated. Preeclampsia has been called the disease of theories because the exact etiology of the disease remains largely unknown (Founds et al., 2011). Currently, there is evidence to support that the disease is heterogeneous in nature and varying subtypes of the disease may exist (Barton, & Sibai, 2008; Founds, et al., 2011). Pathophysiologic, genetic, metabolic, immunologic, and inflammatory causes have all been postulated (Charlton, Tooher, Rye, & Hennessy, 2014; Founds, et al., 2011; Ilekis, Reddy & Roberts, 2007). Due to the heterogeneous nature of the disease, it is important to consider that the pathophysiology may also be different amongst subtypes (Barton, & Sibai, 2008). Blood type has also been postulated to increase risk of both cardiovascular disease and preeclampsia (Biswas et al., 2013; Etemadi et al., 2015; Franchini, & Liumbruno, 2013; Gong et al., 2014; He et al., 2012; Jassim, 2012; Lee et al., 2012). Due to this shared risk factor, blood type, the following study was designed in order to explore the association between maternal blood type and preeclampsia subtype.

More data is needed to better clarify differences in biophysical and biochemical markers of women affected by the early and late preeclampsia subtypes. Data from this study has the potential to allow for more effective diagnosis, treatment, and management of patients with preeclampsia through a better understanding of risk factors of each preeclampsia subtype. Since

both cardiovascular disease and preeclampsia may be associated with blood type, further examination of risk factors shared between the two disease processes is necessary in the hope of establishing a clearer pathophysiologic basis for preeclampsia (Biswas et al., 2013; Bushnell et al., 2014).

The purpose of this study is to investigate the association between maternal ABO blood type and preeclampsia subtype with an aim at improving prediction and diagnosis as well as further exploring risk factors shared with cardiovascular disease. Furthermore, since preeclampsia is said to be heterogeneous in nature, a secondary aim of this study is to better describe variables that increase risk of each subtype. A case-control study was conducted to investigate the magnitude of association between ABO blood type and the development of each preeclampsia subtype. The study compared women with both subtypes of preeclampsia to a control group of women without preeclampsia.

This chapter will thoroughly describe the research design and sampling method used in this study. Furthermore, this chapter describes operational definitions utilized for each variable as well as the eligibility criteria utilized for both preeclampsia subtypes as well as the control groups. Finally, measurement tools, the data management plan as well as an outline of how data was analyzed is described.

Research Design

Case control studies provide the opportunity for retrospective assessment of an outcome, examining differences in predictor variables between cases, those with the outcome criteria and controls. Case control studies are valuable in instances when it is not ethical or feasible to conduct an experiment (Shadish et al., 2002). This design has also been shown to be efficient and less expensive than other designs particularly when studying rare outcomes (Hulley et al.,

2013). Although preeclampsia is not rare, this study will be specifically assessing the early onset subtype of preeclampsia, which is relatively uncommon compared to its late onset counterpart. In their study, MacKay, Berg & Atrash (2001) established the prevalence of early onset preeclampsia to be 0.8%.

Although case control studies cannot yield information on incidence, they provide an excellent methodology by which to obtain descriptive information about those cases that meet outcome criteria, and allows scientists to estimate the strength of association between the independent and dependent variables (Hulley et al., 2013; Lewallen, & Courtright, 1998). In case control methodology the outcome variable is typically dichotomous (Shadish et al., 2002). Although the main association of interest in this study is ABO blood type, the case control design will allow for the assessment of other variables that may influence the development of each subtype of preeclampsia. A case control study designed to assess the association between blood type and preeclampsia subtype will provide data that may be foundational to more costly and time-consuming longitudinal studies that could assess the shared relationship between preeclampsia and ABO blood phenotype and increased future cardiovascular risk (Lewallen, & Courtright, 1998).

Weaknesses of the design include an increased risk for bias that must be addressed (Hulley et al., 2013; Lewallen, & Courtright, 1998). Sampling bias occurs when cases are unrepresentative of the risk factor being studied. In this study, there is limited concern over sampling bias as all pregnant women are followed regularly for prenatal care and are routinely screened for preeclampsia per standard obstetrical practice. During the chart review, if a subject was found not have attended prenatal care, they were not included in the study. The predictor variable being studied (ABO phenotype) is represented in the entire population and is commonly

tested in all pregnant clients at their first prenatal visit and again at delivery. Additionally, due to the retrospective nature of case control studies there may be concerns with measurement error, subsequently clear descriptions of how each variable was measured are described in this chapter (Hulley et al., 2013). All data was extracted from electronic health databases so there was no concern over inaccuracy related to poor maternal recall and the majority of the data was recorded prior to the occurrence of the outcome under study. Finally, strict criteria were used to define both case and controls groups (Lewallen, & Courtright, 1998).

Conceptual Framework

There are four major human blood groups A, B, O and AB (Seyfizadeh et al., 2014). Current research supports that ABO blood phenotypes are now far more useful than in hematology and transfusion medicine alone (Franchini & Lippi, 2015). It seems that blood phenotype may be linked to risk of cancer, cardiovascular disease as well as a variety of infectious disease (Biswas et al., 2013; Franchini & Lippi, 2015). ABO antigens are expressed differently on the red cell surface as well as in a “variety of human cells and tissues, including epithelium, sensory neurons, platelets, and vascular endothelium” (Franchini & Lippi, 2015, p. 1). Each blood type has differing levels of coagulation factors, such as VWF and Factor VIII. Non-O groups have approximately 25% higher levels of VWF than type O individuals (Franchini & Lippi, 2015). Individuals with non-O blood phenotypes have been shown to be at increased risk for venous thrombosis, arterial thrombosis, myocardial infarction and ischemic stroke (Franchini & Lippi, 2015). Thus, the link between alteration in hemostasis seen in certain blood types as well as preeclampsia supports the biologic plausibility of this study.

Feminism provides an ideal philosophical framework by which to explore this problem. Although this study is highly pathophysiologic, the lens of the researcher frames the research

questions posed, how data is extracted and interpreted. With that said, there is also an engendered component to cardiovascular disease. Women experience different cardiovascular signs, symptoms and risk factors than their male counterparts. Studying sex specific cardiovascular risks factors such as preeclampsia may help to improve opportunities for early detection and prevention of heart disease in young women.

Research Question and Hypothesis

The primary aim of this study was to assess the association between ABO blood type and preeclampsia subtype. After a thorough review of the literature, variables were identified and the following research questions were formulated. The research questions addressed in this study included:

Research Question 1

What are the maternal characteristics of women with preeclampsia and with each preeclampsia subtype?

Hypothesis 1: Individuals with early onset preeclampsia will have more traditional cardiovascular risk factors such as, higher body mass index (BMI), chronic hypertension and diabetes. Additionally, more subjects in the early onset subtype will be of African American race, lower socioeconomic class.

Research Question 2

Is there an association between preeclampsia subtype and ABO blood phenotype?

Hypothesis 2: Women with non-O blood types will have greater odds of early-onset preeclampsia, compared to those with O blood type.

Research Question 3

What is the association between preeclampsia subtype and Rh type?

Hypothesis 3: Rh factor will not be associated with preeclampsia or preeclampsia subtype.

Research Question 4

What is the association between ABO blood type, platelet count at > 28 6/7 weeks gestation and preeclampsia development?

Hypothesis 4: ABO blood type will be associated with platelet count at \geq 28 6/7 week in each preeclampsia subtype.

Research Question 5

What is the association between ABO blood type and fetal growth restriction in preeclamptic women?

Hypothesis 5: ABO blood type will be associated with fetal growth restriction in women with preeclampsia.

Research Question 6

What factors are significantly associated with preeclampsia and preeclampsia subtype?

Hypothesis 6: Traditional clinical and sociodemographic risk covariates such as increased BMI, nulliparity, lower socioeconomic status, Black race, extremes in maternal age will increase odds of preeclampsia and early onset preeclampsia subtype. Non-O blood types will have increased odds of preeclampsia and preeclampsia early onset subtype.

The findings from the study could contribute to a more clear understanding of the pathophysiologic basis of each preeclampsia subtype as well as a better understanding of the association between preeclampsia and future cardiovascular disease risk. Additionally, although currently there is no reliable risk-screening model for preeclampsia, this study may provide knowledge about biophysical characteristics of women with each preeclampsia subtype so that health care providers can consider the need for early pregnancy surveillance in certain subgroups of women.

Measurement Tools

Gel Agglutination

When blood type is determined, technologists are assessing the presence of specific antigens on the red blood cell (RBC) and antibodies in the plasma. When the A antigen is present the client is type A, when the B antigen is present the client is type B and when both are present the client has AB blood type. When neither A or B antigen are present the client is typed as O. The ABO type is unique in that there is an inverse relationship between the antigens found on the RBCs and the antibodies found in the plasma/serum. Each blood specimen is tested for both antigens and antibodies (personal communication, B. Steiber, January 29, 2016).

In the study site laboratory, gel agglutination is used as their primary testing methodology. Certified Clinical Laboratory Scientists complete all testing. Gel methodology has been shown to be more sensitive than tube methodology, as the reactions are stronger (personal communication, Steiber, B, January 29, 2016). Gel agglutination assay is used “for the detection of A, B, and D and for ABO serum grouping, indirect antiglobulin tests (IATs), and direct antiglobulin tests (DATs)” (Langston, Procter, Cipolone, & Stroncek, 1999, p. 300). Six microtubules are held in a cassette. Each microtubule has gel in a column that may be neutral or contain a reagent. Reactants are then added to each microtubule (Langston, Procter, Cipolone, & Stroncek, 1999). The RBC’s are tested with two antibody reagents for anti-A and anti-B, each of which has specificity for one antigen. Antibodies in the plasma/serum are tested with RBC reagents known to be A1 cells and B cells.

Using the same sample of blood as above, Rh factor testing is completed at the same time as ABO blood typing described above and the appropriate anti-Rho(D) reagent is included in the

gel cassette. The presence or absence of Rh antigen on the sample's red blood cells is determined by testing the sample with antibody reagent anti-D.

Regardless of the test performed, the gel cassette is spun for 10 minutes in the centrifuge. This is necessary so that the RBCs, whether agglutinated or not, layer in the appropriate area. The strength of agglutination reaction is then graded (+1, +2, +3, +4) by the Certified Clinical Laboratory Scientist in order to determine the presence or absence of agglutination in each of the microtubules. The presence of agglutination determines a positive reaction (personal communication, Steiber, B, January 29, 2016).

The majority of prenatal ABO testing is routine. For routine samples, the Ortho ProVue® is used to complete gel agglutination testing. Prior to utilizing the Ortho ProVue®, the machine is validated by comparing results from the ProVue® to manually tested specimens which were completed according to Blood Bank protocols. The instrument is tested daily with reagents as well as patient specimens in order to achieve 100% accuracy in ABO and Rh blood typing.

Platelet Measurement

In the study site laboratory, platelet counts are completed using an automated blood cell analyzer called the Sysmex XE-series. Utilization of impedance technology has resulted in a dramatic improvement in precision in platelet counts. The Sysmex counts platelets using electronic resistance detection enhanced by hydrodynamic focusing (WellSpan Lab Services, 2012). The study sites laboratory services, cellular microscopy policy CM-IOP-27 outlines principles, care and procedures governing the Sysmex XE series.

Variables

Preeclampsia

Diagnostic criteria of preeclampsia is defined by ACOG (2013) as the new onset of hypertension (≥ 140 mm Hg systolic or ≥ 90 mmHg diastolic) on two occasions at least 4 hours apart after 20 weeks, and proteinuria (> 300 mg in a 24-hour urine collection). In the absence of proteinuria the patient may have any one or more of the following in order to meet preeclampsia diagnostic criteria:

- platelets $< 100,000$ microliter
- serum creatinine > 1.1 or doubling of serum creatinine in the absence of renal disease
- elevated concentrations of blood liver transaminases to twice normal levels
- pulmonary edema
- new onset of cerebral or visual disturbances (ACOG, 2013, p. 4).

Early and late onset. Myatt et al. (2014) reports that a better understanding of preeclampsia subtypes will lead to a more clear understanding of the pathophysiology of preeclampsia and subsequently improved diagnostic prediction. In this study, two preeclampsia subtypes were assessed, early and late onset. Early onset preeclampsia is defined by ACOG (2013) and the ISSHP as occurrence of preeclampsia before or at 33 weeks gestation, while late onset preeclampsia occurs at 34 weeks or later (Lisonkova, & Joseph, 2013; Myatt et al, 2014). Additionally, since perinatal morbidity is much higher when delivery occurs prior to 34 weeks, using this gestational age for designation of subtypes proves important (Myatt et al., 2014).

Previous research on early and late preeclampsia subtypes utilized timing of delivery to classify subjects, or did not describe how subjects were classified into each subtype. Myatt et al. (2014) recommends using onset of preeclampsia clinical symptoms rather than timing of delivery to define each subtype as symptoms may have been clinically present days to weeks before delivery. Prenatal records of each case were reviewed in order to assess gestational age when

preeclampsia diagnostic criteria was documented. Since it is accepted practice to record blood pressures and urine protein at all obstetrical visits, both the prenatal record as well as the hospital inpatient record were reviewed as most cases of preeclampsia were confirmed in the inpatient setting. The exactness of this may be affected due to timing of antenatal visits during pregnancy (Myatt et al., 2014). Since this study involves gestational age as a variable it is important to elucidate method and accuracy of dating. It is common practice at the study site for all women to have first trimester dating ultrasounds to confirm dating. Cases in which preeclampsia occurred in the postpartum period were not included in this study as this may be a separate subtype of the disease.

Major Independent Variables

ABO blood type. Myatt et al. (2014) calls for researchers to begin to explore differences in laboratory findings between preeclampsia subtypes. ABO blood type was chosen as a variable in this study due to the cardiovascular and thrombotic risk associated with certain blood types. There are four major human blood groups A, B, O and AB (Franchini & Lippi, 2015; Seyfizadeh et al., 2014). Blood type is ordered to be drawn as part of prenatal labs on all pregnant clients at their first prenatal visit. ABO was documented as a categorical variable.

Rh factor. Rh factor is the major Rh antigen and when it is present the client is deemed Rh positive, when the Rh antigen is not present the client is Rh negative. The Rh antigen is present in 85% of the population in the United States (Blood Typing Systems, 2013). When blood type is drawn as part of prenatal labs on all pregnant clients at their first prenatal visit, Rh status is included. Rh factor was documented as a dichotomous categorical variable.

Platelets. Platelets are a component of the blood that aid in the clotting process. According to ACOG (2013) preeclampsia diagnostic criteria, in the absence of proteinuria,

platelet levels less than 100,000 per microliter can be diagnostic of preeclampsia, if hypertensive criteria exist. In preeclampsia, thrombocytopenia reveals systemic disease. Platelet and thrombin activation, have been listed as possible pathogenic mechanisms in the development of preeclampsia (Han et al., 2014). In this study, maternal platelet count was documented at three different time periods throughout the subjects pregnancy, less than 20 6/7 weeks gestation (first prenatal lab work), between 21 and 28 6/7 weeks gestation, and greater than 29 weeks gestation (admission for delivery). Platelet count was documented as a continuous variable.

Maternal body mass index (BMI). Prepregnancy BMI has been linked to both preeclampsia and CVD risk (Bodnar et al., 2005; Roberts et al., 2011). Additionally, women with non-O blood types have been shown to have a greater number of traditional cardiovascular risk factors, such as elevated BMI (He et al., 2012). BMI is calculated at the prenatal teaching or first obstetrical visit and is calculated by the electronic health record by taking the persons weight in kilograms divided by the square of their height in meters. Weight is typically measured on a standing scale and height may be self-reported or confirmed by the nurse on the standing scale if the patient is unsure of their height at the time of the intake visit. If a prepregnancy BMI or weight was documented in the prenatal history and physical, this data was utilized. Otherwise only weights measured prior to the end of the first trimester (<13 weeks) were used to calculate maternal BMI's so as to obtain a BMI as close to a prepregnancy as possible. BMI was documented as a continuous variable.

Race. There are differing distributions of blood type by racial and ethnic group (Racial and Ethnic, 2013). Risk of preeclampsia is also shown to be increased in certain race (Lisonkova, & Joseph, 2013; Paré et al., 2014)). Below are the racial groups listed in the hospital database. Each participant's race is self-declared and was abstracted from the Premier® database. This

variable was recoded due to small numbers in many of the racial categories to include White, Black and Other.

- A. Asian
- B. Black or African American
- C. Native Hawaiian or Other Pacific Islander
- D. White
- E. Declined-Other
- F. Declined
- G. Unknown to patient

Maternal socioeconomic class. Socioeconomic status may reflect the access to resources of the women in this study. Other studies have used years of education and income as a proxy's for socioeconomic status (Shavers, 2007). Due to its availability in the database utilized in this study, maternal insurance type was used as a proxy for maternal socioeconomic status. Insurance type was collected as a categorical variable. The following insurances types were listed in the Premier[®] database (1) Commercial Indemnity (Private) (2) Managed Care Non-Cap (Private); (3) Medicaid Managed Care Non-Cap (Public) (4) Medicaid Traditional (Public) (5) Medicaid Traditional (TR) (Public) (6) Medicare Traditional (Public) 7) Self Pay (8) Unknown (9) Other Government Payers (Public). These were recoded into a dichotomous variable, public and private insurance.

Maternal age. Maternal age was calculated as the number of years completed at the time of delivery of the pregnancy. Maternal age was listed on the electronic delivery found in the electronic health record. Age was documented as a continuous variable. Lisonkova, & Joseph, (2013) list extremes in maternal age (<18 or >35) as a risk factor for preeclampsia. In teen pregnancy, researchers have hypothesized that the risk of preeclampsia is elevated due to the immaturity of uterine and cervical blood supply. This in turn may result in increased prostaglandin production and subsequent inflammatory response, which may affect blood

pressure (Aliyu et al., 2010). Researchers have also hypothesized that poor dietary intake of certain nutrients such as vitamins C and E, calcium, zinc as well as essential fatty acids, which have been associated with preeclampsia, particularly in developing countries, could be causative of preeclampsia in teens. In teens, their own growth and bodies' need for nutrients may result in nutrient deprivation of the fetus (Aliyu et al., 2010). Aliyu et al. (2010) also adds that teenage mothers are often nulliparous, and non-white, both of which are risk factors of preeclampsia, which in turn could exaggerate the risk of preeclampsia in this age group (Aliyu et al., 2010). Alternatively, it has been proposed that the increased risk of preeclampsia in older mothers may be due to aging endothelium which does not adapt as well to the hemodynamic demands placed on the maternal vascular system during pregnancy (Rich-Edwards et al., 2014)

Smoking. Interestingly, smoking has been shown to be protective of preeclampsia and may confer a 50% reduction in risk (Hutcheon et al., 2011; Savitz, Danilack, Engel, Elston, & Lipkind, 2014). This association is dose dependent and if smoking is stopped early in pregnancy, there is no protective effect. This association was not seen with snuff (Hutcheon et al., 2011). This variable was collected by review of the prenatal record in the electronic health record and was dichotomized for data collection, as either "yes" or "no." Women who reported not smoking at their prenatal intake visit were considered nonsmoking. If a woman had a positive smoking status documented in her prenatal record by her health care provider at the first prenatal visit, then women were considered smokers.

Parity. Nulliparity is a risk factor of preeclampsia. The "primipaternity hypothesis suggests that risks of preeclampsia are increased among women who have limited exposure to their partner's sperm" (Hutcheon et al., 2011' p. 394). Although there has been some debate over this hypothesis, supporters site higher rates of preeclampsia in women who consistently use

barrier contraception, and the three fold higher risk of preeclampsia in women who conceived following intracytoplasmic sperm injection for azoospermia, with sperm obtained surgically as evidence of this hypothesis (Hutcheon et al., 2011). Nulliparous women have three times higher risk of preeclampsia than do multiparous women. (Hutcheon et al., 2011). The lower rates of preeclampsia seen in multiparous women further support this hypothesis (Hutcheon et al., 2011). Parity will be documented as a dichotomous categorical variable, nulliparous or multiparous. Nulliparous women were those who had no documented history of a term or preterm birth in the electronic delivery log.

Gestational age at birth. At the study site, first trimester ultrasound is routinely performed in order to calculate estimated date of confinement. First trimester ultrasound, improves gestational age accuracy and subsequently allowed for more accurate identification of preeclampsia subtype. As per standard of practice, first trimester ultrasound is obtained to confirm dating by measuring fetal crown rump length (Myatt et al., 2014). If a dating ultrasound completed before 7 weeks gestation calculates a due date that is ≥ 3 days different from the due date that was calculated using the last menstrual period, then the due date was changed in the electronic health record to correspond with the ultrasound report. If a dating ultrasound completed before 10 weeks gestation calculates a due date that is ≥ 5 days different from the due date that was calculated using the last menstrual period, then the due date will be changed to correspond with the ultrasound report. If a dating ultrasound completed before 13 weeks gestation calculates a due date that is ≥ 7 days different from the due date that was calculated using the last menstrual period, then the due date will be changed to correspond with the ultrasound report (personal communication, S. Bennett, February 9, 2016). If a patient presents after 13 weeks and dating has not yet been confirmed, biometry based on fetal biparietal

diameter, abdominal circumference, and femur length is utilized to calculate gestational age. In this case, if the due calculated is ≥ 11 days different from the due date which was calculated using the patients last menstrual period, then ultrasound dating would be used (Myatt et al., 2014). Gestational age was extracted from the delivery record. This variable will be documented as a continuous ratio level variable. If gestational age was in question at delivery, the case was not included in the study. Based off assigned estimated date of delivery, an obstetrical wheel was used to confirm gestational age at onset of preeclampsia symptomology in order to appropriately assign each case to the correct subtype.

Sex of the infant. There is a strong incidence of sexual dimorphism particularly related to disorders of an inflammatory nature (Myatt et al., 2014). A male fetus has been associated with increased risk of preeclampsia ; however, a female fetus has been associated with the early subtype (Myatt et al., 2014). Lisonkova, & Joseph, (2013) reported an association between pregnancy with a male fetus and late onset preeclampsia. In this current study, the sex of the infant was extracted from the electronic delivery record and was coded as a dichotomous categorical variable.

Infant birthweight. Myatt et al. (2014) calls for birth weight to be recorded in all studies on preeclampsia. Fetal growth restriction (FGR) is a common complication of preeclampsia, often associated with the more severe early onset subtype (Kucukgoz Gulec et al., 2013). A growth-restricted infant is characterized by “a reduced birthweight in relation to its length” (Owen, Ogah, Bachmann, & Khan, 2003, p. 411). More specifically, FGR is documented when an infant’s “birth weight and/or birth length is below the 10th percentile for gestational age” (Zohdi et al., 2012 p. 2). All infants are weighed shortly after birth on electronic scales by registered nurses. Birth weight was documented in the electronic health record in

grams, it was initially measured as a continuous ratio level measurement. Infant birthweight was then recoded into birthweight percentiles to assess for rates of growth restriction within subgroups and controls. A fetal biometry calculator which takes into account gestational age and birthweight was used to calculate birthweight percentile. The calculator can be accessed at <http://perinatology.com/calculators/exbiometry.htm>. The calculator utilizes the Hadlock (1991) equation. This variable was then dichotomized in order to represent those infants with growth restriction ($< 10^{\text{th}}$ percentile) and those without growth restriction ($> 10^{\text{th}}$ percentile) (Unterscheider et al. 2013).

Comorbid conditions. Comorbid illnesses to include chronic hypertension (CHTN), preexisting diabetes (DM) and gestational diabetes (GDM) will be collected as dichotomous categorical variables. Chronic hypertension, preexisting diabetes and gestational diabetes are known risk factors for both CVD as well preeclampsia (Lisonkova, & Joseph, 2013; Paré, 2014). In other studies, ABO blood type has been shown to increase risk for diabetes and other cardiovascular disease risk factors (Chen et al., 2014; Gong et al, 2014b; Karabuva, Carević, Radić, & Fabijanić, 2013; Lee et al., 2012). Subsequently, ABO blood type may mediate the development of these comorbid conditions which in turn may increase risk for preeclampsia. In this current study, CHTN, DM and GDM were initially identified by ICD 9 code and then confirmed upon chart review.

Subjects and Setting

Location

Due to accessibility, the electronic health record (EHR) from a large suburban ethnically diverse community hospital in south central Pennsylvania was used for data collection. The city where the hospital is located has a population of 43,935. In 2015, 3,164 births occurred at this

institution. There is only one other small community hospital in this county. This hospital is considered a tertiary referral center for several other small hospitals in surrounding counties subsequently; the majority of high-risk pregnancies are delivered at this hospital due to its NICU and high-risk obstetrical facilities. Thus, utilizing other hospitals in the area would not increase access to clients with the desired outcome. Because the study site is a referral center, more cases of early onset preeclampsia delivered at this institution, as these cases often require early delivery of the pregnancy and utilization of NICU services that are not offered by other hospitals in the area.

Inclusion and Exclusion Criteria

Inclusion criteria for controls included:

- Female clients
- Singleton pregnancies
- Presented for delivery at the study site between November 2014 and March 2016
- ABO blood typing complete
- No diagnosis code for preeclampsia
- Live births

Exclusion criteria for controls included:

- Incomplete ABO blood typing
- Women who did not deliver at the study site between the defined time period.
- Women pregnant with multiples
- Women with a history of preeclampsia

Inclusion criteria for cases included

- Female clients
- Singleton pregnancies
- Presented for delivery at the study site between January 2012 and June 2016
- ABO blood typing complete
- Initially must have intrapartum ICD 9 for preeclampsia (Appendix D) documented in their medical record. Then upon chart review must have met ACOG (2013) diagnostic criteria for preeclampsia.

Exclusion criteria for cases will include:

- Incomplete ABO blood typing
- Women who did not deliver at the study site between January 2012 and June 2016
- Women who delivered prior to 20 weeks gestation
- Women pregnant with multiples
- Women who did not meet preeclampsia diagnostic criteria when chart review completed

ICD 9 codes were used to exclude women in both the case and control groups with a multiple gestation (Appendix D). Women did not have more than one pregnancy included in the study. Women who delivered more than one pregnancy during the study period only had the first pregnancy included in the sample.

Although considered risk factors of preeclampsia, the decision was made to include subjects with comorbidities such as diabetes, gestational diabetes and chronic hypertension in both the case and control groups. Typically, with a case control study, you need to exclude diseases known to be associated with the exposure of interest. Since determination of ABO blood type predates any disease process it was determined that individuals with these conditions should remain in both groups. Additionally, literature supports that ABO blood type may predispose individuals to certain comorbid condition and cardiovascular risk factors, which in turn may increase risk of these comorbid conditions (Chen et al., 2014; Etemadi et al., 2015; Gong et al., 2014a; Gong et al. 2014b; He et al., 2012; Karabuva, Carević, Radić, & Fabijanić, 2013; Lee et al., 2012; Zakai et al., 2014). Subsequently, if these subjects were excluded from the sample, there may be a loss of an important subset of individuals whom blood type already matters in terms of their health status and subsequently may bias the results to the null.

Sample Size

Sample size was calculated using openepi.com. OpenEpi® is free, open source software that can be used to calculate epidemiologic statistics (Dean, Sullivan, & Soe, 2004). In order to

complete the sample size calculation “the desired confidence level, power, a hypothetical percentage of exposure among the controls, and either an odds ratio or a hypothetical percentage of exposure among the cases” must be entered into the statistical software (Dean, Sullivan, & Soe, 2004). In OpenEpi® “results are presented using methods of Kelsey, Fleiss, and Fleiss with a continuity correction” (Dean, Sullivan, & Soe, 2004). The results using the Kelsey method were utilized for sample size determination in this study (Appendix E).

Many similar studies reviewed on this topic did not discuss how they calculated sample size. The power selected for use in the sample size equation was from a study by Shamsi et al. (2010) who reported using a power of 80% with an alpha level of 0.05 and an effect size of 0.3.

In the current study, the exposure variable is non-O blood type. The distribution of ABO blood type in the United States for all races is O 44%, A 42%, B 10% and AB 4% (“Racial and Ethnic Distribution,” 2013) with non-O blood types accounting for 56% of all blood types.

A review of the literature was completed in order to find a percentage of non-O blood type exposure among cases of preeclampsia. There were only two articles identified in the literature that clearly listed the prevalence of blood type in cases of preeclampsia Hiltunen et al., (2009) and Spinillo, Capuzzo, Baltaro, Piazzì, & Iasci (1995). Each uses varying definitions of preeclampsia, and none use the strict definitions of early and late preeclampsia utilized in this study. None of the articles reviewed discussed prevalence of blood type by early or late onset preeclampsia subtype.

After reviewing these articles, Hiltunen (2009) was utilized to calculate sample size. Hiltunen (2009) used a total of 248 cases that fulfilled the diagnostic criteria for pre-eclampsia and 679 controls. In this article the sample consisted of preeclampsia cases and then was analyzed by subsamples 1) severe pre-eclampsia, 2) early pre-eclampsia, and 3) pre-eclampsia

with intra-uterine growth restriction (IUGR). Primigravid women with preeclampsia were also analyzed separately. However, Hiltunen (2009) only reported blood type distribution for the entire preeclamptic sample. Hiltunen (2009) reported a 70.96% prevalence of non-O blood type in preeclamptic subjects. Sample size was calculated using Openepi.com. In order to add power to the study, two controls were added for every case (Lewallen, & Courtright, 1998). A sample size of 126 early cases, 126 late cases, and 252 controls, is able to detect an odds ratio of 1.9 with a power of 80% at a significance level of 5%. Subsequently, each subtype is adequately powered to detect differences.

Matching. Finally, the decision was made to not utilize matching in the design of the study. “Matching should be considered only for risk factors whose confounding effects need to be controlled for but that are not of scientific interest as independent risk factors in the study” (Wacholder, Silverman, McLaughlin, & Mandel, 1992, p. 1043). Because all variables considered for matching are clinical risk factors of preeclampsia, the PI chose not to match on these variables, as they were of scientific interest. Additionally, matching can shrink the sample and subsequently create difficulty in meeting sample size requirements; this was of concern particularly in relation to the overall difficulty in finding early onset cases. Since none of the preeclampsia clinical risk factors under study are predictors of exposure to the primary risk factor under investigation (ABO Blood type), other than race, confounding was not a concern (Wacholder et al., 1992). Rose & van der Laan, (2009) cite that confounders can be adjusted for in the analysis if necessary. In this sample, there were no statistically significant differences in race ($p=.145$) and or maternal age ($p=.279$) between the preeclampsia subtypes and controls.

Data Collection

Initially, data was abstracted from the electronic delivery log on all deliveries between November 2014-March 2016. Subjects with multiple gestations were excluded as well as the second delivery of subjects who had more than one delivery during this time period. This group of patients was assessed for preeclampsia cases that met inclusion criteria. From the remaining subjects who had live births a randomly selected control group was created using excel. Recognizing the rarity of preeclampsia, particularly early onset preeclampsia, the search for preeclampsia cases needed to be expanded to include cases identified by the Premier® database using ICD-9 codes for preeclampsia between January 2012-June 2016 (Appendix D).

The electronic health record of each consecutive preeclampsia case was reviewed to ensure that each subject met preeclampsia diagnostic criteria until the sample size requirement was met for both the early and late onset subtypes. Accuracy of data collection surrounding preeclampsia diagnosis can prove difficult and multiple methods exist. A strength of this study is the strict criteria which was utilized in order to determine a case of preeclampsia. Many population birth cohort studies utilize large data sets consisting of birth certificate data and extract data using diagnostic codes. Diagnostic codes can be used to extract data from electronic sources, however, Brown et al. (2013) reported that coding was only accurate 54% of the time in meeting the diagnostic criteria set forth by ACOG surrounding preeclampsia. Since this study is assessing the consequences ABO blood type has on preeclampsia risk, the accuracy of preeclampsia diagnosis is imperative. Accurate classification of subjects improves the chances of finding real exposure differences between groups. The exacting way in which cases were identified improves the statistical power of the analysis particularly when compared to larger studies that may have utilized less sensitive measures for identifying cases of preeclampsia (Klemmensen, Olsen, Østerdal & Tabor, 2007).

Protection of Human Subjects

This investigator, as required by the study site, completed WCG® research training. Prior to beginning data collection, this investigator obtained IRB approval from the study site and a IRB deferral request was submitted and approved by the University of Wisconsin-Milwaukee. The primary investigator also sought approval from the Women's and Children's Service Line Administration at the study site. A HIPPA waiver was requested and approved as this study posed minimal harm to patients. Ethical research principles were followed in order to assure ethical treatment of data.

Procedures

Study site laboratory procedures were assessed and were confirmed that they met quality standards for accuracy in blood typing as well in determining platelet count. Blood bank type and Rh processes were assessed with the manager of the Blood Bank at the study site. Policies and adherence to federal regulations surrounding blood banking were ensured.

Detailed data were collected on a wide variety of biophysical, laboratory as well as socio-demographic variables. Although all variables were extracted from the electronic health record, not all data was extracted using the same methodology. Appendix F outlines from which database each variable was extracted. The electronic delivery log, electronic health record and the Premier® database were all used to extract data. Data extracted from the Premier® database was merged with data from the electronic delivery log in order to create a cohesive data set. Data collected from the Premier® database was collected by a population health data analyst at the study site as access to the Premier® database is restricted. The ICD 9 codes commonly utilized for preeclampsia were searched in the Premier® database in order to extract documented cases of preeclampsia from the electronic health record during the defined time-period.

Preeclampsia diagnosis and gestational age at diagnosis were confirmed through chart review in order to separate preeclamptic subjects by appropriate subtype. The electronic chart of each subject was then reviewed to gather data on early pregnancy BMI, platelet counts during three gestational time periods, infant birthweight, and to confirm smoking status as well as comorbid conditions of interest (GDM, CHTN, DM). Although identified preliminary by ICD 9 code, diagnosis of GDM, DM and CHTN were also confirmed during chart review in both cases and controls. Within the electronic health record, the prenatal chart of each control subject was also reviewed in order to confirm they had no history of preeclampsia.

Since data is often documented in multiple locations within the electronic health record, a subsample of cases and controls were assessed in order to attempt to standardize the data collection process. A data collection procedure was formalized, documented and reviewed with a research assistant. A paid research assistant who is a registered nurse at the study site and familiar with the electronic health record was utilized to collect chart data on control subjects only. She was paid through the Harriet Werley Doctoral Research Award, which the principal investigator received in 2016.

Data Management and Analysis

Patient data was extracted from patient charts, the electronic delivery log as well as the Premier® database and was stored in a password protect hospital based secured share drive. Statistical analysis was performed using SPSS version 23. Initially data was examined for missing data as well as outliers, and data was cleaned. Missing data was replaced using the number 8888. The data was screened and cleaned in order to check for errors. Minimum and maximum values were assessed as well as number of missing cases for each variable. Any errors or inconsistencies in the data were corrected. Assumptions for statistical tests were checked.

The data was analyzed in order to answer each research question.

Research Question 1

What are the maternal characteristics of women with preeclampsia and with each preeclampsia subtype?

Descriptive statistics were performed for each group, early preeclampsia, late preeclampsia and controls and were used to describe the sample. Initially the categorical variables, maternal ABO blood type, Rh factor, parity, smoking, race, socioeconomic status, infant outcome and infant gender were assessed for frequencies and a table was created to compare data on both cases and controls. Continuous variables such as maternal age, infant birthweight, gestational age and BMI were checked for skewness, normality and outliers. Means and standard deviations were calculated on all continuous variables. Chi square test of independence was estimated for categorical variables of interest. Independent-samples *t*-test were run to determine statistically significant differences between the mean scores for continuous variables in the preeclampsia and control groups. One-way ANOVA was utilized when assessing continuous variables against preeclampsia subtypes and controls, in order to determine significant differences in the mean scores across the three groups.

Research Question 2

Is there an association between preeclampsia subtype and ABO blood type?

The main aim of the study is the association between preeclampsia subtype and ABO blood type. Initially, Chi square test for independence was estimated to assess association between ABO blood type, and both preeclampsia and preeclampsia subtypes. This test was also completed excluding individuals with comorbidities from the sample. When assessing ABO blood type and preeclampsia subtype because the table is larger than 2 x 2, Pearson chi square and Cramer's V were reported. The analysis was also performed to assess the association

between Non-O and O blood types and preeclampsia and preeclampsia subtypes. When assessing Non-O and O blood types and preeclampsia a 2 x 2 table was created, subsequently the Yate's continuity correction value and phi coefficient were reported as appropriate.

Next, logistic regression was performed and odds of developing preeclampsia for each preeclampsia subtype was calculated based of ABO blood type. Logistic regression aims to assess the independent contribution of predictor variables in predicting an outcome (Hulley et al., 2013). After assumptions of logistic regression were checked, an unadjusted binary logistic regression was run assessing the association between preeclampsia and ABO blood type. Race was then added to the model and the regression rerun. Odds ratios of each ABO blood type with 95% confidence intervals (CI) were reported.

Next, multinomial logistic regression was utilized to assess the association between ABO blood type and preeclampsia subtypes. This statistical test was chosen because there are more than two nominal outcomes: early preeclampsia, late preeclampsia, and controls. Because, these groups are unordered it makes them ideal for multinomial logistic regression (Kwak, & Clayton-Matthews, 2002). Multinomial regression creates multi-equation models and allows for the comparison of one group to a reference group, in this case the controls (Meyers, Gamst, & Guarino, 2013). The control group was set as the reference category. When running the regression, it was confirmed that all cells in the model were populated. Odds ratios with 95% confidence intervals (CI) were reported for significant blood types only. The regression was then performed adjusting for race.

Research Question 3

What is the association between preeclampsia subtype and Rh type?

In order to answer research question three, Chi square test for independence was estimated in order to assess the association between Rh factor and preeclampsia as well as preeclampsia subtype. When assessing Rh factor and preeclampsia a 2 x 2 table was created, subsequently the Yates continuity correction value and phi coefficient were reported. When assessing Rh factor and preeclampsia subtype, Pearson chi square and Cramer's V were reported as appropriate. This analysis was also complete when excluding subjects with comorbidities.

Research Question 4

What is the association between ABO blood type, platelet count at $\geq 28\frac{6}{7}$ weeks gestation, and preeclampsia development?

A two way between groups ANOVA was performed to assess if ABO blood type moderates the relationship between preeclampsia subtype and platelet count at $\geq 28\frac{6}{7}$ weeks gestation. Levene's test was assessed for equal variances and a more stringent p value was used as appropriate, and *t* values were reported appropriately. Both main and interaction effects are reported. Additionally, mediation analysis was run to determine if the relationship between maternal ABO blood type (predictor) and preeclampsia subtype (outcome) is mediated by platelet count at $\geq 28\frac{6}{7}$ weeks gestation.

Research Question 5

What is the association between ABO blood type and fetal growth restriction in preeclamptic women?

Chi square test for independence was estimated to assess the relationship between fetal growth restriction (FGR) and ABO blood type in preeclamptic women. Binary logistic regression was also performed. Odds ratios and 95% confidence intervals were reported for significant results.

Research Question 6

What factors are significantly associated with preeclampsia and preeclampsia subtype?

In order to address the most influential preeclampsia risk factors associated with each preeclampsia subtype, another regression was run which included all clinical risk factors in the model. The decision was made to not utilize stepwise regression due to the inherent flaws in this method, particularly since clinical risk factors of preeclampsia are well established in the literature and served as a conceptual model for the development of the model (Field, 2015). First, binary logistic regression was run using all clinically significant independent risk factors which data was collected on (Portney & Watkins, 2009). Variables determined in the review of literature as risk factors for preeclampsia were utilized in the model regardless of significance in this study. Binary logistic regression was performed to examine the association between socioedemographic and clinical covariates and preeclampsia including BMI, maternal age at delivery, sex of the neonate, smoking status at antenatal enrollment, maternal race, insurance type, parity and gestational diabetes. P-values ≤ 0.05 were considered statistically significant. Maternal BMI and age were mean centered prior to being included in the regression modeling. Because these variables (BMI and age) cannot have a true value of zero, these variables were mean centered in order to improve the interpretability of the output. Odds ratios with 95% CI's that were statistically significantly associated with preeclampsia were reported. ABO blood type was then added to the model to determine how much variability ABO blood type accounts for in the model. A table was created and odds ratios with 95% CI's that were statistically significantly associated with preeclampsia were reported.

Secondly, multinomial logistic regression was used to examine the association between covariates of interest and preeclampsia subtype. Initially, the same sociodemographic and clinical covariates were included in the model. Odds ratios and 95% confidence that were statistically significantly associated with preeclampsia subtype were reported. Values of $p < 0.05$

were considered significant (Lewallen & Courtright, 1998). Significant variables in the final model were described. Confidence intervals were assessed and significant variables described did not contain the value of 1.0 in the confidence interval (Portney & Watkins, 2009). Since multiple independent variables were included in the regression model, the odds ratio described was corrected due to each individual variables influence on each other, and the adjusted values were reported (Portney & Watkins, 2009). ABO blood type was then added to the model to determine how much of the variability in preeclampsia subtype ABO blood type explained. A table was created and odds ratios with 95% CI's that were statistically significantly associated with preeclampsia subtype were reported.

Supplementary Analysis

Mediation analysis. PROCESS which is a statistical program created by Andrew Hayes was downloaded to SPSS version 23 in order to perform the mediation analysis (Field, 2015). Mediation occurs when a relationship between a predictor and outcome can be explained through a third variable (Fields, 2015). In mediation, the predictor (X) should predict both the outcome (Y) and mediator (M). The mediator (M) also predicts the outcome (Y) variable. Subsequently when a mediator is present, the relationship between the predictor and the outcome may differ (Fields, 2015). Simple mediation analysis was performed to assess if the relationship between ABO blood type (X) and preeclampsia subtype (Y) was related to a third variable BMI (M). Since the literature supports that individuals with certain blood types may be more at risk for certain cardiovascular risk factors such as increased BMI (He et al., 2012), and since obesity is a known risk factor of preeclampsia, it is plausible that increased BMI mediates the relationship between ABO and preeclampsia (Bodnar et al., 2005; Roberts et al., 2011,). A conceptual model was created and both the indirect and direct effects were reported.

Chapter Summary

The goal of this case control study was to assess risk factors of each preeclampsia subtype, looking specifically at the strength of association between ABO blood phenotype and the development of preeclampsia. This chapter defines the variables included in the study, provides an overview of the sample as well as the data collection methods that were utilized in this dissertation study. An overview of data analysis methods was also provided.

Chapter 4

Study Findings

Chapter Introduction

Both blood type and pre-eclampsia subtype have been established as risk factors for cardiovascular disease, yet understanding of the role that blood type may play in shaping pre-eclampsia subtype remains limited. For this reason, examination of whether blood type is associated with pre-eclampsia subtype is needed to clarify whether the onset of specific pre-eclampsia subtypes may represent an important physiologic link between blood type and later life CVD. The purpose of this hospital-based case-control study was therefore to explore the association between preeclampsia subtype and maternal ABO blood phenotype.

First, descriptive statistics and sample characteristics are presented for the preeclampsia group in its entirety, each preeclampsia subtype, and controls. Next, the primary results for each research question are presented. Chi square test for independence were reported in order to examine associations among categorical variables (Research Questions (RQ's) 1, 2, 3, 5). Two way between groups ANOVA was performed (RQ 4) and both main and interaction effects were reported. Both binary logistic and/or multinomial logistic regression were used to address RQs 2, 5, and 6 and odds ratios and 95% confidence intervals were reported. Additionally, in RQ 6, Nagelkerke, Cox, and Snell R^2 (and change in these values) are reported to represent an estimate of variance explained in each model. All analyses were conducted in SPSS version 23. Finally, a supplementary analysis of the data was performed using mediation analysis in order to assess if the relationship between ABO blood type (X) and preeclampsia subtype (Y) was related to maternal BMI (M).

Research Questions and Hypotheses

The following are the research questions addressed in the study:

Research Question 1

What are the maternal characteristics of women with preeclampsia and with each preeclampsia subtype?

Hypothesis 1: Individuals with early onset preeclampsia will have more traditional cardiovascular risk factors such as, higher body mass index (BMI), chronic hypertension and diabetes. Additionally, more subjects in the early onset subtype will be of African American race, lower socioeconomic class.

Research Question 2

Is there an association between preeclampsia subtype and ABO blood type?

Hypothesis 2: Women with non-O blood types will have greater odds of early-onset preeclampsia, compared to those with O blood type.

Research Question 3

What is the association between preeclampsia subtype and Rh type?

Hypothesis 3: Rh factor will not be associated with preeclampsia or preeclampsia subtype.

Research Question 4

What is the association between ABO blood type, platelet count at > 28 6/7 weeks gestation, and preeclampsia development?

Hypothesis 4: ABO blood type will be associated with platelet count at > 28 6/7 week in each preeclampsia subtype.

Research Question 5

What is the association between ABO blood type and fetal growth restriction in preeclamptic women?

Hypothesis 5: ABO blood type will be associated with fetal growth restriction in women with preeclampsia.

Research Question 6

What factors are significantly associated with preeclampsia and preeclampsia subtype?

Hypothesis 6: Traditional clinical and sociodemographic risk covariates such as increased BMI, nulliparity, lower socioeconomic status, Black race, extremes in maternal age will increase odds of preeclampsia and early onset preeclampsia subtype. Non-O blood types will have increased odds of preeclampsia and preeclampsia early onset subtype.

Results

Sample Characteristics

The following are descriptive statistics for each group. The sample included women with the early onset preeclampsia subtype ($\leq 33\frac{6}{7}$ weeks) ($n=126$), the late onset preeclampsia subtype ($\geq 34\frac{0}{7}$ weeks) ($n=126$) and a control group ($n=259$) of normotensive women. Women in the control group did not have a current or previous history of preeclampsia.

Research Question 1

What are the maternal characteristics of women with preeclampsia and with each preeclampsia subtype?

Table 1 provides descriptive statistics for the preeclampsia group in its entirety as well as controls.

Preeclampsia. Although the main aim of this study was to assess preeclampsia subtypes association to ABO blood phenotypes, the preeclampsia group will be described in its entirety in Table 1. Since many previous studies on this association did not assess by preeclampsia subtype, this author felt it important to also evaluate this relationship and describe the sample in this way. The mean age of women with preeclampsia was 27.7 ($SD = 6.0$ years). Women in this group had

a mean BMI 30.34 (8.4 =SD). Subjects were also categorically assigned a weight classification according to their BMI. In the preeclampsia group, 42% of women were considered obese (BMI > 30). The mean gestational age at delivery for the preeclampsia group was 34.8 (SD= 3.9 weeks). The group was mainly White (77.8), 8.5% were Black, 13.7% were other race. The “other race” category consisted of those that identified as Asian ($n=2$) or Native Hawaiian ($n=12$); subjects from these races were a very small component of this “other” category. The majority of the “other” racial category were individuals who either declined to report their race, or reported other race ($n=65$). During the chart review, it was noted that study subjects who declined to document their race or self-classified as other race tended to be Hispanic. Preeclamptic women overall were more often multiparous (54%) and gave birth to more male (53.2%) than female infants (46.8%). The birthweight percentiles of infants born to mothers in this group were also assessed for frequency of growth restriction (<10% percentile). In the preeclampsia group, 36.1% of infants experienced some degree of growth restriction.

Table 1
Characteristics of Preeclampsia Subjects (n=252) versus Controls (n=259)

Characteristic	Preeclampsia		Controls		<i>p</i>
	n	%	n	%	
Race					.595
White	193	77.8	190	74.2	
African American	21	8.5	23	9	
Other Races	34	13.7	43	16.8	
Parity					.032
Nulliparous	116	46	94	36.3	
Multiparous	136	54	165	63.7	
Insurance Type					.621
Public	104	45.6	103	42.9	
Private	124	54.4	137	57.1	
Neonatal Outcome					
Live Birth	249	98.8	259	100	*
Fetal Death	1	0.4	0	--	
Neonatal Death	2	0.8	0	--	
Season of Birth					.495
Winter	72	28.6	90	34.7	
Spring	73	29	66	25.5	
Summer	45	17.9	45	17.4	
Fall	62	24.6	58	22.4	
Infant Sex					.615
Male	133	53.2	131	49.2	
Female	117	46.8	128	50.8	
BMI					<.001
Underweight	7	3.1	9	3.7	
Normal	58	25.9	112	45.7	
Overweight	65	29	63	25.7	
Obese	94	42	61	24.9	
Smoker	59	23.4	45	17.4	.113
ABO Blood Type					.156
A	95	37.7	100	38.6	
B	32	15.1	32	12.4	
AB	13	5.2	5	1.9	
O	106	42.1	122	47.1	
RhD					.874
Neg	29	11.5	32	12.4	
Pos	223	88.5	227	87.6	
FGR	91	36.1	31	12	<.001

BMI= Body Mass Index FGR=Fetal Growth Restriction

* statistical significance not calculated due to low frequencies

Early onset. The data was further analyzed by preeclampsia subtype and is reported in Table 2. The early onset group consisted of women who met preeclampsia diagnostic criteria on or before 33 6/7th week in pregnancy. The mean gestational age at delivery of the early onset preeclampsia group was 31 weeks and 6 days ($SD=3.09$ weeks) however, gestational age at delivery varied from 21 weeks and 6 days to 37 weeks and 4 days (Table 3). Ages of women in the group ranged from 17-46 years of age, with a mean age of 28.2 years ($SD=6$ years). Early pregnancy BMI of women in the group ranged from 16 to 55.3, with a mean BMI of 31.7 ($SD=8.15$) with 51.4% falling into the obese category ($BMI>30$). The majority of the early onset subgroup was White (73.8%). Blacks accounted for 12.7% of the early onset group. The majority of women in the early onset subtype were multiparous (63.5%). These women gave birth to more female infants (54.0%). Although the overwhelming majority of women in this subtype had live births (97.6%), there was one fetal death and two documented neonatal deaths. Fetal or neonatal deaths did not occur in either the late onset subtype or the control group. Over a quarter of women in the early onset subtype were smokers (27%). Blood type distribution in the early onset subtype was as follows, A (31.7%), B (19.8%), AB (4.8%), (O 43.7%). Rh-positive women made up the majority of the subtype (91.3%), with 8.7% of the group having Rh-negative blood types. Growth restriction most effected infants born to mothers with early onset preeclampsia, with 55.6% of infants in this subtype weighing less than the 10 percentile for their gestational age.

Late onset. The late onset subtype consisted of women who had documented preeclampsia diagnostic criteria on or after the 34 0/7 weeks of pregnancy. Characteristics of this subtype are documented in Table 2. The mean gestational age at delivery was 37 weeks and 6 days ($SD=1.89$ weeks), however, gestational age at delivery ranged from 34 weeks and 2 days

to 41 weeks and 3 days. The late onset subtype consisted of more nulliparous women than multiparous women, 55.6% vs. 44.4%. The majority of infants born in the late onset subtype were males (60.8%). Mean maternal age was similar to the early subtype, 27.13 ($SD= 6$ years). Early pregnancy BMI for the late subtype was 29.11 ($SD= 8.45$), BMI's ranged from 17.8 to 66.5. In the late onset subtype, the highest frequency of women had a normal BMI (BMI 18.5-24.9) (35.7%), however this was closely followed in the frequency by women in the obese category (BMI >30) (33%). This subtype was also predominately White (82%), however consisted of 4.1% Black subjects. Blood type distribution for the late onset subtype was as follows A (43.7%), B (10.3%), AB (5.6%) and O (50.5%). Rh-negative women of any blood type made up 14.3% of the group.

Controls. Control subjects ($n=259$) had no current or previous history of preeclampsia, characteristics of this group can be found in Table 2. The mean gestational age at delivery was 39 weeks and 2 days ($SD=1.72$ weeks) with an average birthweight of 3359gms ($SD=543.4$ grams). Many more of the women in the control group were multiparous (63.7%). The ratio of male to female infants was roughly even (49.2% vs 50.8%). Mean maternal age at delivery was similar to the late group, 27.7 years ($SD=5.56$ years). Of all groups, the controls had the lowest mean early pregnancy BMI, 26.5 ($SD=6.8$). BMI in this group ranged from 15.00 to 53.00. Most women in this group fell into the normal BMI category (BMI 18.5-24.9) (45.7%). Similarly, to the other groups, the control group was also predominately White (74.2%). Black subjects represented 9% of the control group. Of all the groups, controls had the lowest percentage of smokers (17.4%). In the United States blood type distribution is as follows, 44% O, 42% A, 10% B, and 4% AB. Distribution of blood type within the control group was as follows, A 38.6%, B 12.4%, AB 1.9% and O 47%. In the control group, 12.7% had Rh-negative

blood types, 87.6% were Rh positive. In the control group, 12% of infants were growth restricted.

Table 2

Characteristics of Early (n=126) Late (n=126) and Control (n=259) Subjects

Characteristics	<i>Early</i>		<i>Late</i>		<i>Controls</i>		<i>p</i>
	n	%	n	%	n	%	
Race							.145
White	93	73.8	100	82	190	74.2	
Black	16	12.7	5	4.1	23	9	
Other Races	17	13.5	17	13.9	43	16.8	
Parity							.001
Nulliparous	46	36.5	70	55.6	94	36.3	
Multiparous	80	63.5	56	44.4	165	63.7	
Insurance Type							.106
Public	61	52.1	43	38.7	103	42.9	
Private	56	47.9	68	61.3	137	57.1	
Neonatal Outcome							
Live Birth	123	97.6	126	100	259	100	*
Fetal Death	1	0.8	0	--	0	--	
Neonatal Death	2	1.6	0	--	0	--	
Season of Birth							.275
Winter	36	28.6	36	28.6	90	34.7	
Spring	40	31.7	33	26.2	66	25.5	
Summer	26	20.6	19	15.1	45	17.4	
Fall	24	19	38	30.2	58	22.4	
Infant Sex							.047
Male	57	45.2	76	60.8	131	49.2	
Female	68	54	49	39.2	128	50.8	
BMI							<.001
Underweight	3	2.8	4	3.5	9	3.7	
Normal	17	15.6	41	35.7	112	45.7	
Overweight	33	30.3	32	27.8	63	25.7	
Obese	56	51.4	38	33	61	24.9	
Smoker	34	27	25	19.8	45	17.4	.088
ABO Blood Type							.062
A	40	31.7	55	43.7	100	38.6	
B	25	19.8	13	10.3	32	12.4	
AB	6	4.8	7	5.6	5	1.9	
O	55	43.7	51	40.5	122	47.1	
RhD							.380
Neg	11	8.7	18	14.3	32	12.4	
Pos	115	91.3	108	85.7	227	87.6	
FGR	70	55.6	21	16.7	31	12	<.001

BMI= Body Mass Index FGR=Fetal Growth Restriction

* statistical significance not calculated due to low frequencies

Comparison of preeclampsia to controls. Table 1 provides Chi square test for independence results for each variable. A Chi-square test for independence (with Yates Continuity Correction) indicated a significant association between parity and preeclampsia, $\chi^2(1, N=511)=4.6, p=.032, \phi=-.099$. Additionally, a Chi-square test for independence (with Yates Continuity Correction) indicated a significant association between growth restriction and preeclampsia, $\chi^2(1, N=511)=39.64, p<.001, \phi=0.283$. An independent samples *t* test was conducted to compare age, BMI, gestational age and birthweight between preeclampsia patients and controls. All variables were significant at the $p<.001$ level except for maternal age which was not significant ($p=.566$) (Table 3).

Table 3

Means of Age BMI, GA and Infant Birthweight Preeclampsia versus Controls

Variable	Preeclampsia (n=252)		Control (n=259)		<i>p</i>
	M (SD)	95% CI	M (SD)	95% CI	
Age	27.7(6)	[26.9, 28.4]	27.9 (SD 5.6)	[27.3, 28.6]	.566
BMI	30.34(8.4)	[29.3, 31.5]	26.8 (SD 7.1)	[25.6, 27.4]	<.001
GA	34.8(3.9)	[34.3, 35.3]	39.3(SD 1.7)	[39.1, 39.5]	<.001
Birthweight	2381.4 (957.3)	[2261.1, 2501.6]	3359(SD 543.4)	[3292.7, 3525.6]	<.001

Note BMI= Body Mass Index; GA =Gestational Age

Comparison of preeclampsia subtypes. Participants were divided into three groups, according to onset timing of preeclampsia (early $\leq 33\ 6/7$ weeks, late $\geq 34\ 0/7$ weeks, and controls (no preeclampsia)). A Chi-square test for independence indicated a significant association between preeclampsia subtype and infant sex $\chi^2(1, n=509)=6.13, p=.047$, Cramer's $V=.11$ as well as between preeclampsia subtype and parity $\chi^2(1, N=511)=14.45, p=.001$, Cramer's $V=.168$. One-way between-groups analysis variance was conducted to explore significant differences in the mean scores of BMI and maternal age across each

preeclampsia subtype and controls. There was a statistically significant difference in BMI for the three groups: $F(2,468) = 15.3, p < .001$. Because a significant difference was found, post hoc comparisons using the Tukey HSD test were performed in order to reveal exactly where differences in BMI exist between groups. Post hoc comparison revealed that mean BMI scores for all groups were significantly different from each other. There was not a statistically significant difference in age across the three groups (Table 4). Since preeclampsia subtypes are defined based on gestational age, which would result in expected differences in mean birthweight and gestational age, these variables were not compared, as this would not provide any clinically significant findings. Mean gestational age and birthweights for each group were reported earlier in the chapter.

Table 4
Means of Age and BMI by Preeclampsia Subgroup

Variable	Early (n=126)		Late (n=126)		Control (n=259)		<i>p</i>
	M (SD)	95% CI	M (SD)	95% CI	M (SD)	95% CI	
Age	28.2 (SD 6)	[27.2, 29.3]	27.13 (SD 6)	[26.1, 28.2]	27.7 (SD 5.6)	[26, 28.4]	.279
BMI	31.7 (SD 8.1)	[30.1, 33.2]	29.11 (SD 8.4)	[27.5, 30.7]	26.5 (SD 6.8)	[25.6, 27.4]	<.001

Note: BMI=Body Mass Index

Comorbidities in each preeclampsia subtype and controls. Data on common comorbidities such as chronic hypertension (CHTN), gestational diabetes (GDM), and preexisting diabetes (DM) were collected on subjects in all three groups (early preeclampsia, late preeclampsia and controls). The early onset preeclampsia subset had subjects with the most comorbidities, CHTN (27%), DM (9.5%) and GDM (19.0%). Of the late onset preeclampsia subtype, 15.1% had CHTN, 10.3% had GDM, and 3.2% had DM. No subjects in the control group had DM and one had CHTN. A Chi square test for independence also identified a significant association between gestational diabetes and preeclampsia subtype $\chi^2(2, N=511) = 7.54, p = .023$, Cramer's $V = .121$.

Table 5

Prevalence of Comorbidities by Early Preeclampsia (n=126), Late Preeclampsia (n=126) and Controls (n=259)

		Preeclampsia Subtype						<i>p</i>
		Early		Late		Control		
		n	%	n	%	n	%	
CHTN	No CHTN	92	73	107	84.9	258	99.6	*
	CHTN	34	27	19	15.1	1	.40	
Diabetes	No Diabetes	114	90.5	122	96.8	259	100	*
	Diabetes	12	9.5	4	3.2	0	0	
GDM	No GDM	102	81	113	89.7	234	90.3	.023
	GDM	24	19	13	10.3	25	9.7	

Note: CHTN=Chronic Hypertension and GDM=Gestational Diabetes

* denotes that significance level not reported due to low frequencies

Research Question 2

Is there an association between preeclampsia subtype and ABO blood phenotype?

In order to assess the association between preeclampsia subtype and maternal ABO blood phenotype a Chi square test for independence was estimated. Although some expected cell frequencies were small, only 16.7% of the contingency table cells had expected cell frequencies

less than 5, subsequently not violating this assumption (Field, 2015). A Chi square test for independence indicated no significant association between ABO blood type and preeclampsia subtype, $\chi^2(6, N=511)=11.99, p=.062$, Cramer's $V=.108$. Additionally, no significant difference was noted when assessing controls versus the preeclamptic group as a whole ($p=.156$). The percentage of Non-O versus O blood types did not significantly differ by preeclampsia subtype ($p=.456$) or within the preeclampsia group as a whole ($p=.29$).

Exclusion of comorbidities. Since many previous studies excluded individuals with comorbid conditions, the decision was made to rerun the analysis without comorbid conditions included in order to be able to compare data with other studies to see if results were consistent. When subjects with comorbidities (CHTN, GDM and preexisting diabetes) are excluded ($n=108$), from the sample, ABO blood type continues not to be significantly associated with preeclampsia $\chi^2(3, n=403)=6.94, p=.72$, Cramer's $V=.132$. However, after excluding subjects with comorbidities from both the control group and each preeclampsia subtype, the Chi-square results were statistically significant indicating an association among ABO blood type and preeclampsia subtype, $\chi^2(6, n=403)=24.06, p=.001$, Cramer's $V=.173$ (Table 6). The early onset subtype was much more likely to have a B blood type (31%) than those with late onset preeclampsia (9.3%) or controls (12%). Frequency of the AB blood type was fairly even between early (4.2%) and late (5.2%) preeclampsia subtypes. The control group consisted of less subjects with the AB blood type than both the early and late subtypes (2.1%)($p=.33$).

When analyzing the sample with comorbidities excluded, non-O versus O blood type, was statistically significantly associated with preeclampsia subtype $\chi^2(3, n=403)=6.92, p=.031$, Cramer's $V=.131$. However, there was not a statistically significant association when analyzing the preeclampsia group $\chi^2(1, n=403)=2.69, p=0.101, \phi=.09$.

Table 6

ABO Blood Type and Preeclampsia Subtype Comorbidities Excluded

		Preeclampsia Subtype						<i>p</i>
		Early (n=72)		Late (n=97)		Control (n=234)		
		n	%	n	%	n	%	
ABO	A	25	34.7	40	41.2	92	39.3	.001
Blood	AB	3	4.2	5	5.2	5	2.1	
Type	B	23	31.9	9	9.3	28	12	
	O	21	29.2	43	44.3	109	46.6	

Binary Logistic Regression. Binary logistic regression was performed to further assess the association between ABO blood phenotype and preeclampsia. Initially the model included ABO blood phenotype as the predictor. Maternal ABO blood type was not significant in predicting preeclampsia status $\chi^2(3, N=511) = 5.35$ $p = .148$, indicating that the model including ABO blood phenotype alone was unable to distinguish between preeclamptic subjects and controls. The model as a whole explained 1% (Cox and Snell R squared) to 1.4% (Nagelkerke R squared) of the variance in preeclampsia status and correctly classified 53.4% of preeclampsia cases. As shown in Table 7, of the four blood types, only one was statistically significantly associated with preeclampsia. Of the blood types, AB was associated with the greatest odds of preeclampsia (OR= 2.99, 95% CI 1.03, 8.67). This indicates that women with an AB blood type had an almost three times the odds of preeclampsia, compared to those with O blood type. This model was re-run including race as a control variable given the relationship between race and blood type. After controlling for race, the results remain consistent; AB blood type was significantly associated with preeclampsia (OR= 3.03, 95% CI 1.04, 8.80).

Table 7
Summary of Logistic Regression ABO Blood Type and Preeclampsia

Predictor	B	SE	OR	95% C.I.	Wald statistic	p
O						.177
A	0.09	0.20	1.09	[0.75, 1.60]	0.21	.648
AB	1.10	0.54	2.99	[1.03, 8.67]	4.08	.043
B	0.31	0.27	1.37	[0.80, 2.34]	1.31	.255

p<.05

Multinomial logistic regression. Multinomial logistic regression was performed to further assess the association between ABO blood type and preeclampsia subtype (early and late). Maternal ABO blood type was not significantly associated with preeclampsia subtype χ^2 (6, N=511) =11.81 *p*=.066, indicating that the model including ABO blood phenotype alone was unable to distinguish between subjects with each preeclamptic subtype and controls. The model as a whole explained 2.3% (Cox and Snell R squared) to 2.6% (Nagelkerke R squared) of the variance in preeclampsia status. As shown in Table 8, of the four blood types, only blood type AB was statistically significantly associated with late onset preeclampsia (OR= 3.35, 95% CI: 1.02, 11.05). Although none of the blood types were statistically significantly associated with early onset preeclampsia, it is important to note that B blood type is trending towards being significantly associated with early onset preeclampsia in this model (*p*=.078). After controlling for race, results remained consistent; only AB blood type was statistically significantly associated with late onset preeclampsia (OR=3.41, 95% CI, 1.03, 11.29).

Table 8
Summary of Multinomial Logistic Regression ABO Blood Type
and Preeclampsia Subtype

Predictor		B	SE	OR	95% C.I.	Wald statistic	p
Early	O						
	A	-0.12	0.25	.89	[0.55, 1.44]	0.23	.629
	AB	0.98	0.63	2.66	[0.78, 9.10]	2.44	.118
	B	0.55	0.31	1.73	[0.94, 3.21]	3.12	.078
Late	O						
	A	0.27	0.24	1.32	[0.83, 2.09]	1.34	.246
	AB	1.21	0.61	3.35	[1.02, 11.05]	3.94	.047
	B	-0.03	0.37	.97	[0.47, 2.00]	0.01	.938

p < .05

Research Question 3

What is the association between preeclampsia subtype and Rh type?

A Chi square test for independence was estimated to assess the relationship between Rh factor and preeclampsia subtype. Rh type was not significantly associated with preeclampsia subtype, $\chi^2(2, N=511) = 1.94$ $p = .38$, Cramer's $V = .062$ by preeclampsia subtype. It was also not significant when assessing preeclampsia versus controls ($p = .874$). After exclusion of comorbidities, Rh type continued not to be significant when assessed by subtypes ($p = .51$) and in the preeclampsia group as a whole ($p = .65$).

Research Question 4

What is the association between ABO blood type, platelet count at > 28 6/7 weeks gestation, and preeclampsia development?

A two way between groups ANOVA was performed to assess if ABO blood type moderates the relationship between preeclampsia and platelet count at >28 6/7 weeks gestation. The two grouping variables (preeclampsia subtype and blood type) as well as the interaction

between the two were used to predict platelet count. The Levene's Test of Equality of Error Variances was significant ($p=.028$), this suggests that the variance of platelet count across groups was not equal, subsequently a more stringent significance level was used ($p=.01$) when interpreting results. There were no statistically significant main effects noted for either blood type or preeclampsia subtype. The interaction effect between maternal ABO blood type and preeclampsia subtype was not significant $F(6, 482) = .845, p=.536$, meaning that the influence of preeclampsia subtype on platelet count does not depend on maternal blood type. Although non-significant, Figure 1 depicts estimated marginal means of platelet counts at greater than 28 6/7 weeks gestation by each preeclampsia subtype and ABO blood type.

Estimated Marginal Means Platelets > 28 weeks by ABO Blood Type

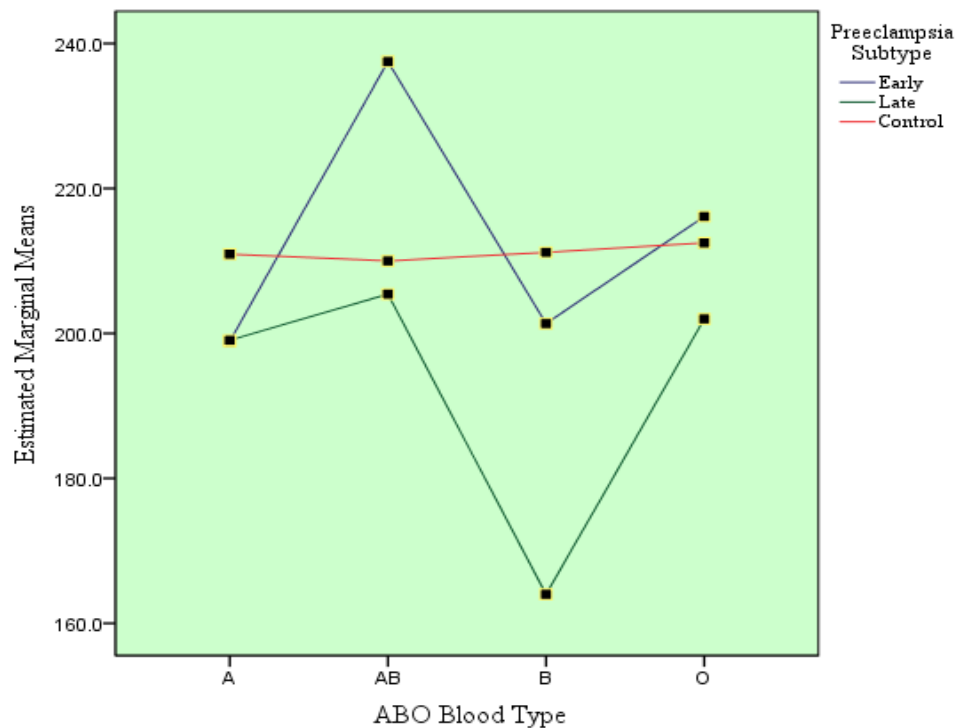


Figure 1 Estimated Marginal Means of Platelets > 28 weeks

Mediation analysis. Mediation analysis was performed using PROCESS 2.16 (Hayes, 2016). PROCESS “uses an ordinary least squares or logistic regression-based path analytic

framework for estimating direct and indirect effects” of variables in a mediated model (Hayes, 2016). Mediation attempts to determine if a relationship between a predictor variable and an outcome variable can be explained by their relationship to a third variable (Field, 2015) (Figure 2). Literature supports that ABO antigens are reflected on the platelet surface, subsequently mediation analysis was run to determine if the relationship between maternal ABO blood type (predictor) and preeclampsia subtype (outcome) is mediated by platelet level at > 28 6/7 weeks gestation. Blood type did not significantly predict preeclampsia subtype $b=1.47, t=.76, p=.44$ additionally, there was no significant indirect effect noted of ABO blood type on preeclampsia subtype through platelet count at >28 6/7 weeks gestation $b=.0008, \text{BCa CI} [-.0013, .0085]$

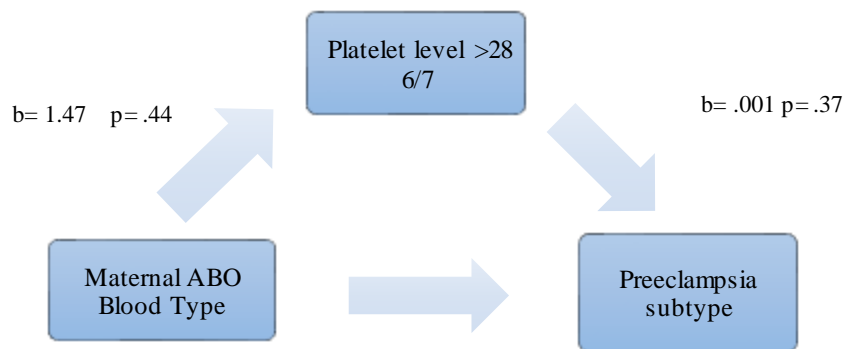


Figure 2 Mediation Analysis Platelets > 28 6/7

Direct effect, $b=-.004, p=.87$
Indirect effect, $b=.0008, 95\% \text{ CI} [-.001, .008]$

Research Question 5

What is the association between ABO blood type and fetal growth restriction in preeclamptic women?

A Chi square test for independence was performed to assess the relationship between ABO blood type and fetal growth restriction (FGR) in preeclamptic women only. This test indicated a significant association between FGR and ABO blood type in preeclamptic subjects $\chi^2(3, n=252) = 19.52, p < .001$, Cramer's $V = .278$. Table 9 depicts frequency of FGR by maternal

ABO blood type in preeclamptic subjects. In preeclamptic women with the B blood type, almost 66% of the subjects had a growth-restricted fetus. No significant association was noted when comparing O versus Non-O blood types ($p=.99$). Logistic regression was performed in order to assess the odds of developing growth restriction amongst women with each preeclampsia subtype. The model including ABO blood type was statistically significant $\chi^2(3, n=252) = 19.04, p < .001$, indicating that the model was able to distinguish between preeclampsia subjects with growth restriction and those without. The B blood type was the only blood type that was statistically significantly associated with growth restriction in preeclamptic women. Women with the B blood type had three times the odds of having a growth-restricted fetus than did women with other blood types (OR=3.44, 95% CI 1.58, 7.50).

Table 9

Association of ABO Blood Type to Fetal Growth Restriction in Preeclamptic Subjects (n=252)

Variable	ABO Blood Type								<i>p</i>
	A		AB		B		O		
	n	%	n	%	n	%	n	%	
FGR	24	25.3	4	30.8	25	65.8	38	35.8	<.001
No FGR	71	74.7	9	69.2	13	34.2	68	64.2	

Note FGR =Fetal Growth Restriction

Research Question 6

What factors are significantly associated with preeclampsia and preeclampsia subtype? preeclampsia subtype?

Initially, binary logistic regression was performed to test the association between preeclampsia and other well-documented risk factors of preeclampsia. An initial model containing eight independent variables (BMI, maternal age at delivery, sex of the neonate, smoking status at antenatal enrollment, maternal race, insurance type, parity and gestational diabetes diagnosis), was statistically significant $\chi^2(9, n=419) = 43.93$ $p < .001$, indicating that the model was able to distinguish between participants with preeclampsia and those without. The model as a whole explained between 10% (Cox and Snell R squared) to 13.3% (Nagelkerke R squared) of the variance in preeclampsia status and correctly classified 64% of preeclampsia cases. The sensitivity of this model or the ability of the model to identify cases of preeclampsia was 51.3%. The specificity of this model or the ability of the model to correctly identify those that did not have preeclampsia was 75%. Four of the variables were statistically significantly associated with preeclampsia, nulliparity (OR=1.68, 95% CI 1.08, 2.61), maternal early pregnancy BMI (OR=1.07, 95% CI 1.04, 1.11), smoking (OR=1.79, 95% CI 1.07, 3.02) and Black race (OR= .456, 95% CI .209, .995).

Binary logistic regression was rerun including ABO blood type into the model. This model contained ABO blood type and the same eight independent variables used in the previous model (BMI, maternal age at delivery, sex of the neonate, smoking status at antenatal enrollment, maternal race, insurance type, parity and gestational diabetes diagnosis). The full model containing all variables including ABO blood type was statistically significant $\chi^2(12, n=419) = 51.01$, $p < .00$, indicating that the model was able to distinguish between participants with

preeclampsia and those without. Including ABO blood type explained an additional 1.5-2% of the variability in preeclampsia group and the model as a whole explained an 11.5% (Cox and Snell R squared) to 15.3% (Nagelkerke R squared) of the variance in preeclampsia status and correctly classified 63.7% of preeclampsia cases. The sensitivity of this model or the ability of the model to identify cases of preeclampsia was 51.3%. The specificity of this model or the ability of the model to correctly identify those that did not have preeclampsia was 74.6%. Four of the variables were statistically significantly associated with preeclampsia, nulliparity, maternal early pregnancy BMI, smoking, and AB blood type (Table 10). The strongest odds of preeclampsia was for AB blood type (OR= 4.93 95% CI 1.26, 19.38), such that individuals who had an AB blood type had almost five times greater odds of preeclampsia, controlling for all other factors in the model. When including ABO blood type into the model, the specificity and sensitivity of the model remained the same and there was not an improvement in the classification of preeclamptic subjects.

Table 10

Summary of Logistic Regression Analysis Preeclampsia with ABO Blood Type

Variable	B	S.E.	Wald	df	p	Odds Ratio	95% C.I.for	
							Lower	Upper
O			5.99	3	.112			
A	-.002	.23	.00	1	.991	1.00	.63	1.57
AB	1.60	.70	5.23	1	.022	4.93	1.26	19.38
B	.29	.33	.76	1	.384	1.33	.70	2.53
BMI	.07	.02	21.17	1	.000	1.07	1.04	1.11
White			6.15	2	.046			
Black	-.78	.41	3.69	1	.055	.46	.21	1.02
Other Races	-.63	.33	3.58	1	.058	.53	.28	1.02
GDM	.10	.35	.08	1	.781	1.10	.56	2.16
Smoking	.58	.27	4.61	1	.032	1.78	1.05	3.01
Nullipara	.53	.23	5.39	1	.020	1.70	1.09	2.65
Age	-.03	.02	.02	1	.144	.97	.93	1.01
Insurance	.006	.24	.00	1	.979	1.01	.63	1.61
Sex of Baby	.12	.21	.32	1	.572	1.130	.74	1.70

Note. CI=confidence interval for odds ratio

Multinomial logistic regression. Multinomial logistic regression was performed to assess the association of well-documented preeclampsia risk factors on each preeclampsia subtype (early and late). This model contained eight independent variables (BMI, maternal age at delivery, sex of the neonate, smoking status at antenatal enrollment, maternal race, insurance type, parity and gestational diabetes diagnosis). The full model containing all variables was statistically significant $\chi^2(16, n=417) = 44.17, p < .000$ and was a good fit based off non-significant Pearson and deviance statistics. The model as a whole explained between 14.2% (Cox and Snell R squared) to 16.4% (Nagelkerke R squared) of the variance in preeclampsia subtype and correctly classified 56.1% of subjects.

Three variables were statistically significantly associated with early onset preeclampsia, smoking, White race and BMI. In this study, White individuals were almost three times more

likely to have early preeclampsia (OR= 2.7, 95% CI: 1.11, 6.72). The effect of smoking was positive, (OR= .53, 95% CI .29, .97), so those that did smoke were roughly 50% less likely to develop early onset preeclampsia. For every one unit increase in BMI there is roughly an 8% increase in risk of early preeclampsia (OR= 1.08, 95% CI 1.04, 1.12).

The association of both parity and BMI were statistically significant with late onset preeclampsia. Nulliparous women had two times greater odds of late onset preeclampsia (OR= 2.1, 95% CI .1.25, 3.65). For every 1-unit increase in BMI there is roughly a 7% increase in odds of late preeclampsia (OR= 1.06. 95% CI 1.03, 1.11).

Multinomial logistic regression was rerun including ABO blood type into the model. This model contained maternal ABO blood type and the same eight independent variables used in the previous model (BMI, maternal age at delivery, sex of the neonate, smoking status at antenatal enrollment, maternal race, insurance type, parity and gestational diabetes diagnosis). The full model containing all predictors was statistically significant $\chi^2(24, N=418) = 74.81$ $p<.001$ and was a good fit based off non-significant Pearson and deviance statistics. Including ABO blood type explained an additional 2.2% -2.6% of the variability in preeclampsia subtype and the model as a whole explained an 16.4% (Cox and Snell R squared) to 18.8% (Nagelkerke R squared) of the variance in preeclampsia subtype and correctly classified 56.8% of subjects (early 24%, late 17.9%, controls 87.9%). When including ABO blood type into the model, the model remained significant however, there was only a small improvement in the classification of subjects with each preeclamptic subtype (56.1% vs 56.8%).

As shown in Table 11, only four of the variables were statistically significantly associated with early onset preeclampsia, AB blood type, smoking, White race and BMI. The strongest association was between AB blood type and early-onset preeclampsia (OR=5.41, 95% CI 1.19,

24.55). This indicated that participants who had an AB blood type have five times greater odds of early onset preeclampsia, controlling for all other factors in the model. In this study, White individuals were almost three times more likely to have early preeclampsia (OR=2.9, 95% CI: 1.16, 7.26). Smoking was also statistically significantly associated with early onset preeclampsia (OR=.528, 95% CI .286, .974). For every one unit increase in BMI there is roughly an 8% increase in risk of early preeclampsia (OR=1.08, 95% CI 1.04, 1.12).

The association of both parity and BMI remained statistically significantly associated with late onset preeclampsia. Nulliparous women had two times greater odds of late onset preeclampsia (OR=2.2, 95% CI .125, 3.65). For every 1-unit increase in BMI there is roughly a 7% increase in odds of late preeclampsia (OR=1.07, 95% CI 1.03, 1.11). AB blood type was trend-level in the late onset group ($p=.053$).

Table 11

Summary of Multinomial Logistic Regression Analysis Preeclampsia Subtype and ABO Blood Type

								95% CI	
Predictor		B	S.E	Wald	df	p	Odds Ratio	Lower	Upper
Early	Blood Type A	.01	.29	.00	1	.982	1.007	.569	1.782
	Blood Type AB	1.69	.77	4.79	1	.029	5.414	1.194	24.554
	Blood Type B	.66	.38	2.99	1	.084	1.927	.916	4.053
	Insurance	-.19	.29	.42	1	.516	.827	.468	1.464
	White	1.07	.47	5.20	1	.023	2.904	1.162	7.258
	Black	.60	.59	1.01	1	.316	1.812	.567	5.790
	GDM	-.22	.41	.29	1	.590	.804	.363	1.780
	Smoking	-.64	.31	4.18	1	.041	.528	.286	.974
	Sex of Baby	.15	.26	.34	1	.559	1.164	.699	1.939
	Nullipara	.28	.28	1.00	1	.317	1.325	.764	2.297
	BMI	.08	.02	18.61	1	.000	1.079	1.042	1.117
	Age	-.02	.03	.37	1	.545	.985	.938	1.034
Late	Blood Type A	-.001	.39	.00	1	.997	.999	.575	1.735
	Blood Type AB	1.52	.79	3.73	1	.053	4.574	.979	21.381
	Blood Type B	-.18	.44	1.60	1	.689	.837	.351	1.997
	Insurance	.21	.30	.51	1	.474	1.239	.690	2.225
	White	.28	.39	.51	1	.475	1.322	.614	2.847
	Black	-1.23	.73	2.85	1	.091	.292	.070	1.219
	GDM	.001	.43	.00	1	.999	1.001	.435	2.303
	Smoking	-.48	.33	2.08	1	.150	.620	.324	1.188
	Sex of Baby	-.40	.26	2.29	1	.131	.674	.404	1.124
	Nullipara	.28	.28	7.67	1	.006	2.148	1.250	3.690
	BMI	.02	.02	12.14	1	.000	1.067	1.029	1.106
	Age	-.04	.03	2.94	1	.086	.957	.910	1.006

Note. CI=confidence interval for odds ratio

BMI and Age are mean centered

Supplementary Analysis

Body mass index. A supplementary analysis was performed in order to assess if BMI mediates the development of preeclampsia. Previous literature supports the relationship between ABO blood type and cardiovascular risk factors, however, due to the small number of subjects in the control group with CHTN (n= 1) and DM (n=0), the mediation effect of these variables could

not be assessed. Mediation analysis was run to determine if the relationship between maternal ABO blood type (predictor) and preeclampsia (outcome) is mediated by maternal early pregnancy BMI. Blood type did not have a significant direct effect on BMI, additionally, there was no significant indirect effect noted of ABO blood type on preeclampsia subtype through BMI $b = -0.02$, BCa CI $[-0.023, .005]$ (Figure 3). Even when excluding women with comorbidities from the sample, no mediation effect was noted.

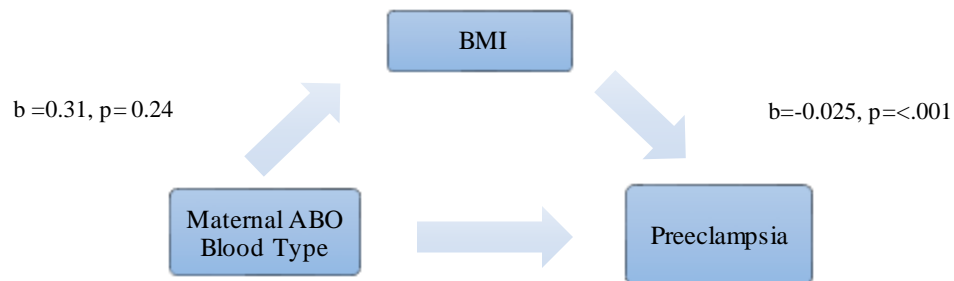


Figure 3 Mediation Analysis BMI

Direct effect, $b = -0.01$, $p = .73$
 Indirect effect, $b = -0.02$, 95% CI $[-0.02, 0.005]$

Chapter Summary

This chapter reports the results of the study. Descriptive statistics for subjects with preeclampsia as well as controls were reported. Descriptive statistics were also reported by preeclampsia subtype. Data was analyzed according to each research question with the main aim of the study to assess the relationship between ABO blood type and preeclampsia subtype. Both logistic and multinomial regression modeling were performed. The findings of this study suggest that blood type may play a role in the development of each preeclampsia subtype particularly in individuals without preexisting and other comorbid conditions of pregnancy. Preeclamptic subjects with B blood type had three times the odds of having a growth-restricted

fetus. Logistic regression, revealed that even when controlling for race, AB blood type was statistically significantly associated with increased odds of preeclampsia and late onset preeclampsia specifically. Finally, the inclusion of ABO blood type in a model with other clinical risk factors of preeclampsia did not improve the specificity and sensitivity of the model and no improvement was seen in the classification of preeclamptic subjects.

Chapter 5

Discussion, Conclusion, Recommendations

Chapter Introduction

The pathophysiology of preeclampsia remains unclear. Scientists believe there may be multiple subtypes of the disease, which have varying pathophysiology. Biomarkers must be explored in order to better elucidate the pathophysiologic basis of each preeclampsia subtype. Assessing the association between commonly collected biomarkers such as blood type and preeclampsia may prove valuable in risk identification. This chapter provides a discussion of the results of this study. A hospital-based case control study of 511 subjects was completed with the main aim of examining the association between maternal ABO blood type and preeclampsia subtype. Each research question is subsequently addressed. The results are compared and contrasted to existing literature on the topic. Finally, strengths and limitations of the study are presented as well as implications for clinical practice and policy and suggestions for further research.

Research Question 1

What are the maternal characteristics of women with preeclampsia and with each preeclampsia subtype?

Description of Study Groups

Clinical risk factors. Although not the primary aim, this study provided rich descriptive data of each preeclampsia subtype. Since many studies do not evaluate preeclampsia in relation to distinct early and late subtypes, this current study provided a unique opportunity to collect data on differences between these two preeclampsia subtypes. Due to the number of clinical and sociodemographic variables on which data was collected, these variables are discussed individually below.

In this study, maternal age was not significant for preeclampsia subtype ($p=.279$) or preeclampsia ($p=.566$). Maternal age continued not to be significant when assessed categorically (<18 years, 18-34 years, >35 years) ($p=.763$). Lisonkova, & Joseph, (2013) cite maternal age <20 years as a risk factor for early onset preeclampsia and reported that older maternal age > 35 years, was associated with preeclampsia, but not a specific subtype. However, Trostad, Magnus, and Stoltenberg (2011) states that research is conflicting on whether maternal age is a risk factor of preeclampsia.

Infant sex. Results of this current study were in line with previous research, more infants born to preeclamptic women were male than controls (53.2% v 49.2%) although this difference was not significant ($p=.615$). However, in this current study there was a significant difference in infant sex when assessed by preeclampsia subtype ($p=.047$). In this study, 54% of the early onset subtype had a female infant versus 39.2% in the late onset subtype and 50.8% in the control group. There seems to be a complex and not well understood interaction between the fetus and maternal physiology. Fetal sex has been shown to have an impact on a variety of maternal obstetrical outcomes (Jaskolka, Retnakaran, Zinman, & Kramer, 2016). A recent systematic review by Jaskolka and colleagues (2016) reported male fetal sex only to be a maternal risk factor for preeclampsia in the non-Asian population. Previous research has shown that carrying a female fetus is associated with early onset or more severe preeclampsia subtypes which is in line with results from this dissertation study (Myatt et al., 2014; Zheng, Deng, Zhong, & Shi, 2016).

Race. Although maternal race was not significant by subtype ($p=.145$), there were far more Black subjects in the early onset subtype (12.7%) than in the late onset subtype (4.1%) and the control group (9%). This may point to the fact that race may influence the severity of the

disease, since the early onset subtype is often considered more severe due to its associated higher rates of maternal and neonatal morbidity and mortality.

Overall data has been conflicting on the significance of race by preeclampsia subtype. Data from this study is in contrast to Trostad et al. (2011) which lists African American race as risk factor of preeclampsia. Pare' et al. (2014) reported that African American race was not a risk factor for early onset preeclampsia. Conversely, Lisonkova, & Joseph (2013) reported that African American race was a risk factor for early onset preeclampsia. Tucker, Berg, Callaghan, and Hsia (2007) reported that although non-Hispanic black women did not have a higher prevalence of preeclampsia than white women did, they did have much higher case-fatality rates. Results on race garnered from this study may be related to the lack of diversity within the sample overall. Community demographics reported on the 2010 census revealed the racial make-up of the community where this current study was completed to be 88.54% White, 5.6% Black and 5.86% other race which includes Asian and Native Hawaiian or Pacifica Islander (Demographics, 2010). Due to the lack of racial diversity in the community overall, a larger sample size may be necessary to detect true subtype differences by race. However, the differences in racial make-up of each preeclampsia subtype and controls in this current study does support that race may in some way mediate severity of the disease.

Parity. Rates of nulliparity were significantly different between preeclampsia subtype and controls ($p < .001$). Interestingly, rates of nulliparity were comparable between early preeclamptic subjects and controls (36.5% v 36.3%), however; subjects with late onset preeclampsia had the highest rates of nulliparity (44%) of all the groups. Rates of multiparity were significantly higher in the early onset subtype (63.5%) than the late onset subtype (44.4%) ($p = .004$). Data from this current study, further supports the heterogeneous nature of this disease,

possibly suggesting that the pathophysiology of late onset preeclampsia may have an immunologic component, possibility related to “primipaternity hypothesis” (Hutcheon et al., 2011, p. 394). Since rates of multiparity were significantly higher in the early onset subtype, it could point to higher rates of preeclampsia recurrence in the early onset subtype. This in turn could suggest a different pathophysiologic basis for the early subtype. Data on preeclampsia recurrence was not collected in this current study; future research should be done assessing the association between preeclampsia recurrence and maternal ABO blood type.

Body mass index. Body mass index was significantly different by preeclampsia subtype. The early onset preeclampsia subtype had the highest mean early pregnancy BMI ($M=31.7$, $SD=8.15$) and 51.4% of subjects in this subtype were in the obese category ($BMI > 30$) compared to the late onset subtype (33%) and control group (24.9%) which were much more comparable in terms of BMI.

It is well documented that obesity substantially increases the risk of preeclampsia; Bodnar et al. (2005) reported that women with a body mass index (BMI) of 30 have threefold higher odds of developing preeclampsia. Roberts et al. (2011) reported that obesity increased risk of both the early and late subtypes of preeclampsia. In this study, data was collected on maternal prepregnancy or early pregnancy (<13 weeks gestation) BMI. Roberts et al. (2011) postulate that since both preeclampsia and obesity share a similar dyslipidemic profile, possibly, obese women with the most abnormal lipids are at the highest risk of preeclampsia. In this study, lipid profiles on each patient were not collected. Research supports that dyslipidemic profiles may differ by blood type (Etemadi et al., 2015; Lee et al, 2012). Future research should aim to compare lipid profiles by ABO blood type of women in each preeclampsia subtype as this may help to further differentiate the pathophysiologic basis of each preeclampsia subtype and

provide some clarity about the association between preeclampsia and long-term risk or cardiovascular disease.

Since obesity is one of the only modifiable risk factors of preeclampsia, this study further supports the need for health care providers to provide education and counseling on preconception and interconception weight loss in order to help women reach a healthy weight prior to pregnancy. Since women with the early onset subtype had higher rates of both multiparity and obesity than both the late onset subtype and controls, it is important for providers to have conversations about interconception weight loss and management in order to prevent complications in future pregnancies. The provision of evidence based and culturally appropriate programs to women of reproductive age on weight management and physical activity may have significant implications for maternal and neonatal obstetrical outcomes.

Smoking. Due to the retrospective nature of this study, it is difficult to determine how much and how often subjects smoked. There were more smokers in both preeclamptic subtypes than in the control group. Rates of smoking between the preeclampsia subtypes and controls was not significant ($p=.088$), subjects with early onset preeclampsia had the highest rates of smoking (27%). In the regression when all other clinical risk factors were included, smoking was protective of early onset preeclampsia, (OR= .53, 95% CI .29, .97), so those that did smoke were roughly 50% less likely to develop early onset preeclampsia. There was no significant association with the late onset subtype. This result was similar to other studies (Hutcheon et al., 2011; Savitz et al., 2014). However, this association has been shown to be dose dependent, and if smoking is stopped early in pregnancy, there is no protective effect. In this current study, smoking data was collected based off documentation of smoking status early in pregnancy so the amount each women smoked was difficult to quantify.

Gestational diabetes. Researchers have documented that preexisting disease such as chronic hypertension and diabetes are risk factors for preeclampsia (Bartsch et al., 2016). As expected, the highest rates of the comorbidities studied (GDM, HTN, DM) were seen in subjects with early onset preeclampsia. Rates of gestational diabetes were significantly different between the preeclampsia subtypes and control group ($p=.023$) with the highest rates of GDM seen in the early onset preeclampsia subtype (19%). In the early onset subtype, 22 subjects delivered between 21 and 28 weeks. Although some subjects in the early onset subtype may have had early prenatal glucose testing due to clinical risk factors such as BMI > 30, history of GDM or previous infant > 8'13oz, because testing for GDM typically occurs at 28 weeks of gestation, it is possible, that due to rates of prematurity in this subtype, numbers of subjects with GDM may have been far higher in this group and gone undiagnosed (WellSpan High Risk Standing Order Set, 2016). Rates of GDM in the late onset and control group were comparable (10.3% v 9.7%). A study by DeSisto, Kim & Sharma (2014) using data from the Pregnancy Risk Assessment Monitoring System (PRAMS) found prevalence of GDM might be as high as 9.2% which was similar to controls in this study (9.7%). Rates of GDM in women with the early onset preeclampsia subtype were over twice that of controls. This supports the need for the provision of pointed patient education at the time of GDM diagnosis on signs and symptoms of preeclampsia, as preeclampsia seems to occur earlier in this patient group.

Chronic hypertension. More subjects in the early onset subtype (27%) had CHTN than in the late onset (15.1%) or the control group (0.4%). Rates of CHTN between the early and late onset preeclampsia subtypes were significantly different ($p=.03$). This further supports the increased severity of the disease in women with CHTN. Providers should be aware of ACOG (2013) and the United States Preventive Task Force (2014) guidelines for the use of aspirin

therapy for preeclampsia prophylaxis in women with CHTN. The use of aspirin therapy beginning as early as 12 weeks gestation may help to decrease risk of preeclampsia in these high-risk women. Additionally, recent research has shown that women with early onset preeclampsia have 7-9 times increased risk of CVD later in life (van Rijn et al., 2013). In this current study, the higher rates of obesity and chronic hypertension seen in the early preeclampsia subtype could further support that the link between early onset preeclampsia and CVD risk may be potentiated by the increased rates of cardiovascular risk factors seen in this subtype rather than physiologic alterations that occur from preeclampsia. Further longitudinal studies are needed in this area.

Neonatal outcome. There were three fetal ($n=1$)/neonatal ($n=2$) deaths in the early onset preeclampsia subtype. However, there were no occurrences of fetal or neonatal death in the late onset or control groups. Subsequently, statistical significance was not reported on this variable. However, it is important to consider the clinical significance of this finding. It is well established that the early onset subtype is associated with significant perinatal morbidity and mortality (Hutcheon et al., 2011; Lisonkova, & Joseph, 2013). This may be related to the earlier gestational age at birth however, the increased morbidity and mortality in the early subtype may also be related to other pathophysiologic changes that occur in subjects with this subtype that require further study.

Seasonality. In this current study, infant month of birth was classified according to season (winter, spring, summer, and fall). The current study did not support the seasonality of preeclampsia and found no difference in the seasonal rates of the disease by subtype ($p=.275$). Literature on preeclampsia supports the seasonality of the disease. In non-tropical climates such as the U.S., increased rates of preeclampsia have been seen in the winter months (Tepoel, Safflas, & Wallis, 2011). Interestingly, in a metaanalysis by Tepoel et al. (2011) three of 14 studies

reviewed also did not support the hypothesis that preeclampsia is seasonal in nature; two of the three were also hospital based case control studies. A larger sample size may be necessary to detect seasonal differences.

Research Question 2

Is there an association between preeclampsia subtype and ABO blood phenotype?

Preeclampsia subtype (early, late) trended towards being significantly associated with ABO blood phenotype when assessed by individual blood type (A, B, AB, O) ($p=.062$). In this current study, when analyzing the frequency of each blood type within each preeclampsia subtype, it was noted that 19.8% of the early onset subtype had a B blood type. This was considerably more than in the late onset preeclampsia subtype (10.32%) and control group (12.36%) and trended towards statistical significance ($p=.06$). Both the AB ($p=.133$) and A blood types ($p=.147$) were also not significantly associated with preeclampsia subtype when analyzed individually. Additionally, this author did not find non-O blood type to be significantly associated with preeclampsia ($p=.29$) or preeclampsia subtype ($p=.46$).

Excluding comorbidities. Interestingly, when excluding individuals with comorbidities such as GDM, CHTN and DM, ABO blood type was significantly associated with preeclampsia subtype ($p=.001$). Subsequently, there seems to be a stronger relationship between blood type and preeclampsia subtype in healthy individuals than in those with comorbidities. It is possible that in healthy individuals, the pathway to preeclampsia development is influenced by blood type's effect on the regulation of biomarkers of coagulation and inflammation whereas the increased risk of preeclampsia in individuals with comorbid conditions may follow a different pathophysiologic pathway. Due to the low number of individuals with CHTN and DM in the

control group, mediation analysis was not performed. In future studies, mediation analysis could be done in order to assess the role various biomarkers play in mediating the development of preeclampsia subtype in women with and without comorbidities and of different blood types. This could be done in order to better assess if there are different pathophysiologic pathways that lead to the development of preeclampsia in previously healthy women.

There have been conflicting results in studies that have explored the association between ABO blood type and preeclampsia. The results of this current study were similar to those of other authors in the literature review (Alpoim et al., 2012; Hiltunen et al., 2009; Hentschke et al., 2014) and did not find non-O blood types to be associated with preeclampsia. Results on the association between blood type and preeclampsia have varied, and it is difficult to synthesize the literature on ABO blood type and preeclampsia as studies have used varying inclusion criteria. Some studies in the literature review chose to exclude subjects with comorbidities or multiparous women from their sample (Alpoim et al., 2011; Hiltunen et al., 2009; Lee, Zhang et al., 2012; Spinillo et al., 1995). In this current study, in order to address the possible confounding effect of comorbid conditions, subjects with comorbidities (CHTN, DM and GDM) were included in both the case(s) and control groups. Lee, Zhang et al. (2012) also chose to include women with preexisting hypertension and diabetes in their study and did not find any evidence of confounding.

Logistic regression. Binary logistic regression was performed to further assess the association between ABO blood phenotype and preeclampsia. Maternal ABO blood type was not significant in predicting preeclampsia, however, AB blood type was statistically significantly associated with the greatest odds of preeclampsia (OR 2.99, 95% CI 1.03, 8.67). Even when adjusting for race, subjects with AB blood type continued to have three times the odds of

developing preeclampsia (OR 3.03, 95% CI 1.04, 8.80). Similar results were reported when examining the association between blood type and preeclampsia subtype. Interestingly, even when controlling for race, AB blood type (OR 3.41, 95% CI, 1.03, 11.29) was statistically significantly associated with the greatest odds of late onset preeclampsia, no blood type was statistically significantly associated with early onset preeclampsia.

This current study supported the findings of other studies on this topic, which also reported that women with AB blood type have the highest odds of preeclampsia of all blood types. In these studies, odds ratios ranged from 1.0 to 2.4 (Alpoim et al., 2012; Clark & Wu, 2008; Hiltunen et al., 2009; Lee & Zhang et al., 2012; Phaloprakarn & Tangjitgamol, 2013). The main aim of these previous studies was to examine the association between ABO blood type and preeclampsia as a homogenous group. To this authors knowledge, this study is the first to describe an association between late onset preeclampsia and AB blood type.

Rationale for the association between preeclampsia and AB blood type may be related to the increased levels of Von Willebrand factor noted in non-O blood phenotypes. Individuals with non-O blood types have been shown to have a 25% increase in VWF and FVIII above their O blood type counterparts and plasma levels of VWF increase by blood type in the following order O>A>B>AB (Alpoim et al., 2012). It is plausible that the higher levels of these coagulation factors may increase the risk of thrombus (Alpoim et al., 2012; Alpoim et al., 2011). Researchers have postulated that the increased risk of thrombus in individuals with non-O blood types may result in increased risk of micro-thrombus in the placental vasculature subsequently decreasing placental blood supply (Alpoim et al., 2011). Future research should be done to examine if levels of VWF vary by blood type and each preeclampsia subtype.

In addition to varying level of coagulation factors, blood type has been shown to play a role in inflammation and immunity as well as the regulation of other biochemical markers (Alpoim et al., 2011; Franchini & Lippi, 2015; Hiltunen et al., 2009; Lee et al., 2012; Phaloprakarn & Tangjitgamol, 2013). Seyfizadeh et al. (2014) reported that serum creatinine levels in blood type AB were higher than all other blood groups. Increasing serum creatinine level is considered a diagnostic marker of increasing preeclampsia severity, and could play a role in the pathophysiology of the disease (ACOG, 2013). Future research should address the association between levels of biomarkers of preeclampsia severity such as serum creatinine, and maternal blood type.

Research Question 3

What is the association between preeclampsia subtype and Rh type?

Rh factor was not significantly associated with preeclampsia ($p=.87$) or preeclampsia subtype ($p=.38$) even when controlling for comorbidities ($p=.51$). However, the early onset preeclampsia group had the lowest frequency of Rh-negative women (8.73%) as compared to the late onset group (14.3%) and controls (12.4%). In this current study, 87.64% of the control group was Rh positive, this is similar to population based results which report that 85% of the population is Rh positive ("Blood Typing Systems," 2010). The results of this current study were in line with Shamsi et al. (2010) and López-Pulles et al. (2010) who both also reported no significant association between Rh factor and preeclampsia. Two studies in the review of literature did report an association between Rh factor and preeclampsia. Sezik et al. (2001) stated that O Rh negative women had a higher risk of HELLP syndrome. Lee and Zhang et al. (2012) also reported that Rh-positive women had a small increased risk of preeclampsia (OR

1.07, 95% CI 1.03-1.10). More research may be necessary to assess if Rh status plays a role in the severity of the disease and in differing populations.

Research Question 4

What is the association between ABO blood type, platelet count at > 28 6/7 weeks gestation, and preeclampsia development?

In this study, blood type did not moderate platelet level at admission for delivery in preeclampsia subtypes and controls. The interaction effect between maternal ABO blood type and preeclampsia subtype was not significant $F(6, 482) = .845, p = .54$, nor were there statistically significant main effects noted for either blood type or preeclampsia subtype. The estimated marginal means of platelet levels at > 28 6/7 weeks gestation in both the early and late preeclampsia subtype followed a similar pattern by blood type. The mean platelet count in women with B blood type was noted to be less than that of other blood types, although not statistically significant. Additionally, although not statistically significant, the higher platelet counts noted in subjects with AB blood type may also be clinically significant (Figure 1).

ABO antigens are expressed on a variety of human cells and tissues including platelets (Karabuva, Carević, Radić, & Fabijanić, 2013). Subsequently, it is important to consider the role blood type may play in mediating platelet count. The endothelial damage that occurs in cases of severe preeclampsia causes increased turnover of platelets resulting in decreased platelet count (Özdemirci et al., 2016). The diagnostic criteria of preeclampsia is defined by ACOG (2013) as the new onset of hypertension (≥ 140 mm Hg systolic or ≥ 90 mmHg diastolic) after 20 weeks gestation on two occasions at least four hours apart, and proteinuria (≥ 300 mg in a 24-hour urine collection). In the absence of proteinuria, if the patient has a platelet count $< 100,000$ per microliter, the diagnosis of preeclampsia can be made. A decrease in platelet count to $< 100,000$ per microliter also marks increasing severity of the disease. Data from this current study

supports the need for future research to explore the relationship between blood type and levels of other laboratory markers associated with severe features of preeclampsia such as platelets, liver enzymes, and serum creatinine.

Furthermore, other laboratory measures of platelets may better test their relationship to both ABO blood type and preeclampsia subtype. Han et al. (2014) and Myatt (2009) also did not find an association between platelet count and development of preeclampsia, but did find that mean platelet volume was significantly greater in women who developed preeclampsia. Other platelet indices, especially mean platelet volume (MPV), may be better indicators of preeclampsia severity than platelet count (Han et al., 2014; Myatt, 2009).

Research Question 5

What is the association between ABO blood type and fetal growth restriction in preeclamptic women?

In this current study, in women with preeclampsia, FGR was significantly associated with ABO blood type ($p < .001$). Of the blood types, subjects with B blood type had the highest rates of FGR. In women with preeclampsia, 66% of those with a B blood type experienced FGR. Preeclamptic women with the B blood type had three times the odds of having a growth-restricted fetus than did women with other blood types (OR 3.44, 95% CI 1.58, 7.50).

Hiltunen et al. (2009) also discovered an association between blood type and growth restriction in preeclamptic women, however in their study; preeclamptic subjects with AB blood type had higher rates of growth restriction. Although fetal growth restriction is no longer considered diagnostic of preeclampsia, it may point to increasing severity of the disease (Fox, Huang & Chasen, 2008). The American College of Obstetricians and Gynecologists (2013) Task Force Statement on Hypertension in Pregnancy recommends that preeclamptic women experiencing growth restriction should be monitored closely. These results further support that B

blood type may in some way be associated with increasing severity of preeclampsia and women with this blood type may require close monitoring of fetal growth as well as other severe features of the disease. Further research should examine the pathophysiologic mechanism that mediates the development of growth restriction in individuals with the B blood type.

Research Question 6

What factors are significantly associated with preeclampsia and preeclampsia subtype?

Preeclampsia. Initially, binary logistic regression was performed to test the association between preeclampsia and other well-documented risk factors of preeclampsia. The initial model containing eight independent variables (BMI, maternal age at delivery, sex of the neonate, smoking status at antenatal enrollment, maternal race, insurance type, parity and gestational diabetes diagnosis) was statistically significant $\chi^2(9, n=419) = 43.93$ $p < .001$. Binary logistic regression was rerun to include ABO blood type and the same eight independent variables used in the previous model. This model was also statistically significant, $\chi^2(12, n=419) = 51.01$, $p < .00$, however, including ABO blood type only explained an additional 1.5-2% of the variability in preeclampsia group. The addition of ABO blood type did not provide much improvement when classifying preeclampsia subjects. However, controlling for all other factors in the model, subjects with AB blood type had almost five times greater odds of preeclampsia (OR=4.93 95% CI 1.26, 19.38). The confidence intervals reported are wide, indicating that the sample size of individuals with AB blood type were rather small. Subsequently, results of this analysis should be interpreted cautiously.

Preeclampsia subtype. Multinomial logistic regression was performed to test the association between preeclampsia subtype and other well-documented risk factors of preeclampsia. The initial model containing eight independent variables (BMI, maternal age at

delivery, sex of the neonate, smoking status at antenatal enrollment, maternal race, insurance type, parity and gestational diabetes diagnosis) was statistically significant $\chi^2(16, n=417) = 44.17, p < .000$. There were different variables statistically significantly associated with each preeclampsia subtype. Smoking, White race, and BMI were statistically significantly associated with early onset preeclampsia, while nulliparity and BMI were statistically significantly associated with late onset preeclampsia.

Multinomial logistic regression was rerun to include ABO blood type as well as the eight clinical risk factors used in the previous model. The model remained significant however; there was only a small improvement in the classification of subjects within each preeclamptic subtype. When all clinical risk factors are included in the model, AB blood type significantly predicts membership in the early onset preeclampsia subtype (OR=5.41, 95% CI, 1.19, 24.55) and was trend-level in the late onset group ($p=.053$). The other variables that were significant prior to the inclusion of blood type into the model remained the same by subtype. This suggests that the utility of blood type as a risk factor for preeclampsia (regardless of onset) is weak after considering the other risk factors collected in the study, which is further supported by the small amount of variability accounted for in the model by ABO blood type. ABO blood type may not be a valuable addition to a preeclampsia-screening algorithm that already includes common clinical risk factors of preeclampsia. With this said, it is important to consider the role ABO blood type has in the regulation of other biomarkers of inflammation and coagulation that may influence the development of each preeclampsia subtype. Than et al. (2011) reported that Placental Protein 13 (PP13) an early biomarker of preeclampsia, binds differently to each ABO blood type. Subsequently, serum levels of PP13 may prove to be a better predictor of

preeclampsia, and this may explain the association between ABO blood type and preeclampsia when other variables are not in the model.

Strengths and Limitations

To this author's knowledge, this current study demonstrated for the first time an association between AB blood type and late onset preeclampsia subtype in women in the United States even after controlling for race. Subjects with AB blood had three fold-increased risk of late onset preeclampsia. Varying definitions of preeclampsia subtypes used in the literature have made it difficult to synthesize the outcomes of studies reviewed. The inclusion of older research in reviews may lead to further difficulty in synthesizing outcomes due to recent changes in preeclampsia diagnostic criteria.

Recognizing the heterogeneity of the disease, Myatt et al. (2014) called for researchers to begin to examine preeclampsia by the subtypes, early and late onset. Myatt (2014) suggests that researchers should carefully examine medical records for the onset of preeclampsia symptomology rather than simply using gestational age at delivery to signify subtype membership. A strength of this study is that preeclampsia was studied by subtype rather than studying preeclampsia as a homogenous group. Additionally, subjects were classified into preeclampsia subtypes based on the documented onset of subjective and/or objective preeclampsia diagnostic criteria rather than gestational age at delivery. Chart review was used to assess for strict ACOG (2013) diagnostic criteria in order to determine subtype membership (early onset $\leq 33\ 6/7$ weeks, late onset $\geq 34\ 0/7$). Other studies have used diagnostic codes to confirm preeclampsia cases, and used gestational age at delivery as a proxy to classify preeclampsia subtype, which could result in subtypes being diluted due to lack of stringent classification criteria. Confirming preeclampsia diagnosis was time consuming, but improved

the overall reliability of the study as compared to studies that utilized diagnostic codes for inclusion without a formal chart review to confirm the diagnosis (Brown et al., 2013; Savitz et al., 2014).

The study was adequately powered to detect differences in O versus non-O blood type by preeclampsia subtype. Many previous studies in this area chose to use subsample analysis to assess differences between preeclampsia subtypes, subsequently achieving the appropriate sample size for both early and late onset subjects is a strength of this study.

In order to further improve the overall reliability of the data gleaned, prior to beginning data collection, the blood bank was visited and their procedures for calibration and documentation of blood type were assessed for quality. Additionally, standardized data collection procedures were utilized when abstracting information from the EHR

It is necessary to address the limitations of this study and the applicability and generalizability of the findings. Many of the variables included in the study were measured by self-report, such as parity and ethnicity. Although this could potentially affect accuracy of the data, collection of data by self-report for these measures is appropriate, as it is standard practice (Gorber, Tremblay, Moher, & Gorber, 2007). It is unknown if height was consistently measured or collected by self-report, as this varies by obstetrical practice, this may affect the reliability of the data on this variable (Gorber et al., 2007). Additionally, due to the retrospective nature of this study, in terms of measurement of variables, weight measurements were not collected from scales that were known to be regularly calibrated. However, all health care personnel who obtained this data are trained on how to obtain these measurements, although it is unknown about the rigor of teaching and monitoring the practices of these tasks.

A small sample from one hospital in one geographic region was utilized for this study, and a majority of the subjects were White so the findings may not be generalizable to other populations. Additionally, due to the fact that the hospital is a referral center, cases, particularly those with the early onset preeclampsia subtype, may have followed a different referral pattern than controls. To this author's knowledge, no other studies have been conducted in the U.S with the primary aim to assess the relationship between preeclampsia subtype and ABO blood type. Since blood type varies by ethnicity this study fills an important gap in the literature.

Recommendations

Recommendations for Clinical Practice

Preventative therapy. Research exploring biophysical characteristics and biochemical markers associated with each subset of preeclampsia is integral to the discovery of the distinct pathophysiologic mechanisms which drive the onset timing and severity of this heterogeneous disease. Without a clear understanding of the pathophysiology of preeclampsia, the creation of targeted therapies has proven difficult. Recent evidence has shown use of aspirin may be helpful in prevention of preeclampsia particularly in high-risk women (Bujold et al., 2010). In patients with preeclampsia, it seems that aspirin improves trophoblast invasion of the uterine spiral arteries as well as the cytokine profile, and may decrease cell death and increase production of placental growth factor (Middeldorp, 2014). Literature supports increased levels of certain inflammatory cytokines and procoagulation factors by blood type (Franchini, Mengoli, & Lippi, 2016), and due to the variation in platelet count and FGR noted by blood type in this study, it may be valuable to assess if aspirin therapy is more effective in high-risk women with certain blood types.

Early identification. In this study, 66% of preeclamptic women with a B blood type experienced FGR; preeclamptic women with B blood type had three times the odds of having a growth-restricted fetus. This suggests that health care providers should carefully assess for FGR in preeclamptic women with the B blood type. Additionally, the trend in lower platelets in the B blood type could also serve as a proxy for risk of increased severity of the disease. This also supports the need to closely monitor pregnant clients with B blood types early in pregnancy for manifestations of preeclampsia. Early in pregnancy, providers may want to begin to educate women with B blood type about the signs and symptoms of preeclampsia and keep this association in mind if these patients report non-descript symptoms that could be associated with preeclampsia. Furthermore, research currently supports that low dose aspirin therapy started earlier in pregnancy (<16 weeks) is more effective particularly in reducing risk of FGR and severe preeclampsia (Roberge et al, 2016). Subsequently, blood type could be useful in identifying women at risk for poor outcomes early in pregnancy and thus allow for earlier initiation of aspirin therapy.

Cardiovascular risk. ABO blood type, specifically non-O blood type, has been shown to increase cardiovascular risk (Etemadi et al., 2015; Gong et al., 2014; He et al., 2012; Jassim, 2012; Lee, Lin et al., 2012). With an understanding that preeclampsia not only effects pregnancy, but also influences the lifelong cardiovascular health of a woman and her offspring, an understanding of risk factors shared between both diseases could provide a foundation for the development of programs and therapies aimed at decreasing risk of long-term cardiovascular sequela in these women (Herrera-Garcia, & Contag, 2014). When adjusting for race only, this study revealed an association between AB blood type and both preeclampsia, and specifically late onset preeclampsia. Cardiovascular research also supported the link between AB blood type

and poor cardiovascular outcomes (He et al., 2012; Karabuva, Carević, Radić, & Fabijanić, 2013; Zakai et al. 2014). Health care providers should consider targeting women with AB blood type earlier in their lifespan in order to assess for cardiac risk and provide cardiopreventative strategies.

ˆ **Maternal weight management.** Although not the main aim of this study, BMI was consistently statistically significantly associated with increased odds of developing preeclampsia and each preeclampsia subtype. With an understanding of the increased maternal and neonatal morbidity and mortality associated with preeclampsia this study further supports the importance of education on and support towards achieving a healthy weight prior to and between pregnancies (Malnory & Johnson, 2011).

Policy Recommendations

Documentation. Although this study yielded interesting statistical results, clinically relevant results were also gleaned during the extensive chart review and data collection process that may have implications for practice. It is well documented that the clinical signs and symptoms of preeclampsia are often non-descript (You, Wolf, Bailey, & Grobman, 2012). Upon chart review, women reported “not feeling right,” and many reported headache as a clinical symptom. Cerebral disturbance as a symptom was often difficult for providers to interpret as many women had a history of migraines that made it difficult to determine if the headache was a marker of increasing preeclampsia severity or simply the patient’s medical history. Additionally, in an effort to confirm preeclampsia diagnosis a 24-hour urine specimen was often ordered. This seemed to delay diagnosis (Townsend, R., O’Brien & Khalil, 2015). Chart review revealed that maternal diagnosis of preeclampsia was not consistently documented in the history and physical of the newborns born to preeclamptic women. With an understanding of the association between

preeclampsia and risk of hypertension in these offspring, documentation of birth history proves important to assessing future health and complications in infants born to preeclamptic women (Founds, 2014; Geelhoed et al., 2010; Herrera-Garcia, & Contag, 2014).

Cardiovascular risk education. Finally, during documentation review, a few charts revealed provider documentation, on conversations with the subject on the association between preeclampsia and long-term cardiovascular risk. The majority of electronic health records of preeclampsia cases which were reviewed did not have documentation on education related to the association between preeclampsia and long-term cardiovascular health. Recent literature has shown that many obstetricians and primary care physicians are not aware of the association between preeclampsia and future cardiovascular disease in previously affected women (Young, Hacker & Rana, 2012). In a study by Young, Hacker & Rana, (2012) “only 9% of internists and 38% of obstetrician-gynecologists were providing cardiovascular risk reduction counseling to women with a history of preeclampsia” (p. 1). The AHA (2014) reported that many more women with a history of preeclampsia experienced a cardiovascular event in the 10 years following delivery than those women with uncomplicated pregnancies (Bushnell et al., 2014). Best practices on when and how to educate women on the association between CVD and preeclampsia are not known. However, based on the risk of poor cardiovascular outcomes in the decade following the index pregnancy, and AHA recommendations for follow up, providers need to ensure women are educated on this association soon after delivery so women can begin close follow up with their PCP and institute cardiopreventive strategies (Celi et al., 2013).

Screening algorithms. It seems that an ideal preeclampsia screening algorithm would need to be done in the first trimester, as any preventive intervention would also need to be provided early in pregnancy when placentation occurs. Since abnormal placentation may be a

key component of the pathophysiology of preeclampsia, the timing of effective screening tests is important to consider (Townsend et al., 2015). Although, providers and policy makers are working to promote early recognition and standardized treatment plans for preeclampsia, currently there is currently no valid screening algorithm for preeclampsia. In this current study, when including ABO blood type into a model with other clinical risk factors of preeclampsia, ABO blood type did not increase the sensitivity or specificity of the model. Furthermore, when in the model with other clinical risk factors of preeclampsia, AB blood type only increased odds of early onset preeclampsia, and each preeclampsia subtype had different variables that were significantly associated with the outcome. Subsequently, it is also important to consider that subtypes of preeclampsia may have differing etiology, and one single screening algorithm may not be appropriate in order to capture all women at risk of preeclampsia. Mikat et al. (2012) suggests that early detection requires three main components, each that complements the other when assessing risk. These components include biophysical markers, biochemical markers, and maternal medical history (Mikat et al., 2012). Mikat et al. (2012) stated that when using maternal history alone for early preeclampsia detection, only 30% of women are detected with a 5% false positive rate. More research is needed in order to discover biomarkers specific to each preeclampsia subtype in order to improve prediction.

Recommendations for Further Research

The primary aim of this study was to determine if maternal ABO blood type was associated with preeclampsia subtype. The subtypes of preeclampsia that were explored were related to timing of onset, early ($\leq 33\frac{6}{7}$ weeks gestation) versus late ($\geq 34\frac{0}{7}$ weeks gestation). There are other preeclampsia subtypes discussed in the literature, such as, proteinuric and non-proteinuric preeclampsia and preeclampsia with FGR and preeclampsia without FGR.

Future research should be done to explore if there is an association between these other preeclampsia subtypes and ABO blood type.

Previous studies on the association between ABO blood type and preeclampsia have been done in various racial and ethnic populations. Articles in the review of literature consisted of studies done in Thai, Hungarian, Turkish, Scottish, Finnish, Swedish, Iranian, Ecuadorian and Brazilian populations (Alpoim et al., 2012; Clark et al., 2007; Hiltunen et al., 2009; Lee, Zhang et al., 2012; Phaloprakarn & Tangjitgamol, 2013; Seyfizadeh et al., 2014). Since blood type varies by ethnicity, it is important to explore this association in various populations.

Additionally, since serum levels of certain inflammatory, thrombotic, and angiogenic markers such as VWF, PP13, and Tumor necrosis factor, that may mediate the development of preeclampsia subtype, vary by blood type and ethnicity, further study is necessary to explore this association. This may help to clarify the role maternal ABO blood phenotype plays in the development of each preeclampsia subtype, and support why results are inconsistent in studies on ABO blood type and preeclampsia, as the association between certain inflammatory cytokines and blood type may vary by ethnicity (Franchini, Mengoli, & Lippi, 2016)

Data on fetal and paternal blood type should be included in future research on the association between maternal ABO blood type and preeclampsia in order to determine if differences in maternal and fetal or paternal blood type mediate an immune reaction which predisposes women to either preeclampsia subtype (Phaloprakarn & Tangjitgamol, 2013). Due to the high rates of multiparity noted in the early onset subtype in this current study, it may also be important for further studies to examine rates of preeclampsia recurrence by ABO blood type.

In this current study, markers of severity such as growth restriction and lower platelet levels were seen more often in individuals with the B blood type. Although early onset

preeclampsia is often considered the more severe of the subtypes, women with both subtypes of the disease can have preeclampsia with severe features (Boyd et al., 2013; van Rijn et al., 2013). Women who have met the basic criteria for preeclampsia are classified as having severe features if their systolic blood pressure is ≥ 160 mmHg or diastolic is ≥ 110 mmHg on two occasions at least 4 hours apart, have platelet counts less than 100,000 per microliter, impaired liver function, renal insufficiency, pulmonary edema, or cerebral or visual disturbance (ACOG, 2013). Further research should be conducted to assess if certain severe features occur more commonly in each ABO blood phenotype.

Summary and Conclusion

Preeclampsia has been studied in biologic, immunologic, genetic and pathophysiologic research (Charlton, Tooher, Rye, & Hennessy, 2014; Founds et al., 2011; Ilekis, Reddy & Roberts, 2007). The disease seems to result from a complex interplay of maternal and fetal factors (Hermes et al., 2013). There is a growing body of evidence that has begun to explore the role of ABO blood phenotypes in the development of disease. Scientists now recognize the influence ABO blood type plays in the body's inflammatory and hemostatic responses (Franchini, & Bonfanti, 2015).

Although there were significant limitations to the study, this study provides a unique contribution to the literature on blood type and preeclampsia. Current research supports that preeclampsia is heterogeneous in nature and must be studied as such (Founds et al., 2011; Myatt et al., 2014). This current study utilized strict definitions that delineated preeclampsia by subtype (early and late), instead of assessing preeclampsia as a homogenous group. Subsequently, this study may better explain blood type's association with each preeclampsia subtype (early and late). Similar to other studies with this aim, subjects with the AB blood type

had roughly three times higher odds of preeclampsia. This study added to literature and reported that subjects with AB blood type had three times the odds of late onset preeclampsia than subjects with other blood types. Since AB blood type is the rarest of all blood types, larger studies done in the United States which utilize strict data collection procedures and assess preeclampsia by subtype are necessary to confirm this relationship. Preeclampsia may provide an early opportunity to identify women at risk for cardiovascular disease (Smith, Pudwell, & Roddy, 2013). Further exploration of risk factors, such as blood type, which are shared between preeclampsia and cardiovascular disease, may help in the development of interventions which could decrease long-term cardiovascular risk in these women.

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Appendix A: U.S Preventive Services Task Force Levels of Evidence

I: Evidence obtained from at least one properly designed randomized, controlled trial or meta-analysis of randomized, controlled trials.

II-1: Evidence obtained from well-designed controlled trials without randomization.

II-2: Evidence obtained from II well-designed cohort or case-control analytic studies, preferably from more than one center or research group.

II-3: Evidence from multiple time series with or without the intervention.

III: Opinions of respected authorities, based on clinical experience, descriptive studies or reports of expert committees

U.S. Preventive Services Task Force. (1996). Guide to clinical preventive services (2nd ed.). Baltimore: Williams and Wilkins.

Appendix B: Evidence Table ABO Blood Phenotype and Preeclampsia

Title of article Author Level of Evidence	Research Aim	Sample	Research Design	Results	Strengths/Limitations
Alpoim et al (2012) Preeclampsia and ABO blood groups: a systematic review and meta-analysis Level I	A systematic review of the literature for published studies investigating whether ABO blood groups could influence PE developing	Two studies met eligibility criteria N=992 women with PE N= 883 healthy pregnant women Total n = 1,875	Systematic review Meta-Analysis	Significant overall effect when comparing pregnant women of blood group AB versus non-AB ones for the risk of PE (OR 2.42; 95 % CI 1.63–3.58) No significant findings for any other blood type	Very small only found two studies that meet inclusion criteria Large group of severe preeclamptic patients in study Utilized ≥ 2 g of proteinuria as diagnostic Diagnostic criteria of PE has changed since publication Reports $\geq 0.3/24$ hours in discussion as inclusion criteria different than stated in inclusion criteria
Alpoim et al (2012) ADAMTS13, FVIII, von	FVIII activity, VWF and ADAMTS13 plasma levels, according to O and “non O” blood	N= 55 pregnant with sPE N=35 normotensive pregnant	case-control study	FVIII activity and VWF levels were significantly higher in sPE	Fairly small sample size. No power analysis Brazilian population

<p>Willebrand factor, ABO blood group assessment in preeclampsia</p> <p>Level II-2</p>	<p>groups, in women with severe PE (sPE).</p>	<p>N= 50 non-pregnant women.</p> <p>Total N= 140</p>		<p>group, as compared to normotensive pregnant women (P=0.01 and P=0.05, respectively)</p> <p>the frequency of O and non O blood groups in sPE and normotensive pregnancies was similar 31% and 69% in sPE; 43% and 57% in normotensive pregnancies P=0.25</p>	<p>4% AB blood type</p> <p>Definition of preeclampsia >2 g proteinuria=not ACOG definition</p> <p>Severe vs mild no longer used in preeclampsia nomenclature</p> <p>Patients after 29 weeks May have lost some sPE patients who delivered prior to 29 weeks</p>
<p>Clark et al (2007)</p> <p>The GOAL study: a prospective examination of the impact of</p>	<p>The impact of FVL/ABO(H) on a composite of all major vascular complications (PET, IUGR, pregnancy loss and VTE)</p>	<p>N=4157 consented to participate.</p>	<p>Prospective cohort study</p>	<p>No association with ABO(H) alone, or any interaction between ABO(H) and FVL was observed.</p>	<p>Scottish population</p>

factor V Leiden and ABO(H) blood groups on haemorrhagic and thrombotic pregnancy outcomes					
Clark & Wu (2008) ABO(H) blood groups and pre- eclampsia. A systematic review and meta-analysis	Examined the literature to see if there is a relationship between ABO(H) and pre-eclampsia, in order to reveal whether there is a true association between the two	17 eligible studies	A systematic review and meta-analysis	No consistent link between blood group AB and pre- eclampsia, with a pooled odds ratio of group AB versus the remainder of 1.02 (95%CI 0.86 to 1.22) Comparing a combined group of non-Os (i.e. AA, AB and BB) with group O gave similar results, with a	No specific features (disease definition, disease severity, date of publication, or ABO(H) distribution in controls) distinguished those few studies giving any form of positive association from the remainder.

				<p>pooled odds ratio of 1.01 (95% CI 0.91 to 1.12), but with some evidence of heterogeneity ($p=0.01$) and individual study estimate inconsistency ($I^2 49\%$)</p>	
<p>Franchini, Mengoli, Lippi (2016) Relationship between ABO blood group and pregnancy complications: a systematic literature analysis</p>	<p>To elucidate the role of ABO antigens in pregnancy-related complications, a systematic review of the literature published in the past 50 years was completed. A meta-analytical approach was also applied to the existing literature on the association between ABO status and pre-eclampsia.</p>	<p>9 studies (7 case control/2 cohort)</p>	<p>Systematic Review and meta-analysis</p>	<p>Blood group O was associated with lower odds of preeclampsia Effect size .77 (95% CI .67-.88) ($p<.001$).</p> <p>For group A the effect size was 1.78 (95% CI: 1.04-3.07), higher prevalence of this blood group among the patients with pre-eclampsia ($p=0.037$). The heterogeneity</p>	<p>Studies included were done in varying ethnic backgrounds, however the ethnic backgrounds were not described in this meta-analysis.</p> <p>Review dated back 50 years.</p>

				was substantial ($I^2=63.7\%$). For group AB the effect size was 1.94 (95% CI: 1.20-3.13), also higher than one ($p=0.007$) The heterogeneity was also substantial ($I^2=64.2\%$).	
Hentschke et al. (2014) Is there any relationship between ABO/Rh blood group and patients with pre-eclampsia	The purpose of this study was to evaluate the association between preeclampsia and blood groups in a group of pregnant women hospitalized in a University Hospital in Porto Alegre, Brazil	N=414 women were diagnosed as preeclampsia/eclampsia N=9611 women were identified to the control group.	Case Control	No difference in blood group distribution $P>0.05$	Brazilian population
Hiltunen et al (2009) Blood group AB and factor V Leiden as risk factors for pre-eclampsia:	ABO blood group, seven thrombophilia associated polymorphisms, and anti-beta2-glycoprotein	248 cases fulfilling strict criteria for pre-eclampsia and 679 controls	A population based nested case-control study	Blood group AB increased the risk for pre-eclampsia as a whole (OR 2.1, 95% CI 1.3-3.5), and in the three subgroups	Population from Finland Done retrospectively on medical records Used strict diagnostic criteria

A population based nested case-control study	I antibodies as risk factors for pre-eclampsia			(OR 2.3, 3.8, 3.4; 95% CI 1.3-3.9, 2.0-7.1, 1.6-7.1).	Noted lower incidence of preeclampsia in their study sample than in overall population
Lee et al (2012) ABO and RhD blood groups and gestational hypertensive disorders: a population-based cohort study	Examined the association between ABO and RhD blood groups and gestational hypertensive disorders in a large population-based cohort	All singleton deliveries in Sweden born to first-time mothers during the period 1987–2002 [total n = 641 926; any gestational hypertensive disorders, n = 39 011 (6.1%); preeclampsia cases, n = 29 337 (4.6%); severe pre-eclampsia cases, n = 8477 (1.3%)].	Cohort study	Compared with blood group O, all non-O blood groups had modest but statistically significantly higher odds of preeclampsia. Blood group AB had the highest risk for pre-eclampsia (OR = 1.10, 95% CI 1.04–1.16) and severe pre-eclampsia (OR = 1.18, 95% CI 1.07–1.30).	Population from Sweden Utilized ICD 9 codes to identify subjects-outcome Strict inclusion criteria Included gestational hypertension
López-Pulles et al. (2010) Assessment of Genetic Contributions	To identify the immunogenetic factors that trigger the beginning of preeclampsia and eclampsia	142 pregnant women in Obstetrics and Gynecological Hospital Isidro Ayora in Quito, who are grouped into two different groups, diseased and healthy.	A retrospective, case-control study	No statistical difference when comparing the blood type and Rh factor of the two groups ($p > 0.05$).	Ecuadorian Women Small sample size Several blood types not represented.

to Risk of Preeclampsia in Ecuadorian Women					
Phaloprakarn & Tangjitgamol (2013) Maternal ABO blood group and adverse pregnancy outcomes	To determine the relationship between maternal ABO blood group and risk of adverse pregnancy outcomes. preeclampsia, gestational diabetes mellitus (GDM), preterm delivery, low birth weight (LBW) small for gestational age (1stSGA) infants	5320 singleton pregnant women 350 (6.6%), preeclampsia, 333 (6.3%) GDM 543 (10.2%) Btw 6/08-7/10 1 st PNC < 14vweeks No known underlying disease or condition that could affect the pregnancy outcome	Retrospective cohort	Women with A or AB blood types, were found at increased risk of preeclampsia compared with O type individuals; adjusted relative risks were 1.7 (95% confidence interval (CI), 1.3 to 2.3; P¼0.001) for A phenotype and 1.7 (95% CI, 1.1 to 2.6; P¼0.01) for AB phenotype	Thai population Included GDM, LBW & SGA and preterm labor as outcomes Retrospective, potential risk factors may not have been identified Did not include Rh-population
Seyfizadeh et al (2014) Is there association between ABO blood group and the risk factors of unfavorable	Examine the relationship between blood group and pregnancy blood levels of Fasting blood sugar Hgb HCT Urea	N= 792 Healthy pregnancy women Tabriz health centers (Iran)	Prospective Cohort	The serum creatinine of AB group was significantly higher than the other 3 groups p<0.005)	Confounders not discussed Statistical Demographics of study populations not shown Completed in Iran

outcomes of pregnancy?	<p>Creatinine RBC's</p> <p>To determine if blood group has an association with some of the risk factors of unfavorable outcomes in pregnancy</p>			<p>Serum urea higher in AB group than other 3 groups ($p < 0.005$)</p>	
<p>Sezik et al. (2002)</p> <p>Distribution of ABO and Rh blood groups in patients with HELLP syndrome.</p>	<p>Evaluate the relationship between HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome and the maternal blood groups</p>	<p>N=547 severe preeclampsia divided by blood groups</p>	<p>Retrospective Cohort</p>	<p>O negative risk for HELLP-OR 3.1 CI (1.28–7.43) ($p < 0.01$)</p> <p>Did not detect increased incidence of HELLP syndrome in patients with the blood group 0 (independent of the Rh type)</p>	<p>Turkish Women</p>
<p>Than et al (2011)</p> <p>PP13, Maternal ABO Blood Groups and the Risk Assessment of</p>	<p>To test whether PP13-binding to erythrocytes, maternal blood-group has an effect on serum PP13 and its performance as a predictor of</p>	<p>maternal serum PP13 in Caucasian (n = 1078) and Hispanic n = 242 women were analyzed according to blood groups</p>	<p>Cross-sectional</p>	<p>Women with preeclampsia associated with IUGR had the lowest PP13 MoMs in the first trimester and the highest MoMs</p>	<p>Placenta sample from women in Budapest Hungary</p>

Pregnancy Complications	preeclampsia and IUGR			<p>in the second and third trimesters</p> <p>In Caucasian women: AB blood type had lowest serum levels of PP13 in the first trimester.</p> <p>Blood group B had the highest median PP13 MoMs throughout pregnancy</p> <p>In Hispanic women: Same as Caucasian cohort</p> <p>PP13 had differential binding to RBCs according to ABO blood types. PP13-binding was</p>	
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				<p>similar in blood groups A and O, the weakest in blood group B, and the strongest in blood group AB in comparison to other blood groups</p> <p>By adjusting to ABO blood group, the prediction accuracy of the PP13 test is improved for preeclampsia, IUGR and preeclampsia with IUGR</p>	
Witsenburg et al. (2005)	The aim of this study was to determine whether elevated factor VIII levels are associated with uteroplacental insufficiency, in	<p>N= Controls 272 women</p> <p>N=Plasma samples of 75 women with a history of pregnancy complicated by pre-eclampsia, HELLP syndrome, pregnancy</p>	Case Control	<p>VIII:C levels were similar at 123 IU/dl in both the patient group and the controls. In a logistic regression model,</p>	<p>Netherlands</p> <p>Elevated factor VIII levels were associated with a</p>

	particular pre-eclampsia, HELLP syndrome or pregnancy-induced hypertension and intrauterine growth retardation	induced hypertension or intrauterine growth restriction		after adjusting for age and blood group, no effect of factor VIII:C levels on the risk of pregnancy complications was observed, with the exception of IUGR with (OR 2.9, CI 1.0—8.7) or without hypertension (OR 2.0, CI 0.7—6.4).	two- to three-fold increased risk of IUGR. No observed effect from ABO-blood group, but this is acquired rather than genetically determined. Limitations of study not addressed Very small sample size
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Title of article Author Level of Evidence	Research Aim	Sample	Research Design	Results	Strengths/ Limitations
<p>Biswas, Ghoshal, Halder, Mandal (2013)</p> <p>Distribution of ABO blood group and major cardiovascular risk factors with coronary heart disease.</p> <p>Level II-2</p>	<p>The purpose of this study was to establish whether ABO blood group is related to coronary heart disease in an individual in Asian Indian Bengali population of eastern part of India</p>	<p>N=250 CHD patients and 250 age and sex matched healthy subjects</p>	<p>Case - control</p>	<p>The distribution of ABO blood groups in patients versus control group was A in 24.00 versus 21.60%, B in 30.80 versus 32.40%, O in 38.40 versus 21.60%, and AB in 6.80 versus 24.40%. The analysis showed significant difference in frequency of O (OR = 1.857, 95%CI = 1.112-3.100, P = 0.018) and AB (OR = 0.447, 95%CI = 0.227-0.882, P = 0.020) blood group between healthy controls and CHD individuals</p> <p>AB blood group decreases the risk of CHD in healthy controls, and it might be due to the higher concentration of high density lipoprotein cholesterol (HDL-c), while the</p>	<p>Asian Indian Bengali population</p>

				O blood group increases the risk of CHD due to lower HDL-c levels in Bengali population of eastern part of India.	
Chen et al. (2014) Analysis of circulating cholesterol levels as a mediator of an association between ABO blood group and coronary heart disease. Level II-2	To estimate the mediation effect size between cardiovascular disease and blood type	6476 consecutive patients	Case Control	Subjects of non-O type had higher levels of total cholesterol, low-density lipoprotein cholesterol, and non-high-density lipoprotein cholesterol (mean [SEM] in mmol/L: 4.931[0.021], 3.041 [0.018], and 3.805 [0.020] in non-O type compared with 4.778 [0.026], 2.906 [0.021], and 3.669 [0.024] in O type; $P=3.8 \times 10^{-7}$, $P=1.5 \times 10^{-7}$, and $P=3.1 \times 10^{-7}$, respectively). 10% of the effect of non-O type on coronary artery disease susceptibility was mediated by increased low-density lipoprotein cholesterol level ($P=7.8 \times 10^{-4}$) and 11%	Used mediation analysis Which revealed LDL as a mediator of CHD risk on patients with Non O blood type Did not discuss limitations of the study Done in Southern China

				of the effect of non-O type on myocardial infarction risk was mediated by raised low-density lipoprotein cholesterol level ($P=2.0 \times 10^{-3}$)).	
<p>Etemadi et al. (2015)</p> <p>Mortality and cancer in relation to ABO blood group phenotypes in the Golestan Cohort Study</p> <p>Level II-3</p>	<p>To study the association between ABO blood groups and overall and cause-specific mortality in the Golestan Cohort Study</p>	<p>50,045 people 40- to 70-years old</p>	<p>Population based cohort design</p>	<p>Non-O blood groups were associated with significantly increased total mortality (hazard ratio (HR) = 1.09; 95% confidence interval (CI): 1.01 to 1.17) and cardiovascular disease mortality (HR = 1.15; 95% CI: 1.03 to 1.27)</p> <p>plasma total cholesterol and LDL in those with blood group A</p>	<p>Did not check for differentiation between Type A subtypes.</p> <p>Study population in northeastern Iran</p> <p>No direct analysis of the mediation effect of biochemical changes in the ABO mortality association.</p>

					left censoring of the mortality data
<p>Gasso et al. (2012)</p> <p>A common variant of the ABO gene protects against hypertension in a Spanish population.</p> <p>Level II-2</p>	<p>The objective of this study was to establish whether genetic polymorphisms that could be related to angiotensin-converting enzyme (ACE) levels are associated with hypertension.</p>	<p>269 hypertensive patients and 254 healthy controls.</p>	<p>Case Control</p>	<p>Only one polymorphism of the ABO gene (rs495828) presented nominal pointwise $P < 0.05$ values (odds ratio = 0.33, 95% CI 0.19-0.58, $P = 6 \times 10^{-5}$) and achieved $P < 3.8 \times 10^{-3}$, the nominal P-value considered significant after Bonferroni correction. Analysis of the genotype frequencies showed that the model that correctly explained the observed association was the recessive model (odds ratio = 0.03, 95% CI 0.01-0.15, $P = 1 \times 10^{-6}$). These results indicate that genetic variants that could be related to ACE activity are good predictors of hypertension, and identify ABO as a good candidate</p>	<p>Further studies are required to confirm this association.</p>

				gene for genetic studies of hypertension risk.	
<p>Gong et al. (2014). Relation of ABO blood groups to the severity of coronary atherosclerosis: an Gensini score assessment.</p> <p>Level II-2</p>	<p>Investigated the relation of the ABO blood groups to the severity of coronary atherosclerosis assessed by Gensini score (GS)</p>	<p>2919 consecutive patients undergoing coronary angiography were enrolled</p>	<p>Cross-sectional cohort study</p>	<p>The frequency of blood group A was significantly higher in the upper GS tertiles (24.4% vs. 28.2% vs. 29.5%, $p = .032$).</p> <p>Blood group A was independently associated with GS ($\beta = 0.043$, $p = .017$). Group A remained significantly associated with mid-high GS (OR = 1.44, 95% CI 1.16-1.80, $p = .001$),</p> <p>Group O was showed as a protective factor (OR = 0.77, 95% CI = 0.65-0.92, $p = .004$).</p>	<p>Single center study</p> <p>The crosssectional nature of the study limits the ability to infer a causal relation between ABO blood group and cardiovascular events</p> <p>Large Chinese cohort</p>
<p>Gong et al. (2014) High-sensitivity C-reactive protein mediates in part the impact of ABO blood group on coronary artery disease</p>	<p>CRP might play an interesting role in the association of ABO group with CAD or MI</p>	<p>3806 consecutive patients who underwent selective</p>	<p>Cross-sectional study</p>	<p>There is a difference in blood type distribution between controls and patients with CAD ($p =$</p>	<p>Used mediation analysis</p>

Level II-3		coronary angiography for being suspected or known coronary atherosclerosis		<p>0.005) and patients with MI ($p= 0.047$)</p> <p>Non-O blood group remained significantly association with CAD and MI (OR = 1.49, 95% CI 1.20–1.84, $p < 0.001$; OR = 1.24, 95% CI = 1.04–1.47, $p = 0.017$), respectively after adjustment for common cardiovascular risk factors</p> <p>The effect of non-O blood type on CAD and MI susceptibility is mediated partly by hs-CRP levels.</p>	Large Chinese cohort
<p>He et al. (2012)</p> <p>ABO blood group and risk of coronary heart disease in two prospective cohort studies.</p> <p>Level I</p>	investigate the associations between ABO blood group and CHD risk in prospective cohort studies.	(the Nurses' Health Study [NHS] including 62 073 women and the Health Professionals Follow-up Study [HPFS] including 27 428 men)	Meta-analysis of two large Prospective cohort study	<p>ABO blood group was significantly associated with the risk of developing CHD in both women and men (log-rank test; $P=0.0048$ and 0.0002, respectively).</p> <p>In the combined analysis adjusted for cardiovascular risk factors, compared with</p>	United States

				<p>participants with blood group O, those with blood groups A, B, or AB were more likely to develop CHD (adjusted hazard ratios [95% CI] for incident CHD were 1.06 [0.99-1.15], 1.15 [1.04-1.26], and 1.23 [1.11-1.36], respectively). Overall, 6.27% of the CHD cases were attributable to inheriting a non-O blood group. Meta-analysis indicated that non-O blood group had higher risk of CHD (relative risk =1.11; 95% CI, 1.05-1.18; P=0.001) compared with O blood group.</p>	
<p>Jassim W.E. (2012) Association of ABO blood group in Iraqis with hypercholesterolaemia, hypertension and diabetes mellitus. Level II-2</p>	<p>Investigated the possible association of diabetes mellitus, hypercholesterolaemia and hypertension with ABO type.</p>	<p>920 patients with diabetes mellitus, hypertension and hypercholesterolemia</p>	<p>Case control</p>	<p>Levels of total cholesterol, glucose and systolic/diastolic blood pressure were all significantly higher in male and female patients in blood group O than other groups, with a decreasing trend</p>	<p>Iraqi population</p>

				from group A to B then AB. Similar trends by blood group were seen for the healthy controls although the differences were less marked	
Karabuva, Carević, Radić & Fabijanić (2013) The association of ABO blood groups with extent of coronary atherosclerosis in Croatian patients suffering from chronic coronary artery disease. Level II-2	Examined the relationship between ABO blood groups and extent of coronary atherosclerosis in patients with chronic coronary artery disease (CAD), 2) compare ABO blood groups distribution in CAD patients and general population, 3) examine possible differences in traditional risk factors frequency in CAD patients with different ABO blood group	N= 646 chronic CAD patients	Cohort	No association between ABO blood groups and the extent of coronary atherosclerosis in Croatian CAD patients is observed. Observation that AB blood group might possibly identify Croatian males at risk to develop the premature CAD has to be tested in larger cohort of patients.	Croatian population
Lee et al. (2012)	Aimed to investigate the association between	277 consecutive subjects (men	Case Control	Patients with CAD showed a significantly different blood group distr	Taiwanese population.

<p>Association of blood group A with coronary artery disease in young adults in Taiwan.</p> <p>Level II-2</p>	<p>the ABO blood groups and the risk of coronary artery disease (CAD) and myocardial infarction (MI) in a young Taiwanese population</p>	<p>younger than 45 years and women younger than 55 years) who underwent coronary angiography (136 with documented CAD and 129 without CAD) at our center, between 2005 and 2008</p>	<p>tribution (O, 30.1%; A, 39.7%; B, 26.5%; AB, 3.7%) than that shown by the controls (O, 42.6%; A, 24.0%; B, 27.1%; AB, 6.2%; $p=0.032$). Patients with blood group A had a greater risk of CAD and MI than those with non-A blood groups (OR=2.08, 95% CI=1.23-3.54; OR=2.21, 95% CI=1.19-4.09, respectively). After adjustment for common cardiovascular risk factors such as age, gender, hypertension, cigarette smoking, diabetes mellitus, body mass index, family history of CAD, and lipid profiles; blood group A remained significantly associated with an increased risk of CAD and MI (OR=2.61, 95% CI 1.11-6.14, $p=0.028$; OR=3.53, 95% CI=1.21-10.29, $p=0.021$, respectively).</p>	<p>Angiographically determined CAD</p> <p>Focused on younger patients</p> <p>Single-center design retrospective</p> <p>Small sample size, incomplete lipid profile data, and lack of detailed genotype information of the ABO alleles</p>
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<p>Reilly et al. (2011)</p> <p>Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies.</p> <p>Level II-2</p>	<p>Tested whether genetic factors distinctly contribute to either development of coronary atherosclerosis or, specifically, to myocardial infarction in existing coronary atherosclerosis.</p>	<p>N= patients who had angiographic CAD and myocardial infarction (n=5783)</p> <p>N=those who had angiographic CAD but no myocardial infarction (n=3644).</p>	<p>Case control</p>	<p>In the comparison of patients with angiographic CAD who had myocardial infarction versus those with angiographic CAD but no myocardial infarction, there was a novel association at the ABO locus ($p=7.62 \times 10^{-9}$). The ABO association was attributable to the glycotransferase-deficient enzyme that encodes the ABO blood group O phenotype previously proposed to protect against myocardial infarction.</p>	<p>CAD in patients of European ancestry genetic predispositions promote the development of coronary atherosclerosis whereas others lead to myocardial infarction in the presence of coronary atherosclerosis. The relation to specific CAD phenotypes might modify how novel loci are applied in</p>
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					<p>personalized risk assessment and used in the development of novel therapies for CAD.</p> <p>CAD was defined by angiography</p> <p>Multistudy design</p> <p>Misclassification possible as patient with CAD might develop MI</p>
Sode, et al. (2013) Risk of venous thromboembolism and myocardial infarction associated with factor V Leiden and prothrombin mutations and blood type.	Examined if ABO blood type alone and in combination with mutations in factor V Leiden R506Q and	Genotype of 66,001 white participants for ABO blood type, factor V Leiden R506Q	Prospective Cohort	Multivariable adjusted HRs for myocardial infarction by genotypes did not differ from 1.0.	White and Danish population

Level II-2	prothrombin G20210A is associated with the risk of venous thromboembolism and myocardial infarction in the general population.	and prothrombin G20210A.			Used ICD 9 codes to define MI Large sample
Wauters E. et al. (2013). Influence of 23 coronary artery disease variants on recurrent myocardial infarction or cardiac death: the GRACE Genetics Study. Level II-2	Evaluated whether these 23 loci also predispose to recurrent MI or cardiac death following an acute coronary syndrome (ACS).	2099 ACS patients enrolled in the Global Registry of Acute Coronary Events (GRACE) UK-Belgian study	Prospective Cohort	C-allele carriers of the rs579459 variant, which is located upstream of the ABO gene and correlates with blood group A, were independently associated with recurrent MI [multivariable-adjusted hazard ratio (HR) 2.25, CI = 1.37-3.71; P = 0.001] and with recurrent MI or cardiac death [multivariable-adjusted (HR) 1.80, CI = 1.09-2.95; P = 0.021] within 5 years after an index ACS. The association of rs579459 was replicated in 1250 Polish patients with 6 months follow-up after an index ACS [multivariable-adjusted (HR) 2.70, CI =	European population

				1.26-5.82; P = 0.011 for recurrent MI]. Addition of rs579459 to a prediction model of 17 clinical risk factors improved risk classification for recurrent MI or cardiac death at 6 months as calculated by the integrated discrimination improvement method (P = 0.037), but not by C-statistics (P = 0.096).	
Zakai et al. (2014) ABO blood type and stroke risk: The Reasons for Geographic And Racial Differences in Stroke Stud Level II-2	Assess the association of blood type with stroke and whether blood type contributes to racial disparities in stroke in the United States.	30,239 participants	Case-cohort	Blood type AB is associated with an increased risk of stroke that is not attenuated by conventional stroke risk factors, and factor VIII levels were associated with 60% of the association. While blood type AB is rare in the US population, it is a significant stroke risk factor and may play an important role in stroke risk in these individuals.	Blacks and Whites from the United States

Appendix D: ICD 9 Codes

Diagnosis	ICD 9 Codes
Preeclampsia	642.41/642.42/ 642.0/642.1/ 642.2/642.3/642.4/642.5/642.6 642.7/642.9 Premier queried using 4 digit ICD 9 codes, but asked system to include all cases with 5 digit codes which are inclusive of the 4 digit ICD 9 codes listed above.
Gestational Hypertension	642.31/642.32
Severe Preeclampsia	642.51/642.52
Eclampsia	780.39
HELLP syndrome	642.51/642.52
Multiple Gestation	651.01/651.11/651.21/651.31/651.41/651.51/ 651.61/651.71/651.81/651.91
Diabetes	250.00
Chronic Hypertension	642.22/642.21/642.02/642.01/642.72/642.71

Appendix E: Sample Size Calculation Using Hiltunen (2009)

Two-sided confidence level(1-alpha)	95
Power(% chance of detecting)	80
Ratio of Controls to Cases	2
Hypothetical proportion of controls with exposure	56
Hypothetical proportion of cases with exposure:	70.96
Least extreme Odds Ratio to be detected:	1.92

	Kelsey	Fleiss	Fleiss with CC
Sample Size - Cases	126	123	133
Sample Size - Controls	251	245	265
Total sample size:	377	368	398

References

Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-15
 Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 & 3.19

CC = continuity correction

Results are rounded up to the nearest integer.

Results from OpenEpi, Version 3, open source calculator--SSCC

Appendix F: Database by Variable

Variable	Level of Measurement	Database
ABO blood type <ul style="list-style-type: none"> • A • B • AB • O 	Categorical	Delivery Log
Platelets <ul style="list-style-type: none"> • < 20 weeks gestation • >20 weeks -28 weeks • Admission for delivery 	Continuous	< 20; > 20-28 weeks -Chart Review
Rh Factor <ul style="list-style-type: none"> • Rh - • Rh + 	Categorical	Delivery Log
Early Pregnancy BMI	Continuous Also assessed at the categorical level	Chart Review
Infant Birthdate	Collected as Continuous Recoded into categorical levels by season	Delivery Log
Comorbid Disease <ul style="list-style-type: none"> • DM • CHTN • GDM 	Categorical	Premier ® (ICD 9/10 Codes) Confirmed during chart review
Parity <ul style="list-style-type: none"> • Nulliparous • Multiparous 	Categorical	Delivery Log
Age at delivery	Continuous	Delivery Log
Infant Outcome <ul style="list-style-type: none"> • Live Birth • Fetal Death • Neonatal Death 	Categorical	Delivery Log

Socioeconomic status (insurance type proxy) <ul style="list-style-type: none"> • Public Insurance • Private Insurance 	Categorical	Premier®
Race <ul style="list-style-type: none"> • White • Black • Other 	Categorical	Delivery Log
Smoking <ul style="list-style-type: none"> • Non smoking • Smoking 	Categorical	Premier or Delivery Log using ICD 9-10 code and Chart Review
Gestational age at birth	Continuous	Delivery Log
Infant sex <ul style="list-style-type: none"> • Male • Female 	Categorical	Delivery Log
Infant birthweight-grams	Continuous Recoded to assess fetal growth restriction. Categorical (> 10% <10%)	Delivery Log and Chart Review
Preeclampsia <ul style="list-style-type: none"> • Early onset $\leq 33\frac{6}{7}$ weeks • Late onset ≥ 34 weeks 	Categorical	Cases identified through Premier® using (ICD 9/10 Codes) Onset timing confirmed with Chart Review

Curriculum Vitae
Adriane Burgess MSN, RNC-OB, CCE

EDUCATION

Ph.D. in Nursing, University of Wisconsin-Milwaukee **Expected May 2017**
Dissertation: *Maternal ABO blood phenotype and factors associated with preeclampsia subtype*

Master of Science in Nursing, Nursing Education Drexel University **2012**

Bachelor of Science in Nursing, Villanova University **May 1997**

PROFESSIONAL EXPERIENCE

TEACHING EXPERIENCE

Pennsylvania State University-Harrisburg **August 2015-Present**
Instructor

- Coordinates classroom and clinical learning
- Designs online and face to face course content
- Monitor and assess student progress in the accelerated degree program
- Supports student success as Academic Success Coach
- Builds relationships with clinical sites
- Teaches Fundamentals, Medical Surgical Nursing, Research, Professional Development and Maternal Newborn Nursing.

Elsevier

Maternal Newborn Subject Matter Expert –Sherpath **April 2016-October 2016**

- Design module outlines for the creation of an online maternal newborn course
- Assign appropriate content to maternal newborn course modules
- Create student learning objectives using appropriate levels of Bloom's taxonomy
- Work with instructional design team to create a complete maternal newborn course within Sherpath

Notre Dame of Maryland University **April 2013-July 2015**

Assistant Professor

- Designs course content
- Participates in and chairs school of nursing committees
- Monitor and assess student progress in the classroom and clinical setting
- Facilitates coordination of clinical sites
- Coordinates classroom and clinical learning to meet course objectives\
- Teaches in both online and classroom settings
- Designs and runs simulation using standardized and high fidelity simulators

- Experience teaching Maternal Infant Nursing, Nutrition, Informatics, Family Nursing and Holistic Health Assessment

York College of Pennsylvania

Adjunct Faculty, Maternal Infant Nursing

August 2012-March 2013

- Organize and implement obstetric simulation
- Perform student evaluations
- Monitor and assess student progress in the clinical setting
- Coordinate clinical learning to be in line with course objectives

Harrisburg Area Community College (HACC), Lancaster, PA

Adjunct Faculty, Maternal Infant Nursing

Fall 2012

- Monitor students during postpartum simulation
- Complete patient assignments for each nursing student
- Monitor students while they completed patient care.
- Grade care plans and written assignments.
- Mentor students to achieve course objectives

Kaplan

April 2011-March 2013

NCLEX Faculty

- Educate students on the Kaplan decision tree.
- Mentor student's bi annually to pass NCLEX.
- Enact curriculum according to Kaplan standards.
- Utilize a variety of teaching strategies in a classroom setting with traditional, non-traditional and international students.

Harrisburg Area Community College (HACC), York, PA

Clinical Instructor

Summer 2007

- Monitored students in skills lab and assisted them in learning basic physical assessment skills, including vital signs, auscultation, palpation and taking a complete history and physical.
- Completed patient assignments for each nursing student and monitored students during patient care.
- Graded completed assignments such as diagnosis cards and nursing syllabus requirements.

CLINICAL EXPERIENCE

York Hospital, York, PA

July 2007-Present

Clinical Research Specialist

September 2016-Present

- Responsible for collection and analysis of data for quality projects which support the Women's and Children's Service Line
- Collaborate with interdisciplinary teams to complete research articles and projects
- Provide research expertise on the design and implementation of research projects

- Create and manage a dashboard of quality measures specific to the Women's and Children's Service Line

Clinical Education Specialist

Coordinator of Childbirth and Family Education September 2009-September 2016

- Plan and implement curriculum for antenatal education programs
- Create a variety of course offerings to meet the needs of the community
- Manage and evaluate all teachers and office secretary
- Review and revise programmatic educational materials
- Coordinate programming with OB/GYN offices within the health system
- Complete prenatal teaching history and physical appointments on newly pregnant patients

Maternity/Antepartum/Gynecological Nurse March 2011-August 2013

- Monitor and assess patient and newborn.
- Administer medications per physician order.
- Interpret fetal heart tracings in the ante partum patient
- Educate patients and families on care of the newborn and maternal self-care
- Monitor patients throughout their recovery for both vaginal and surgical deliveries.
- Utilize computer documentation throughout the nursing process

Perinatal Nurse Coordinator July 2007-September 2009

York Hospital Maternal Fetal Medicine

- Monitored and assessed high-risk antepartum patients for fetal and maternal well-being and need for impending delivery.
- Educated patients concerning disease processes.
- Helped to improve neonatal outcomes of patients with a history of preterm delivery through weekly surveillance and medication administration.
- Assessed patient's history and physical to determine their need for ultrasound, genetic counseling and antenatal testing.

St Joseph Medical Center, Baltimore, MD **2001-2008**

Childbirth Educator 2002-2008

- Empowered families through the provision of education on labor and birth.
- Provided families with education on how to care for a newborn.
- Educated eleven to thirteen year olds on babysitting techniques.
- Created educational materials for the courses taught.

Labor and Delivery Nurse 2001-2007

- Monitored and assessed patient and fetus throughout labor and delivery and recovery.
- Interpreted fetal heart tracings in the antepartum and throughout labor.
- Scrubbed and circulated in the operating room for cesarean deliveries.

Sinai Hospital, Baltimore, MD **1998-2001**

Labor and Delivery Nurse

- Job duties consist of the same components as my position in Labor and Delivery at St. Joseph Medical Center.

St Joseph Medical Center, Baltimore, MD

1997-1998

Medical-Surgical Nurse 5W

- Monitored and assessed patients with a variety of illnesses and surgical procedures, including gynecological patients.
- Performed pre-operative and discharge teaching as needed.

LICENSES

Registered Nurse

Maryland
Pennsylvania

Expires **December 31, 2017**
Expires **April 30, 2018**

CERTIFICATIONS/TRAININGS

Nursing

National Certification Corporation (NCC)
Inpatient Obstetrics (RNC-OB)

Expires **December 31, 2016**

Other

American Heart Association
Basic Cardiac Life Support (BCLS)

Expires **June 31, 2018**

Prepared Childbirth Educators
Certified Childbirth Educator (CCE)

Expires **December 31, 2019**

Maryland Faculty Academy for Simulation Teaching in Nursing

Completed **March 2015**

Safe Sitter
Certified Safe Sitter Instructor

AWHONN

AWHONN Intermediate Fetal Monitoring Course

Completed **November 2012**

HONORS/SCHOLARSHIPS/AWARDS

Sigma Theta Tau International, Honor Society of Nursing
Eta Eta Chapter, York College

Inducted **2011**

Kaplan NCLEX Faculty Quarterly ACE award recipient	Winter 2012
Sigma Theta Tau-Eta Eta Graduate Nurse Scholarship	Fall 2013
MHEC New Nurse Faculty Fellowship Recipient	Fall 2013
Milton and Joan Morris Fellowship Recipient	Summer 2014
NEF Scholarship Award Recipient	Summer 2015
Barbara L. Tate Scholarship	Summer 2016
Harriet H. Doctoral Student Nursing Research Award	Summer 2016

INVITED PRESENTATIONS

Buchko, B.; Gutshall, C.; Brown, M.; **Burgess, A.**; Cadawas, T.; Hammers, F.; Hersey, C.; Hess, C.; Hollenbach, P.; McWilliams, V.; Puller, R.; Quickel, H.; Snyder, D. (June 25, 2012). "A Renewed Commitment to Improving Quality and Efficiency of Postpartum Education During Hospitalization", accepted for podium presentation at the 2012 AWHONN, National Convention, Washington DC.

Burgess, A. & Williams, W. (June 21, 2013). "Countdown 2: DRIVE: A Program Implementation", accepted for podium presentation at the 2013 Safe Kids Worldwide, Childhood

Burgess, A. (November 11, 2014). "Can we prevent infant sleep related deaths? What you need to know now" Holy Spirit Hospital. Camp Hill, PA.

Brennan, J., Brinkley, E. & **Burgess, A.** (November 21, 2014). "The Unexpected Benefit of Evaluating Assessment Skills of RN-BSN Students Using Standardized Patients" presented at AACN 2014 Baccalaureate Education Conference.

Consolini, M. & **Burgess, A.** (March 4, 2015). "The Link between High Risk Pregnancy and Early Onset Cardiovascular Disease" WellSpan Health-York Hospital

Burgess, A. (May 2015). "Pearls of Wisdom" Invited speaker, Class of 2015 Undergraduate Nursing Pinning Ceremony. Notre Dame of Maryland University.

Burgess, A. (February 1, 2016). Doctoral Nursing Student Organization "Publishing During Your Program." Presented at Doctoral Nursing Student Organization University of Wisconsin-Milwaukee.

Burgess, A., Stover, S., Yingling, C. & Consolini, M. (November 4th, 2016). "The Postpartum Preeclampsia Visit as a Method to Address Cardiovascular Risk in Preeclamptic Women," Geisinger Nursing Research Conference.

PANEL PARTICIPANT

Go Red For Women: Ladies Fun Night Out. (February 27, 2015). WellSpan Health, York, PA.

POSTERS

Burgess, A. & Brinkley, E. (June 14, 2014) "A Picture Is Worth A Thousand Words. Caring Through Cultural Competence Using PhotoVoice" poster presented at Institute for Educators at the University of Maryland School of Nursing Teaching and Technology: What's Now, What's Next

Moore, D. & **Burgess, A.** (October 20, 2014) "A LEAN look at Prenatal Teachings" poster presented at WellSpan Leadership Forum

Burgess, A., Brinkley, E. & Murphy-Buc, H. (June 12, 2015) "NCLEX Action Plan: Student and Faculty Collaboration to Promote Self-Motivation for NCLEX Preparation," Institute for Educators at the University of Maryland, School of Nursing "Readiness for Practice: What's Trending?"

Burgess, A. & Shiffer, W. (June 12, 2015). The Importance of High Touch in a High Tech World: Using a Doula to Teach Labor Support to Undergraduate Nursing Students," Institute for Educators at the University of Maryland, School of Nursing "Readiness for Practice: What's Trending?"

Murphy-Buc, H., Brinkley, E. & **Burgess, A.** (June 12, 2015). "Peer-Editing: Building Research and Writing Skills for Professional Nursing Practice." Institute for Educators at the University of Maryland, School of Nursing "Readiness for Practice: What's Trending?"

Murphy-Buc, H., Brinkley, E. & **Burgess, A.** (February 4, 2016). "Teaching and Learning Through Caring Science: NCLEX Action Plan for Student Success," AACN 2016 Faculty Development Conference.

Moore, D. & **Burgess, A.** (November 4, 2016). "Prenatal Teaching: Improving the Quality of the First Antenatal Visit," Geisinger Nursing Research Conference.

PUBLICATIONS

Burgess, A. (2014). An Evolutionary Concept Analysis of Labor Support. *International Journal of Childbirth Education*, 29(2), 64-72.

Burgess, A. (2015) Interconnectedness: The Grandparents Role in Childbearing and Parenting. *International Journal of Childbirth Education*, 30(1), 68.

Burgess, A. & Founds, S. (2016) Nursing Care for Cardiovascular Implications of Preeclampsia. *MCN: The American Journal of Maternal Child Nursing*, 41(1), 8-15.

Burgess, A. (accepted) Intergenerational reflections on birth: Story as a method to enhance meaning making in pre licensure nursing students. *Journal of Perinatal Education*.

Burgess, A, Murphy-Buc, H., & Brennan, J. (accepted). Using a Complex Patient Management Scenario to Help Bridge the Education Practice Gap. *Nursing Education Perspectives*

PROFESSIONAL AFFILIATIONS

Sigma Theta Tau International, Eta Eta Chapter
Association of Women's Health, Obstetric and Neonatal Nurses (AWHONN)
Midwest Nursing Research Society (MNRS)

SERVICE ACTIVITIES

York Hospital

- Member, Zika Registry Project 2016-present
- Member, Perinatal CET 2016-present
- Designer Postpartum Preeclampsia Follow Up Program 2015-present
- Member, Prenatal Teaching Redesign Committee 2013-2016
- Member, Cribs for Kids Committee 2010-present
- Member, York County Safe Kids Coalition 2010-2016
- Countdown 2:Drive Project Coordinator 2011-2016
- Member, Postpartum Education Committee 2010-2011

Notre Dame of Maryland University

- Member, Undergraduate Circle 2013-July 2015
- Chair, Student Circle 2014-July 2015
- Member, Professional Development Circle 2013-July 2015
- Member University Parking Appeals Board 2013-July 2015
- Member, Faculty Search Committee February 2015-July 2015

The Pennsylvania State University

- Speaker Series Committee October 2015-September 2016
- Math Dosage Template Committee November 2015-December 2015
- Skills Blueprint Committee April 2016-September 2016
- Facilitator 400 Level Focus Group Fall 2016
- Academic Success Coach Committee Fall 2016

Association of Women's Health and Neonatal Nurses

- South Central Pennsylvania Planning Committee

2015-present