UNIVERSITY OF CALIFORNIA, SAN DIEGO

SAN DIEGO STATE UNIVERSITY

# Indicators of Maternal Risk for Hypertensive Disorders of Pregnancy: Analysis of the MotherToBaby Cohort Data, 2004-2014

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy

in

Public Health (Epidemiology)

by

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2016

The Dissertation of Maria Perpetua Gemima De Ocampo is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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2016

# DEDICATION

This dissertation is dedicated to my mother. Her determination and perseverance through challenges life has presented to her are truly inspirational.

I would also like to dedicate this dissertation to my brother. One can only aspire to match his boundless generosity and irrepressible optimism.

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## FIELDS OF STUDY

Major Field: Public Health (Epidemiology)

ABSTRACT OF THE DISSERTATION

Indicators of Maternal Risk for Hypertensive Disorders of Pregnancy: Analysis of the MotherToBaby Cohort Data, 2004-2014

by

Maria Perpetua Gemima De Ocampo

Doctor of Philosophy

University of California, San Diego, 2016
San Diego State University, 2016

Professor Christina D. Chambers, Chair

Objective: Hypertensive disorders of pregnancy are leading causes of morbidity and mortality in all pregnancies in the United States. The objective of this dissertation was to examine the impact of risk factors, including the use of antidepressants (chapter 1), folic-acid containing supplements (chapter 2), and cigarettes (chapter 3), on the development of gestational hypertension and preeclampsia.

Methods: Data was collected from pregnant women who participated in the MotherToBaby cohort studies from 2004 to 2014. Maternal information was collected at intake, every three months until the birth of the baby and once after birth. Data collected at these interviews included demographics, medical history, lifestyle factors, substance

use, medication use, fetal sex, and study outcomes. Unadjusted and adjusted odds ratios (OR) and their 95% confidence intervals (CI) were generated using logistic regression to assess the association between the risk factors and the outcomes.

Results: A total of 3,474 women were included in the study. In chapter 1, women who continued to use antidepressants after 20 weeks of gestation were at higher risk for gestational hypertension (aOR: 1.83; 95% CI: 1.05, 3.21) after adjustment. Women who used serotonin-norepinephrine reuptake inhibitors were at significantly increased risk for gestational hypertension, but not preeclampsia. Findings from chapter 2 indicated no significant association between folic acid-containing supplement use and hypertensive disorders of pregnancy. Interestingly in chapter 3, smokers had decreased risk (aOR: 0.41; 95% CI: 0.21, 0.79) for gestational hypertension after adjustment. No significant association was observed between smoking and preeclampsia. After stratifying by timing of supplements use, smokers who used folic-acid containing supplements after recognition of pregnancy were at an even more decreased risk for gestational hypertension (aOR: 031; 95% CI: 0.13, 0.75).

Conclusion: Hypertensive disorders of pregnancy are poorly understood and remain major causes of maternal morbidity and mortality worldwide. Identifying risk factors for these disorders is important in planning intervention strategies. Additionally, further research is needed to examine the biological mechanisms by which risk factors contribute to the development of these disorders.

# INTRODUCTION

Hypertensive disorders of pregnancy remain some of the leading causes of maternal morbidity and mortality in the United States (US). Reported incidence rates for the years 2009-2010 indicate that 4.1% of all pregnant women develop gestational hypertension (GH) and 3.8% develop preeclampsia (PE) in the general US population.[1,2] In the study population for this dissertation, which covered the years 2004-2014, overall rates for GH were 6.9% and 4.1% for PE, which were generally consistent with previous studies especially in light of the characteristics of the women in the study sample.[3–5]

Furthermore, rates of GH and PE have steadily increased over the years. Data from the National Hospital Discharge Survey from 1987 to 2004 showed a 25% increase in PE and a 184% increase in GH reports over this time period.[6] The rise in PE and GH cases was attributed to the increased rates of known risk factors, such as obesity, diabetes, multiple births, and advanced maternal age at pregnancy.[6] The authors further argued that changes in diagnostic guidelines in 1996 and 2002 resulting in narrower definitions of PE may have in turn contributed to the sharp increase seen in GH diagnoses.[6–8] For the purposes of this dissertation, GH was defined as new onset of hypertension after 20 weeks of gestation, and PE was defined as GH with proteinurea after 20 weeks of gestation. These definitions are consistent with the diagnostic guidelines for the period covered by this study.

The data used to examine GH and PE for this dissertation came from the MotherToBaby pregnancy studies. The MotherToBaby network provides free health counseling and information service to thousands of pregnant women and healthcare

providers across North American every year. Pregnant women who contacted MotherToBaby were also screened for enrollment in MotherToBaby US and Canada and MotherToBaby California. In this dissertation, women who were enrolled and met all study requirements from January 1, 2004 to June 30, 2014 were included (N=3,474). The majority of women included in this cohort were Caucasian, had some college or higher education, and took folic acid-containing supplements during pregnancy, which differs somewhat from rates in the general population.[9,10] Additionally, the proportion of women who had comorbidities, such as asthma and autoimmune diseases, which have been associated with increased risk for PE,[11–13] were also higher compared to the general population.[9,14–16] As many women who contacted the MotherToBaby network were referred to this service by their physician, it could also be assumed that many of these women received some prenatal care during their pregnancy. Furthermore, as only women who contacted the MotherToBaby service were included in the study, a self-selection bias may have occurred. These factors combined may call into question the representativeness of the study population and may limit the generalizability of findings in this dissertation. Special care was taken in examining these important factors. Potential confounding by study population characteristics were examined in terms of their association between the exposures of interest and the outcomes. Changes in practice over the course of the study period from 2004 to 2014 were also taken into account by controlled for the enrollment year of study participants.

Risk factors for hypertensive disorders of pregnancy have been identified in previous studies.[17,18] In this dissertation, modifiable risk factors, including use of

antidepressants, folic-acid containing supplements, and cigarettes, were addressed. These factors were chosen because of their prevalence in use during pregnancy and the important role each play in the development of GH and PE as suggested by previous studies. It has been reported that as high as 13% of pregnant women use antidepressants during pregnancy with some studies indicating increased risk for GH and PE.[3,19,20] Other studies indicated a protective effect against hypertension in pregnancy with use of folic acid or folic acid-containing supplements.[21,22] Cigarette use has also been shown to be a preventive factor against hypertensive disorders of pregnancy.[23,24] Yet, even with these studies, gaps in knowledge still persist, such as how GH and PE are affected by timing of exposures or by interactions between exposures.

Limitations in previous studies were also addressed in this dissertation. Unlike other studies which relied on data from healthcare databases or collected information retrospectively, data in this study was collected over the course of pregnancy and once after the birth of the child. Information, such as start and stop times of antidepressant and supplement use were collected throughout pregnancy, whereas information from other studies using healthcare databases may only have medication dispensing information, and not actual reports of maternal use.[19,20,25] Data on cigarette use, including start and stop times and amount of cigarettes used, were also collected throughout pregnancy which may be more reliable compared to other studies that collected this information after pregnancy.[23,26] This dissertation examined how each of the exposures of interest contributed to the likelihood of developing GH and PE among women who participated in the MotherToBaby pregnancy studies.

Other limitations in the study should be noted in interpreting results. Due to the small number of cases reported by exposure groups, it is possible that spurious associations were found between study exposures and outcomes. In contrast, the small number of the study sample could have also contributed to non-significant findings. This is especially relevant in the second and third chapters where there was insufficient power partly due to the low prevalence of the control group (i.e. folic acid-containing supplement nonusers) or low prevalence of exposure (i.e. cigarette use). Nevertheless, it is important to consider these study findings in the context of the overall literature in these topics. Although the studies were underpowered, the magnitude and the direction of results were in agreement with other larger studies, which should strengthen the validity of the dissertation findings.

In summary, the hypotheses of the study were examined in the MotherToBaby cohort. Unique characteristics of this population may limit external validity and other limitations may have biased study results. However, inherent strengths in a prospective design, as well as other strengths of the study, such as adjusting for important confounders, support the soundness of the findings. In chapter 1, results suggested an 83% increased risk for GH in women who continued to take antidepressants after 20 weeks of gestation. Almost a five-fold increase in GH risk was observed in women who used serotonin-norepinephrine reuptake inhibitors. Antidepressant use also seemed to increase risk for PE, which is consistent with previous studies;[19,20,25] however, this was not a significant association due in part to insufficient power. Low power could have also explained for the non-significant associations found between the use of folic-acid

containing supplements and GH and PE in chapter 2; although, the magnitude of effect and the direction of the association, in relation to PE risk, was similar to other reports.[21,22,27] In chapter 3, a significant risk reduction for GH was found among pregnant smokers, which was in line with what has been found in previous studies.[23,24] This association was strongest in smokers who began using folic acid-containing supplements after recognition of pregnancy. An inverse association was also found with cigarette use and PE risk, similar to other studies;[23,24] but, this was not significant. This may be due to a number of factors including, underreporting of cigarette use and low power. Future research on the mechanisms by which the exposures examined in this dissertation contribute to the pathogenesis of GH and PE could be informative in planning for prevention strategies for women at risk for these conditions.

# CHAPTER 1

## Risk for Gestational Hypertension and Preeclampsia in Women Who Discontinued or Continued Antidepressant Medication Use during Pregnancy

ABSTRACT

Purpose: To examine the association between discontinued and continued use of antidepressants and risk for gestational hypertension (GH) and preeclampsia (PE).

Methods: Data from the MotherToBaby cohort studies from 2004-2014 were analyzed to compare women who discontinued antidepressant use <20 weeks gestation (discontinuers) and women who continued antidepressant use ≥20 weeks gestation (continuers) to nonusers for risk of GH (blood pressure ≥140/90 mmHg on two or more occasions at ≥20 weeks of gestation) and PE (GH with proteinuria). Maternal data, including exposures and study outcomes, were collected through multiple phone interviews. Medical records were used to validate outcomes. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using polytomous logistic regression. Risk for GH and PE were also assessed within antidepressant drug classes.

Results: 3,471 women were eligible for analysis. Unadjusted OR for GH showed significant (OR: 2.03; 95% CI: 1.19, 3.44) risk for continuers and non-significant association for discontinuers compared to nonusers. Continuers were still significantly at risk for GH (aOR: 1.83; 95% CI: 1.05, 3.21) after adjustment. No significant associations with PE were found for discontinuers or continuers. Additional analyses by drug class showed that continued use of serotonin-norepinephrine reuptake inhibitors (SNRI) increased risk for GH (aOR: 4.96; 95% CI: 1.33, 18.56), but not PE. Selective serotonin reuptake inhibitors or other antidepressants use were not associated with increased risks for GH or PE.

Conclusions: Results suggest continuers are at risk for GH, particularly SNRIs users. No significant association was found among discontinuers. Antidepressant users were not at significant risk for PE.

# INTRODUCTION

Depression occurs in 12-25% of pregnant women in the US.[28,29] An estimated 3-13% of pregnant women suffering from depression use medication to manage their condition; the most common type of medication being selective serotonin reuptake inhibitors (SSRIs).[30,31] Previous studies suggest an increased risk for gestational hypertension and preeclampsia for antidepressants users.[3,19,20,25,32] However, there is not a clear consensus as other studies suggest no association.[3,32] A more in-depth understanding of antidepressant use as a risk factor for hypertension is essential as both GH and PE are major contributors to maternal morbidity and mortality in the US and are associated with adverse infant health outcomes, such as intrauterine growth retardation, low birth weight, and preterm birth.[5] The mechanisms responsible for the pathogenesis of hypertensive disorders of pregnancy remain unclear. Maladaptation of immune responses, abnormal placental development, inadequate trophoblast invasion, and oxidative stress are some key factors that have been identified.[33,34] Risk factors, including genetic, behavioral, and environmental, have shown to trigger development of these conditions.[17,35,36]

In previous studies, the class of antidepressant medication used exacerbated the risk for GH and PE.[3,19,20,32] The use of SSRIs, serotonin-norepinephrine reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs) have been shown to contribute to the risk for PE.[3,19,20] and the use of SSRIs has been shown to be a contributor to the development of GH.[37] Timing of antidepressant use has also shown to be a significant risk factor for PE, but not for GH.[3] A study of 5,731 women who participated in the Slone

Epidemiology Center Birth Defects Study in 1998-2007 showed that those who continued to use SSRIs past the first trimester had 4.86 (95% CI: 2.70, 8.76) greater risk for PE compared to nonusers.[3] Another study that included 69,448 pregnancies of women with depression, identified from healthcare utilization databases in British Columbia, showed a similar elevated risk for PE with continued use of antidepressants.[19]

Many of the previous studies that examined antidepressant use and hypertensive disorders of pregnancy used maternal reports only or data from healthcare databases, which only provide information on dispensed medications and may not accurately capture actual drug use or the exact timing of medication use. Although our study also relied on maternal reporting, data on exposures were collected prospectively multiple times during pregnancy. Maternal reports on study outcomes were also later validated using the participants' medical records. Furthermore, this study was able to provide a closer examination of potential confounders, such as change in paternity and presence of autoimmune diseases and how they impact the relation between antidepressant exposure and GH and PE.

The purpose of this study was to examine the extent to which use and timing of antidepressants in pregnancy increased the risk for GH and PE while adjusting for potential confounding factors. A secondary aim of the study was to examine specific antidepressant drug classes in relation to risk of GH and PE.

# METHODS

## Data Sources

Participants in this study were enrolled in the MotherToBaby (MTB) US and Canada and MTB California cohort studies which have been ongoing since 1998. MTB US and Canada included pregnancy studies on asthma, autoimmune diseases, and vaccines. The MTB network, a program of the Organization of Teratology Information Specialists (OTIS), is a toll-free telephone counseling service that provides information and risk assessment for pregnant women regarding exposures in pregnancy. There are 13 MTB sites in North America that provide assistance to approximately 80,000 pregnant women and healthcare providers each year. Pregnant women who called the MTB phone line were screened for eligibility for the MTB pregnancy outcome studies. Eligible women were referred to the MTB research center at the University of California, San Diego. Women were found eligible for the studies if they resided in the US or Canada, were no more than 20 weeks in gestation at the time of enrollment, and had no prior diagnosis of any major birth defects in the current pregnancy at the time of enrollment. Informed consent was obtained from all women who participated in these studies.

After enrollment, women completed a comprehensive intake interview and information on the following were collected: demographics, partner information, pregnancy history, last menstrual period, prenatal tests, assistive reproductive methods, previous pregnancy outcomes and complications, family health history, mother's medical history, use of prescription and over-the-counter medications, recreational drugs, dietary supplements, herbal products, and alcohol and tobacco use. Follow-up interviews were

conducted approximately every three months until the end of the pregnancy. Information on exposures collected at intake was updated at each successive interview, and information on new exposures, prenatal tests, and any new pregnancy complications was obtained. An outcome interview was conducted at the end of the pregnancy. At this time, women were asked about pregnancy complications since the last interview and pregnancy outcomes. Postnatal follow-up of live born infants continued for a minimum of one year after birth. Additional data, such as birth outcomes, were also collected from medical records obtained from the obstetrician, birth hospital, and the child's pediatrician. These medical records were also used to validate maternal reports of exposures, outcomes, ultrasounds, prenatal test, maternal medical history, pregnancy complications, delivery, birth outcomes, and newborn complications.

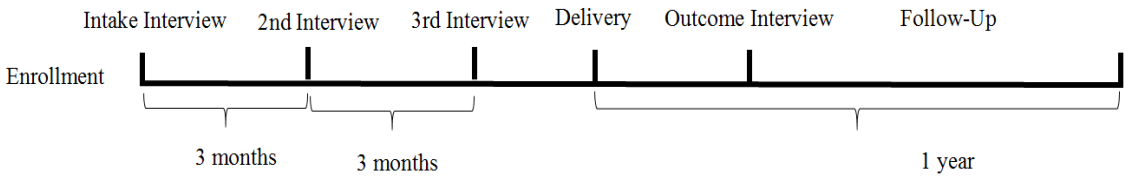



Figure 1.1: MTB cohort study timeline

Study Population

Participants included in this study were enrolled in the MTB US and Canada or MTB California cohort studies from January 1, 2004 to June 30, 2014 and had completed their outcome interview no later than June 30, 2014. To be included in this analysis, women must have had a live singleton birth, had no pre-existing chronic hypertension,

have completed the study requirements, and had available data on study outcomes and the exposure of interest.

## Exposure Definition

The main exposure of interest, antidepressant use and timing of use during gestation, was collected at multiple time points beginning at intake and every three months until delivery and once after birth of the child, reflecting the last weeks of pregnancy (see Figure 1.1). Participants in this study who did not use antidepressants at any time during pregnancy were classified as nonusers. Participants who reported use of antidepressants were classified as discontinuers (women who discontinued use <20 weeks of gestation) or continuers (women who continued use ≥20 weeks of gestation). Few women began using antidepressant medication after conception (N=59). These participants were classified as either continuers or discontinuers in analysis, depending on when and if they discontinued use of antidepressants based on definitions stated above. Use of antidepressants by drug class was classified as selective serotonin reuptake inhibitors (SSRIs) alone, serotonin and norepinephrine reuptake inhibitors (SNRIs) alone, and "other" antidepressants alone. Other antidepressants included serotonin antagonist reuptake inhibitors, monoamine oxidase inhibitors, tricyclic antidepressants and tetracyclic antidepressants. Women who used two of more combined antidepressants were excluded from the subanalysis as we would not be able to determine individual effects of the use of multiple medications. However, these women were included in the full multivariate analysis which examined the overall impact of timing of medication use.

## Outcomes Definitions

Outcomes were self-reported by the participants at any interview after 20 weeks of gestation. Women who had both GH and PE were classified for analysis in the PE group. GH was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two or more occasions after 20 weeks of gestation without the presence of proteinuria, and PE was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two or more consecutive readings 4 or more hours apart with proteinuria of 0.3 g or greater after 20 weeks of gestation.

## Data Validation

### *Cohort Study Data*

Regular data quality assurance procedures were performed by MTB study staff. These procedures included medical record abstractions to validate maternal reports of exposures, study outcomes, pregnancy outcomes, and prenatal exams. Quality assurance checks also extended to data entered in the MTB databases. A majority of all women in the MTB cohort studies had available medical records. For a smaller proportion of women (19%), medical records either could not be obtained or had not yet been received by the cutoff date for this study and therefore interview records were the only source of data.

### *Outcomes Data*

A secondary validation of medical and interview records for 644 women was completed for this study to verify data on the outcomes. Women in this subsample

included all those who reported having GH or PE (N=316) and a small group who did not report having GH or PE (N=328). Because of changes in data collection over time, maternal reports of pre-existing hypertension recorded in the databases were also validated for this study. Medical records for all women who reported having pre-existing hypertension (N=203) were reviewed to confirm their hypertension diagnosis prior to 20 weeks of gestation; this included women in the subsample who reported developing GH or PE and women who did not report developing the outcomes (see Figure 1.2 for further details). Chronic hypertension was defined as maternal report of unresolved pre-existing hypertension within a year of enrollment in the study, any antihypertensive medication use before 20 weeks of gestation, or blood pressure ≥140/90 mm Hg on two or more occasions before 20 weeks of gestation. Women found to have pre-existing chronic hypertension were excluded from the final study population (N=124).

To validate the outcomes and examine the predictive values, mother's medical and interview records were compared against the data entered in the MTB databases. For 80% of the women sampled, available medical and interview records were examined. For the rest of the subsample, no medical records were available, and therefore interview records were the only source of data reviewed (N=132, including 39 with PE, 28 with GH, and 65 non-cases). Women whose medical and interview records did not indicate GH or PE were classified as non-cases for analysis. Women whose medical records supported diagnosis of GH or PE were classified as cases. Self-reports of GH or PE were classified as cases if no medical records were available.

Instances where data in interview records did not agree with data from the medical records were evaluated on a case-by-case basis. There were 27 instances where medical records did not match data from the interview records, including 10 where the mother reported an outcome that was not supported by medical records and 17 where medical records indicated a diagnosis of GH or PE that was not reported by the mother. In the end, the positive predictive values were 83.8% (95% CI: 78.8%, 88.0%) and 98.2% (95% CI: 94.8%, 99.6%) for GH and PE, respectively. The lower positive predictive value for GH was due to the fact that many women who were originally classified in the database as having GH were excluded after a review of their medical records showed evidence that these women had pre-existing chronic hypertension. The negative predictive values were 96.8% (95% CI: 94.5%, 98.3%) for GH and 98.8% (95% CI: 97.3%, 99.5%) for PE.

## Covariates

The following potential confounders were assessed in this study: maternal age and pre-pregnancy body mass index (BMI) (all continuous); education, race/ethnicity, parity, previous spontaneous abortion or stillbirth, gravidity, current asthma status, diabetes status, autoimmune disease status, alcohol use during pregnancy, cigarettes smoked per day at conception, folic acid and prenatal vitamin use, caffeine use, partner change since the last pregnancy, fetal sex, and aspirin and caffeine use at any time during pregnancy (all categorical).

## Statistical Analysis

Analyses were conducted using SAS v.9.3 (SAS Institute Inc, Cary, NC). Descriptive statistics were examined using the mean and the standard deviation for continuous variables, and frequency and percent for categorical variables. Comparisons of the population characteristics were analyzed using ANOVA for continuous variables and chi-square test for categorical variables ($p < 0.05$). Polytomous logistic regression was used to estimate the unadjusted and adjusted odds ratios (OR, aOR) of the associations between timing of antidepressant use and the outcomes, and their 95% CIs. Model 1 examined the unadjusted association between the main exposure of interest and the outcomes. Model 2 adjusted for confounders identified in the study. A confounder was included in the adjusted model if the magnitude of the point estimates changed by ≥10% when that variable was added to the model. In addition to covariates included in Model 2, Model 3 adjusted for significant risk factors for hypertensive disorders of pregnancy that were identified from the literature, including race/ethnicity, parity, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, and gravidity.[38–44] Model 4 adjusted for all covariates included in Models 2 and 3, enrollment year and cohort study (MTB US and Canada vs. MTB California). Subanalyses by drug class were also examined for risk of GH and PE using the same approach. Subjects with missing values for the selected covariates were excluded from the models.

This study was approved by the Human Research Protections Program of the University of California, San Diego.

# RESULTS

There were 3,471 women included in the final sample. Of this sample, 12.6% (N=436) were obtained from the MTB California cohort study, and 87.4% (N=3,035) were obtained from the MTB US and Canada cohort studies. Of those included in the MTB US and Canada cohort studies, 11.8% were drawn from those enrolled in MTB asthma studies (N=410), 43.0% were drawn from MTB autoimmune studies (N=1,492), and 57.1% were drawn from MTB vaccine studies (N=1,983). In the overall final sample, 2,813 women had medical records (81%) and 658 (19%) did not have available medical records.

Among the 3,471 women who met the inclusion criteria for the study, 129 developed GH without PE and 141 developed PE. Table 1.1 shows the characteristics of the participants by exposure category. Women in the overall sample were predominately White, non-Hispanic who completed at least some college. Folic acid or prenatal vitamin use was high in this cohort, with most initiating use before pregnancy. This may reflect the fact that many women included in the cohort were referred to the MTB network by their physician and received prenatal care during pregnancy. Antidepressant discontinuers and continuers were older, had higher pre-pregnancy BMI, had higher proportion with asthma, had lower proportions who were primigravid, and had higher proportion who reported cigarette use during pregnancy compared to nonusers. In addition, discontinuers had higher proportions with diabetes type 1 and 2 compared to nonusers and continuers. Table 1.2 shows that the majority of both discontinuers (55.9%; N=57) and continuers (62.8%; N=157) used SSRIs with depression as the most common indication for

medication use. As expected, the mean duration of antidepressant use was significantly higher among continuers compared to discontinuers.

Table 1.3 shows the unadjusted and adjusted models examining the association between the continuation/discontinuation of antidepressant use and the outcomes. In Model 1, continuers had significantly elevated risks for GH (OR: 2.03; 95% CI: 1.19, 3.44) but not PE (OR: 1.16; 95% CI: 0.62, 2.19) compared to nonusers. Discontinuers had non-significantly lower risk for GH (OR: 0.85; 95% CI: 0.26, 2.71) and non-significantly higher risk for PE (OR: 1.75; 95% CI: 0.79, 3.84) compared to nonusers. After examining each variable listed in Table 1.1, diabetes status and pre-pregnancy BMI were found to confound the association between continuation/discontinuation of antidepressant use and the outcomes (data not shown). After adjusting for these variables in Model 2, risk was significantly elevated for GH (aOR: 1.94; 95% CI: 1.14, 3.31) and was also elevated, but non-significant, for PE (aOR: 1.16; 95% CI: 0.61, 2.19). After adjusting for all covariates in Model 4, the risk for GH continued to be significantly elevated (aOR: 1.83; 95% CI: 1.05, 3.21).

Analyses examining the effects of specific antidepressant drug classes, accounting for continuation/discontinuation of medication use, on the development of GH and PE are shown in Table 1.4. Only women who were taking SNRIs, SSRIs, or other antidepressant drug class alone were included. Women who took more than one type of antidepressants drug class were excluded (N=49). In comparison with nonusers, SSRI continuers were 2.05 (95% CI: 1.08, 3.90) more likely to develop GH, but not PE in unadjusted analyses. After adjusting for all confounders in Model 4, the odds of developing GH were reduced

and no longer significant. For SNRI continuers, the unadjusted odds of developing GH were 4.96 (95% CI: 1.43, 17.29) compared to nonusers. The odds remained significantly elevated (Model 4 aOR: 4.96; 95% CI: 1.33, 18.56) after adjusting for race/ethnicity, parity, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, pre-pregnancy BMI, enrollment year, and cohort study. SNRI discontinuers had elevated risk for GH before and after adjustment; however, these results were not significant. Women who took other antidepressant drug classes alone were not at significant risk for GH. Women who discontinued other antidepressants were also not at significant risk for GH or PE after adjustment for all covariates. PE risk for women who continued use of other antidepressant drug classes could not be determined because there were no cases of PE in this group.

Further analyses were completed to assess how other factors impact the risk for the outcomes. Data for the following analyses are not shown. The total length of time antidepressants were used in gestational weeks was examined to determine the effects of duration of use. No significant association was found between duration of use and PE before or after adjustment. In contrast, women who used antidepressants for at least 20 weeks in duration were significantly at risk for GH. Additionally, there were 59 women who began using antidepressant after becoming pregnant, and 24 of these women began using medication only after 20 weeks of gestation. This subsample of late users was analyzed to examine if their risk for GH and PE differed from those who began before 20 weeks and continued medication use after 20 weeks of gestation. Risk for GH and PE were raised before and after adjustment, but were not significant.

# DISCUSSION

Findings from this study suggest that continued antidepressant use ≥20 weeks of gestation may increase a woman's risk for GH. This is similar to previous studies that showed an elevated risk for GH.[3,32,37] In two of these studies, timing of antidepressant use was examined.[3,37] In Toh et al., the unadjusted estimate showed a significant relationship between continued medication use and GH.[3] However, adjusted analysis controlling for mother's medical history, demographics, medication use, and substance use yielded non-significant results (OR: 1.88; 95% CI: 1.00, 3.53).[3] Unlike the Toh et al. study which collected maternal information six months after the birth of the child, our study collected information on medication use at multiple time points during pregnancy and once after delivery which should have minimized information bias.

Additional analysis in our study showed that the association between antidepressant use and GH was stronger in women who continued to use SNRIs alone. Upon closer examination of the association between SNRI use and GH, it should be noted that only 4 antidepressant users developed the outcome. Because of the low number of outcome events, it is possible that the study found spurious associations between continued use of SNRIs and GH. These findings differ somewhat from previous studies. A case-control study of the Quebec Pregnancy Registry did not find significant associations between SNRI use and pregnancy-induced hypertension.[37] However, study authors also pointed out that the non-significant findings may be due to statistical power issues in the study. Antidepressants, SNRIs in particular, have been thought to contribute to high blood pressure due to resulting increased serotonin and norepinephrine levels in

the body from medication use.[45–47] It is possible that increased activity of these key neurotransmitters may have contributed to development of GH for women in this study.

No significant risk for PE was observed in women who continued use of antidepressants in this study, which differs from previous studies that showed significant increased risks for PE.[3,19,20,25] Similar to our study, Thombre et al. also did not find a significant association between antidepressant use and PE; however, timing of medication use was not considered in analysis.[32] There was also no significant risk of PE when duration of use and antidepressant use only after 20 weeks of gestation were examined in our study. Although not significant, findings from the analysis of late users (those who used antidepressants only after 20 weeks of gestation) showed greater magnitude of risk for PE, which may suggest that timing of medication use may have a larger impact on the development of PE. Low power for detecting significant associations for PE may account for our non-significant results.

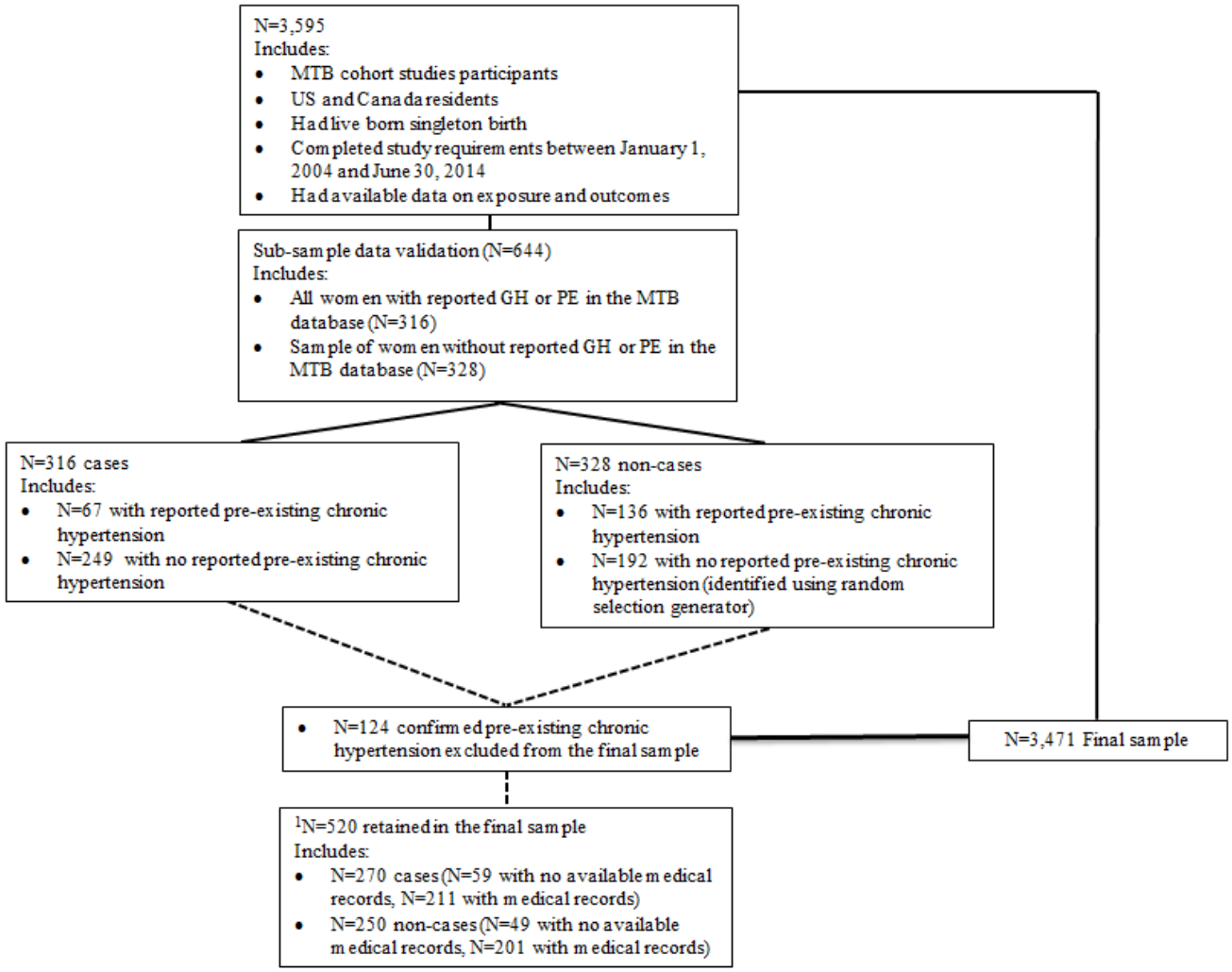
Another limitation in this study was that we were unable to adjust for depression or other underlying mood disorders in the models. Since current depression status or other mood disorders were not consistently collected in the MTB cohort studies, it was difficult to systematically identify women with these disorders in the reference group, and therefore, we were unable to fully examine the influence of mood disorders in the findings of this study. Available data on antidepressant drug indication showed that a majority of antidepressant users were prescribed medication for depression, yet our analysis results indicated varying effects by different drug classes, which may suggest that depression is not the only factor driving the increased risk for hypertensive disorders

of pregnancy. However, it is also possible that these variations in findings could be associated with depression severity. Depression has been shown to have an impact on the development of PE.[39–41] Avalos et al. found that depressed women who used antidepressants in the second trimester of pregnancy were at 70% greater risk for developing PE in comparison to women who did not use antidepressants and did not have depression.[25] Previous studies by Palmsten et al. have also supported increased risk for PE with use of specific antidepressant drug classes, such as SSRI, SNRI and TCA for only women with depression.[19,20] It should be noted that unlike these two previous studies which used information from hospital and pharmacy databases, our study used multiple interviews with women to collect information on medication use throughout pregnancy, which may have enhanced information regarding drug use. Another possibility for our divergent results is the misclassification of the outcomes, which were based on self-reports. For a small fraction of women included in the study, no medical records were available to validate the reports of the outcomes. However, for most women in the study, medical records were used to confirm development of GH or PE in pregnancy. In addition, consistent interview methods throughout pregnancy and after birth to collect information on study outcomes should have minimized underreporting in this population.

Results of this study showed that women who continued antidepressant use in pregnancy were at greater risk for developing GH. Although not significant, analysis also suggested an increased risk for developing PE with continued antidepressant use. We recommend that women considering use of antidepressants in pregnancy consult with their doctors to weigh the risks and benefits of continuing with their medication. Further

studies should examine how potential confounders, such as depression and other underlying mood disorders and their severity, impact the risk for GH and PE.

Chapter 1, in full, has been submitted for publication of the material as it may appear in the Archives of Women's Mental Health, De Ocampo, Maria P.G.; Araneta Maria Rosario G.; Macera, Caroline A.; Alcaraz, John E.; Moore, Thomas R.; Chambers, Christina D., Springer. The dissertation author was the primary investigator and author of this paper.




1. There were 27 instances in which mother's self-report of the outcomes did not agree with information in medical records.

Figure 1.2: Flowchart of data validation of study outcomes

Table 1.1: MotherToBaby cohort study participant characteristics by continuation/discontinuation of antidepressant use (N=3,471)

| **Characteristics** | **Nonusers** (N=3,119) N (%) | **Discontinuers** (N=102) N (%) | **Continuers** (N=250) N (%) | **P-value** |
|---|---|---|---|---|
| Outcomes | | | | |
| None | 2,887 (92.6) | 92 (90.2) | 222 (88.8) | <0.01 |
| Gestational Hypertension | 109 (3.5) | 3 (2.9) | 17 (6.8) | |
| Preeclampsia | 123 (3.9) | 7 (6.9) | 11 (4.4) | |
| Education | | | | |
| ≤HS grad | 360 (11.5) | 18 (17.6) | 14 (5.6) | <0.01 |
| Some college | 604 (19.4) | 26 (25.5) | 62 (24.8) | |
| College grad | 1,179 (37.8) | 32 (31.4) | 99 (39.6) | |
| Post grad | 974 (31.2) | 26 (25.5) | 73 (29.2) | |
| Missing | 2 (0.1) | 0 (0.0) | 2 (0.8) | |
| Race/Ethnicity | | | | |
| Caucasian | 2,336 (74.9) | 74 (71.2) | 217 (86.8) | <0.01 |
| Black | 118 (3.8) | 7 (6.7) | 3 (1.2) | |
| Hispanic | 396 (12.7) | 17 (16.3) | 19 (7.6) | |
| Asian/Pacific Islander | 181 (5.8) | 4 (3.8) | 5 (2.0) | |
| Native American/AK Native | 21 (0.7) | 0 (0.0) | 2 (0.8) | |
| Other | 64 (2.1) | 2 (1.9) | 4 (1.6) | |
| Missing | 1 (0.0) | 0 (0.0) | 0 (0.0) | |
| Parity | | | | |
| Nulliparous | 1,640 (52.6) | 46 (45.1) | 118 (47.2) | 0.07 |
| Primiparous | 969 (31.1) | 31 (30.4) | 80 (32.0) | |
| Multiparous | 509 (16.3) | 25 (24.5) | 52 (20.8) | |
| Missing | 1 (0.0) | 0 (0.0) | 0 (0.0) | |
| Gravidity | | | | |
| 1 | 1,214 (38.9) | 34 (33.3) | 82 (32.8) | <0.01 |
| 2 | 967 (31.0) | 24 (23.5) | 70 (28.0) | |
| ≥3 | 938 (30.1) | 44 (43.1) | 98 (39.2) | |
| Previous spontaneous abortion or stillbirth[1] | | | | |
| Yes | 775 (40.7) | 28 (41.2) | 75 (44.6) | 0.61 |
| No | 1,130 (59.3) | 40 (58.8) | 93 (55.4) | |
| Asthma | | | | |
| Yes | 518 (16.6) | 25 (24.5) | 70 (28.0) | <0.01 |
| No | 2,601 (83.4) | 77 (75.5) | 180 (72.0) | |
| Diabetes (Type 1 and 2) | | | | |
| Yes | 29 (0.9) | 4 (3.9) | 2 (0.8) | <0.01 |
| No | 3,090 (99.1) | 98 (96.1) | 248 (99.2) | |
| Autoimmune diseases[2] | | | | |
| Yes | 1,257 (40.3) | 44 (43.1) | 91 (36.4) | 0.39 |
| No | 1,862 (59.7) | 58 (56.9) | 159 (63.6) | |
| Aspirin use | | | | |
| Yes | 199 (6.4) | 5 (4.9) | 23 (9.2) | 0.18 |
| No | 2,920 (93.6) | 97 (95.1) | 227 (90.8) | |
| Number of cigarettes per day at conception | | | | |
| 0 | 2,911 (93.3) | 89 (87.3) | 219 (87.6) | <0.01 |
| 1-9 | 120 (3.7) | 9 (8.8) | 20 (8.0) | |
| ≥10 | 88 (2.8) | 4 (3.9) | 11 (4.4) | |
| Alcohol use during pregnancy | | | | |
| No alcohol | 1,645 (52.7) | 53 (52.0) | 128 (51.2) | 0.97 |
| Occasionally | 1,378 (44.2) | 47 (46.1) | 115 (46.0) | |
| Once a week or more | 96 (3.1) | 2 (2.0) | 7 (2.8) | |

1. Excludes primigravid women
2. Autoimmune diseases include rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, psoriasis, Crohn's disease, multiple sclerosis, lupus, antiphospholipid syndrome, celiac disease, connective tissue disease, fibromyalgia, Sjorgen's syndrome, ulcerative colitis, inflammatory bowel disease, autoimmune liver disease, autoimmune thyroid dysfunction, and iritis

Table 1.1 (continued): MotherToBaby cohort study participant characteristics by continuation/discontinuation of antidepressant use (N=3,471)

| **Characteristics** | **Nonusers** (N=3,119) N (%) | **Discontinuers** (N=102) N (%) | **Continuers** (N=250) N (%) | **P-value** |
|---|---|---|---|---|
| Caffeine use during pregnancy | | | | |
| Yes | 2,615 (83.8) | 88 (86.3) | 215 (86.0) | 0.55 |
| No | 504 (16.2) | 14 (13.7) | 35 (14.0) | |
| Timing of folic acid/prenatal vitamin use | | | | |
| Before conception | 1,841 (59.0) | 45 (44.1) | 178 (71.2) | <0.01 |
| After conception or never | 1,278 (41.0) | 57 (55.9) | 72 (28.8) | |
| Partner change | | | | |
| Yes | 216 (6.9) | 10 (9.8) | 23 (9.2) | 0.18 |
| No | 2,858 (91.6) | 88 (86.3) | 216 (86.4) | |
| Missing | 45 (1.4) | 4 (3.9) | 11 (4.4) | |
| Fetal sex | | | | |
| Male | 1,581 (50.7) | 47 (46.1) | 140 (56.0) | 0.17 |
| Female | 1,534 (49.2) | 55 (53.9) | 110 (44.0) | |
| Missing | 4 (0.1) | 0 (0.0) | 0 (0.0) | |
| Cohort Study | | | | |
| MTB California | 325 (10.4) | 35 (34.3) | 76 (30.4) | <0.01 |
| MTB US and Canada | 2,794 (89.6) | 67 (65.7) | 174 (69.6) | |
| | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | **P-value** |
| Age (years)[3] | 32.0 (5.0) | 32.6 (5.4) | 33.1 (4.9) | <0.01 |
| Pre-pregnancy BMI[4] | 24.8 (5.7) | 25.8 (5.9) | 25.7 (5.7) | <0.01 |

3. Missing=3
4. Missing=25

Table 1.2: Use of antidepressants in the MotherToBaby cohort studies (N=352)

| **Characteristics** | **Discontinuers** (N=102) N (%) | **Continuers** (N=250) N (%) | **P-value** |
|---|---|---|---|
| Antidepressant class used | | | |
| SSRI alone | 57 (55.9) | 157 (62.8) | <0.01 |
| SNRI alone | 17 (16.7) | 21 (8.4) | |
| Other alone | 22 (21.6) | 29 (11.6) | |
| Combined use (≥2 antidepressant drug class) | 6 (5.9) | 43 (17.2) | |
| Indication | | | |
| Depression | 58 (56.9) | 188 (75.2) | <0.01 |
| Anxiety | 23 (22.5) | 46 (18.4) | |
| Other[1] | 12 (11.8) | 7 (2.8) | |
| Missing | 9 (8.8) | 9 (3.6) | |
| | Mean (SD) | Mean (SD) | |
| Duration of use (gestational weeks) | 8.2 (5.7) | 31.9 (11.3) | <0.01 |

1. "Other" indications included sleep aid, disease management, and pain management

Table 1.3: Risk association of gestational hypertension and preeclampsia and continuation/discontinuation of antidepressant medication among participants of the MotherToBaby cohort studies (2004-2014)

| Outcomes | N | N (%) with outcome | Model 1 OR[1] (95% CI) | Model 2 OR[2] (95% CI) | Model 3 OR[3] (95% CI) | Model 4 OR[4] (95% CI) |
|---|---|---|---|---|---|---|
| **Gestational Hypertension** | | | | | | |
| Nonuser | 3,119 | 109 (3.5) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Discontinuer | 102 | 3 (2.9) | 0.85 (0.26, 2.71) | 0.70 (0.21, 2.31) | 0.68 (0.21, 2.27) | 0.69 (0.21, 2.81) |
| Continuer | 250 | 17 (6.8) | 2.03 (1.19, 3.44) | 1.94 (1.14, 3.31) | 1.82 (1.05, 3.15) | 1.83 (1.05, 3.21) |
| **Preeclampsia** | | | | | | |
| Nonuser | 3,119 | 123 (3.9) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Discontinuer | 102 | 7 (6.9) | 1.75 (0.79, 3.84) | 1.60 (0.72, 3.31) | 1.67 (0.73, 3.84) | 1.69 (0.73, 3.91) |
| Continuer | 250 | 11 (4.4) | 1.16 (0.62, 2.19) | 1.16 (0.61, 2.19) | 1.28 (0.67, 2.45) | 1.30 (0.67, 2.51) |

1. Model 1: unadjusted
2. Model 2: adjusted for diabetes status and pre-pregnancy BMI
3. Model 3: adjusted for race/ethnicity, parity, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, pre-pregnancy BMI, and gravidity
4. Model 4: adjusted for race/ethnicity, parity, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, pre-pregnancy BMI, gravidity, cohort study and enrollment year

Table 1.4: Association of gestational hypertension and preeclampsia and continuation/discontinuation of use of antidepressant by drug class among participants of the MotherToBaby cohort studies (2004-2014)

| GESTATIONAL HYPERTENSION | | | | | | |
|---|---|---|---|---|---|---|
| **Exposure** | **N** | **N (%) with outcome** | **Model 1 OR[1] (95% CI)** | **Model 2 OR[2] (95% CI)** | **Model 3 OR[3] (95% CI)** | **Model 4 OR[4] (95% CI)** |
| SSRI alone | | | | | | |
| Nonuser | 3,119 | 109 (3.5) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Discontinuer | 57 | 1 (1.8) | 0.48 (0.07, 3.51) | 0.41 (0.05, 3.14) | 0.36 (0.05, 2.83) | 0.36 (0.05, 2.84) |
| Continuer | 157 | 11 (7.0) | 2.05 (1.08, 3.90) | 1.96 (1.02, 3.76) | 1.83 (0.94, 3.57) | 1.88 (0.96, 3.71) |
| SNRI alone | | | | | | |
| Nonuser | 3,119 | 109 (3.5) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Discontinuer | 17 | 1 (5.9) | 1.77 (0.23, 13.48) | 1.48 (0.19, 11.52) | 1.66 (0.20, 13.45) | 1.68 (0.21, 13.68) |
| Continuer | 21 | 3 (14.3) | 4.96 (1.43, 17.29) | 4.87 (1.38, 17.21) | 4.77 (1.28, 17.72) | 4.96 (1.33, 18.56) |
| Other alone | | | | | | |
| Nonuser | 3,119 | 109 (3.5) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Discontinuer | 22 | 1 (4.5) | 1.47 (0.20, 11.12) | 1.10 (0.14, 8.76) | 1.10 (0.14, 8.86) | 1.10 (0.14, 8.84) |
| Continuer | 29 | 2 (6.9) | 1.96 (0.46, 8.35) | 1.92 (0.45, 8.31) | 1.92 (0.44, 8.43) | 1.86 (0.42, 8.19) |
| **PREECLAMPSIA** | | | | | | |
| **Exposure** | **N** | **N (%) with outcome** | **Model 1 OR[1] (95% CI)** | **Model 2 OR[2] (95% CI)** | **Model 3 OR[3] (95% CI)** | **Model 4 OR[4] (95% CI)** |
| SSRI alone | | | | | | |
| Nonuser | 3,119 | 123 (3.9) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Discontinuer | 57 | 3 (5.3) | 1.28 (0.40, 4.15 | 1.23 (0.37, 4.10) | 1.16 (0.34, 3.94) | 1.13 (0.33, 3.86) |
| Continuer | 157 | 4 (2.5) | 0.67 (0.24, 1.81) | 0.66 (0.24, 1.81) | 0.70 (0.25, 1.96) | 0.67 (0.24, 1.90) |
| SNRI alone | | | | | | |
| Nonuser | 3,119 | 123 (3.9) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Discontinuer | 17 | 1 (5.9) | 1.56 (0.21, 11.93) | 1.40 (0.18, 10.81) | 1.80 (0.22, 14.75) | 1.73 (0.21, 14.27) |
| Continuer | 21 | 2 (9.5) | 2.93 (0.67, 12.89) | 2.95 (0.67, 13.06) | 3.64 (0.80, 16.70) | 3.49 (0.76, 16.09) |
| Other alone | | | | | | |
| Nonuser | 3,119 | 123 (3.9) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Discontinuer | 22 | 3 (13.6) | 3.91 (1.14, 13.45) | 3.40 (0.94, 12.30) | 4.01 (1.01, 15.84) | 3.92 (0.99, 15.63) |
| Continuer | 29 | 0 (0.0) | Cannot estimate | Cannot estimate | Cannot estimate | Cannot estimate |

1. Model 1: unadjusted
2. Model 2: adjusted for diabetes status and pre-pregnancy BMI
3. Model 3: adjusted for race/ethnicity, parity, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, pre-pregnancy BMI, and gravidity
4. Model 4: adjusted for race/ethnicity, parity, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, pre-pregnancy BMI, gravidity, cohort study and enrollment year

# CHAPTER 2

## Risk for Gestational Hypertension and Preeclampsia and Timing of Folic Acid Supplement Use

ABSTRACT

Objective: To assess the effects of early and later use of folic acid-containing supplements on the risk for gestational hypertension (GH) and preeclampsia (PE).

Methods: Exposures and outcomes data were obtained through interviews and review of participant's medical records from the MotherToBaby (MTB) cohort studies across the United States and Canada. Folic acid-containing supplement use was classified as early users, late users and nonusers. GH and PE outcomes were self-reported and validated with medical records. Demographics, medical history, lifestyle factors, substance use, and fetal sex were assessed as potential confounders. Unadjusted and adjusted risks for GH and PE were examined using odds ratios and 95% confidence intervals.

Results: 3,474 women were included in the study. There were 2,901 early users, 488 late users and 85 nonusers of folic acid or folic acid-containing multivitamins in the study. Compared to nonusers, the risk of GH were 1.04 (95% CI: 0.32, 3.34) for early users and 0.92 (95% CI: 0.26, 3.22) for late users. After adjustments for all confounders the odds remained non-significant. In examining risk for PE, early (OR: 0.50; 95% CI: 0.21, 1.17) and late users (OR: 0.83; 95% CI: 0.33, 2.06) had non-significantly lower odds compared to nonusers. Similar non-significant results were observed after adjustment in the final models.

Conclusion: Findings from this study suggest that folic acid-containing supplements do not significantly mitigate the risk for developing GH or PE. The timing of the initiation of use also did not affect risk for GH or PE.

## INTRODUCTION

The benefits of taking folic acid supplements during pregnancy to prevent birth defects, such as neural tube defects and cleft palate, are well documented.[48,49] Some evidence have indicated that folic acid-containing supplements may also lower the risk for hypertensive disorders of pregnancy.[22,50–53,27,54–56] Hypertensive disorders of pregnancy, such as preeclampsia and gestational hypertension, affect as many as 81.4 per 1,000 deliveries in the US, and are common causes of maternal morbidity and mortality.[57,58]

It has been suggested that folic acid and folic acid-containing multivitamins may reduce the risk of GH and PE by lowering plasma homocysteine concentrations in pregnant women.[59,60] Hyperhomocysteinemia induces maternal endothelial dysfunction leading to the development of hypertensive disorders.[61] Several studies that have examined the association between the use of folic acid and risk for GH and PE observed significantly lowered risk for women who took folic acid supplements or had high dietary folate intake in pregnancy.[22,50–53,27,54–56] However, some of these studies only used maternal self-reports to confirm the outcomes.[53,54] In addition, many were conducted outside the US making generalizations to the US population problematic due to possible differences in dietary folate intake and variations in food fortification policies.[22,50,52,54–56] Yet, other studies have also indicated that folate alone is ineffective in preventing PE.[50] Previous studies that have used larger samples of women to examine the effects of folic acid and folate on hypertensive disorders did not show significant risk reduction.[62–65] Furthermore, studies of dietary folate intake conducted after mandated food fortification

in 1998 in the US did not indicate added benefit in preventing hypertensive disorders in pregnancy.[62,63]

To our knowledge, only four studies have examined supplement use at the periconception and postconception time periods with respect to risk for hypertensive disorders of pregnancy.[22,50,51,27] The effect of when women initiated supplement use on risk for disease development varied in these studies. One study did not show significant difference between beginning folic acid-containing multivitamins before or after conception.[27] Other studies showed significantly reduced risk with supplement use at the periconceptional period, with one indicating significant findings only in women with body mass index (BMI) less than 25kg/m$^2$.[22,50,51] These studies mainly focused on the effects of supplement use on PE, and not GH. Given the inconsistent findings in the literature, our study sought to examine the effects of using folic acid and folic acid-containing supplements on the development of GH and PE. Our study expanded on previous work by examining whether there were varying effects in timing of supplement use on the risk for GH and PE.

## METHODS

### Data Sources and Study Population

All participants in this study were recruited from the MotherToBaby (MTB) network, a program of the Organization of Teratology Information Specialists (OTIS) which has 13 sites across North America. MTB US and Canada pregnancy studies include ongoing research on asthma, autoimmune diseases, and vaccines. Approximately

80,000 pregnant women and healthcare providers call the OTIS toll-free number for free counseling and risk assessment for various exposures during pregnancy each year. Pregnant women who called were simultaneously screened for eligibility in MTB US and Canada and MTB California cohort studies. Women were eligible for the studies if they were US or Canadian residents, were no more than 20 weeks in gestation, and had no prior diagnosis of any major birth defects in their current pregnancy.

Women completed a comprehensive intake interview that included data collection on demographics, previous pregnancy history and outcomes, last menstrual period, prenatal tests, lifestyle factors, family health history, mother's medical history, and other pregnancy exposures, including supplement use. Follow-up interviews were conducted approximately every three months until the end of the pregnancy, and information on exposures was updated including changes in substance use, medication use and supplement use. Information on new prenatal tests and pregnancy complications were also collected. At the end of the pregnancy, an outcome interview was completed, and information on pregnancy complications and outcomes were collected. In addition to interviews with the mothers, information from medical records was also obtained from the obstetrician, birth hospital and child's pediatrician. These medical records were used to validate maternal reports of study outcomes, medical history, ultrasounds, pregnancy complications, prenatal tests, delivery outcomes, and newborn complications.

Women were included in this study if they enrolled and completed an outcomes interview for the MTB US and Canada or MTB California cohort studies from January 1,

2004 to June 30, 2014, had a live singleton birth, had no pre-existing chronic hypertension, and had available data on study outcomes and the exposure of interest.

This study was approved by the Human Research Protections Program of the University of California, San Diego.

## Exposures

Information on folic acid and folic acid-containing supplements were obtained at intake, follow-up, and outcome interviews. Data included supplements used, start and stop times, and dosage. For this study, the exposure was categorized into 3 three levels: early users (women who reported starting use of supplements prior to or up to 4 weeks after their last menstrual period), late users (women who reported starting use of supplements only after 4 weeks after their last menstrual period), and nonusers (women who did not report using supplements at any time during pregnancy). These cutoffs were determined based on critical time windows for implantation and placentation linked to the pathogenesis of hypertensive disorders of pregnancy.[61]

## Outcomes

In this study, the outcomes were self-reported by participants at any interview after 20 weeks of gestation. Reported outcomes were validated using medical records from the obstetrician or delivery hospital. GH was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two or more occasions after 20 weeks of gestation without the presence of proteinuria, and PE was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two or more

consecutive readings 4 or more hours apart with proteinuria of 0.3 g or more after 20 weeks of gestation. Women who had both GH and PE were classified in the PE group in analysis.

## Data Validation

### *Cohort Study Data*

Regular data quality assurance procedures were performed by MTB study staff. These procedures included medical record abstractions to validate maternal reports of exposures, study outcomes, pregnancy outcomes, and prenatal exams. Quality assurance checks also extended to data entered in the MTB databases. For most women in the MTB cohort studies, medical records were available and validated by the study staff. Medical records for 19% of the study population were not available at the time of the study, and therefore maternal self-reports were the only source of data for the outcomes. A comparison of the characteristics of women with medical records compared with women without medical records showed that women in the former group were older, were more likely to be White, nonsmoker, nulliparous and primigravid, and were more likely to have an autoimmune disease and to have given birth to a boy. Women without medical records were more likely to be diabetic and to have had a partner change, had higher pre-pregnancy body mass index, and were less likely to drink alcohol in pregnancy compared to women with medical records.

*Outcomes Data*

A secondary validation of medical and interview records for a subsample of 644 women was completed for this study to verify data on the outcomes. Women in this subsample included all those who reported having GH or PE (N=316) and a small group who did not report having GH or PE (N=328). Because of changes in data collection over time, maternal reports of pre-existing hypertension recorded in the databases were also validated for this study. Medical records for all women who reported having pre-existing hypertension (N=203) in the overall sample were reviewed to confirm their hypertension diagnosis prior to 20 weeks of gestation; this included women who reported developing GH or PE and women who did not report developing the outcomes (see Figure 2.1 for further details). Chronic hypertension was defined as maternal report of unresolved pre-existing chronic hypertension within a year of enrollment in the study, any antihypertensive medication use before 20 weeks of gestation, or blood pressure ≥140/90 mm Hg on two or more occasions before 20 weeks of gestation. Women found to have pre-existing chronic hypertension were excluded from the final study population (N=124).

To validate the outcomes and examine the predictive values, mother's medical and interview records were compared against the data entered in the MTB databases. For 80% of the women sampled, available medical and interview records were examined. For the rest of the subsample, no medical records were available, and therefore interview records were the only source of data reviewed (N=132, including 39 with PE, 28 with GH, and 65 non-cases). Women whose medical and interview records did not indicate

GH or PE were classified as non-cases for analysis. Women whose medical records supported diagnosis of GH or PE were classified as cases. Self-reports of GH or PE were classified as cases if no medical records were available.

Instances where data in interview records did not agree with data from the medical records were evaluated on a case-by-case basis. There were 27 instances where medical records did not match data from the interview records, including 10 where the mother reported an outcome that was not supported by medical records and 17 where medical records indicated a diagnosis of GH or PE that was not reported by the mother. In the end, the positive predictive values were 83.8% (95% CI: 78.8%, 88.0%) and 98.2% (95% CI: 94.8%, 99.6%) for GH and PE, respectively. The lower positive predictive value for GH was due to the fact that many women who were originally classified in the database as having GH were excluded after a review of their medical records showed evidence that these women had pre-existing chronic hypertension. The negative predictive values were 96.8% (95% CI: 94.5%, 98.3%) for GH and 98.8% (95% CI: 97.3%, 99.5%) for PE.

Covariates

The following variables were assessed as possible confounders: maternal age and pre-pregnancy BMI (both continuous); education, race/ethnicity, parity, previous spontaneous abortion or stillbirth, gravidity, current asthma status, diabetes status, autoimmune disease status, alcohol use during pregnancy, cigarettes smoked per day at conception, aspirin use at any time during pregnancy, antidepressant use during

pregnancy, caffeine use, partner change since the last pregnancy, and fetal sex (all categorical).

## Statistical Analysis

Analyses were conducted using SAS v.9.3 (SAS Institute Inc., Cary, NC). Comparisons of the participant characteristics by use of folic-acid containing supplements were analyzed using ANOVA for continuous variables and chi-square test for categorical variables ($p < 0.05$ was considered to be statistically significant). To assess the unadjusted and adjusted odds ratios and their 95% CIs between timing of supplement use and the outcomes, polytomous logistic regression was employed. Model 1 examined the unadjusted association between the main exposure of interest and the outcomes. Model 2 adjusted for confounders identified in the study. A confounder was included in Model 2 if the magnitude of the point estimate for the main exposure changed by ≥10% when that variable was added to the model. In addition to covariates included in Model 2, Model 3 adjusted for significant risk factors for hypertensive disorders of pregnancy that were identified from the literature, including asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, previous spontaneous abortion or stillbirth, and antidepressant use.[11,50,51,53,27,66] Model 4 adjusted for all covariates included in Models 2 and 3, plus enrollment year and the specific cohort study (MTB US and Canada vs. MTB California).

# RESULTS

There were 3,474 women included in the study. Women enrolled in the MTB California pregnancy studies made up 12.6% (N=438) of the total study participants. Of the study participants who were enrolled in MTB US and Canada pregnancy studies, 43.0% (N=1,493) were drawn from those enrolled in autoimmune disease studies, 11.8% (N=410) were drawn from those enrolled in asthma studies, and 57.3% (N=1,983) were drawn from those enrolled in vaccine studies.

There were 2,901 women who were early users and 488 women who were late users of folic acid-containing supplements, and 85 women who were nonusers during their pregnancy (Table 2.1). Early and late users were significantly younger, more likely to be nulliparous, primigravid, and had asthma compared to nonusers. A greater proportion of early users also had at least a college education, and more likely to be a nonsmoker and Caucasian compared to late users and nonusers. Late and nonusers had slightly higher rate of diabetes, higher use of antidepressants, and had higher pre-pregnancy BMI compared to early users. Nonusers also had higher proportions that used aspirin and experienced a partner change with their current pregnancy. Of those who had a previous pregnancy, higher proportions among supplement users reported having a previous spontaneous abortion or stillbirth compared to nonusers.

Nonusers had the highest incidence of PE (N=6; 7.1%; 95% CI: 1.6%, 12.5%), followed by late users (5.9%), and early users (3.7%). Unadjusted models did not indicate significant associations between early use (OR: 0.50; 95% CI: 0.21, 1.17) or late use (OR: 0.83; 95% CI: 0.33, 2.06) of folic acid-containing supplements and PE compared to

nonuse. After examining each covariate listed in Table 2.1, education, race/ethnicity, parity and pre-pregnancy BMI were identified as confounders. When these covariates were included in Model 2, the risk for PE was reduced among early users (aOR: 0.46; 95% CI: 0.19, 1.11) and later users (aOR: 0.60; 95% CI: 0.23, 1.57); however, results were not significant. Adjusted models continued to show a non-significant lowered risk for PE among both groups of folic acid-containing supplement users after controlling for all variables in Model 4.

No significant association with GH was observed in either early users (OR: 1.04; 95% CI: 0.32, 3.34) or late users (OR: 0.92; 95% CI: 0.26, 3.22) in comparison to nonusers (Table 2.2). Full adjusted models also did not indicate a protective benefit for GH with the use of folic acid supplements in either early (aOR Model 4: 1.08; 95%CI: 0.32, 3.63) or late (aOR Model 4: 0.95; 95% CI: 0.26, 3.48) users.

## DISCUSSION

Previous research indicates that the use of folic acid and folic acid-containing supplements during pregnancy offers numerous health advantages for women, including some that have shown reduced risk for hypertensive disorders of pregnancy.[22,50–53,27,54–56] In the current study, we did not observe a significant risk reduction for hypertensive disorders of pregnancy with the use of folic acid-containing supplements.

The two studies which examined the association between folic acid supplements and GH had conflicting results.[53,65] A population-based study conducted in two provinces in China did not find a protective effect against GH or PE with the use folic acid

supplements.[65] One limitation of this study was that the authors were unable to adjust for important confounders, such as maternal smoking, which could have affected findings; and as it was conducted in specific regions in China, generalizability to the US population may be limited due to differences in dietary habits, food fortification policies, and environmental factors. In contrast, a study of 2,100 women from the Slone Epidemiology Center Birth Defects found that the use of folic acid-containing supplements lowered the odds of developing GH by 45% (adjusted relative risk [aRR]: 0.55; 95% CI: 0.39, 0.79). Women with PE were combined with GH cases in this analysis. When GH without PE was examined, women who used folic acid had 47% (aRR: 0.53; 95% CI: 0.36, 0.80) reduced risk for GH.[53] A protective effect against PE alone was not observed in the study. Unlike the Slone Epidemiology Center Birth Defects study, which collected information 6 months after delivery, our study collected maternal data at several interviews througout pregnancy and once after birth. This should have minimized information bias, including information on supplement use, which are inherently collected retrospectively. Another point that should be considered in our study is the potential misclassification of the outcomes. For approximately 19% of women included in the study, medical records were not available and thus the only source of the outcome measures was maternal reports. If nondifferential misclassification had occurred, this could have biased the estimate away from the null. Were it differential misclassification, a bias either away or towards the null would have resulted.

Although our study results did not show significant associations between folic acid use and PE, our findings seemed to suggest lower odds of disease occurrence with

supplement use. Our non-significant findings also suggested that earlier use of folic acid-containing supplements may be more advantageous compared to later initiation. The lack of significant findings was likely due to the small sample of women who did not take supplements during pregnancy. Among nonusers, 3 developed GH and 6 developed PE. Nonetheless, the direction of effect was consistent with previous studies.

Folic acid-containing supplements have been shown to reduce the risk of PE in women.[22,50–53,27,54–56] Similar to women in our study, many of the women in previous studies were using folic acid supplements and/or folic acid-containing multivitamins, making it difficult to isolate the effects of folic acid alone from the effects of other vitamins taken during pregnancy. Evidence in the literature indicate an association between the use of vitamins and mineral supplements and decreased risk for PE.[50,67] A randomized controlled trial of 283 women that compared the use of 1000mg/day of vitamin C and 400 IU/day of vitamin E with the use of a placebo found that a significantly smaller proportion of women developed PE in the treatment group compared to the placebo group.[67] Other studies also indicated that deficiencies in some micronutrients, such as vitamin D, magnesium, copper, iron, and zinc, may predispose women to PE.[68,69] Yet, other studies have suggested that increased intake of some of these micronutrients, including vitamin D and magnesium, do not have a preventive effect against the disease.[64]

Another limitation of our study was the possibility of residual confounding. Women's diet during pregnancy is an important factor to consider in the relation between folic acid and hypertensive disorders. Unfortunately, our study did not collect

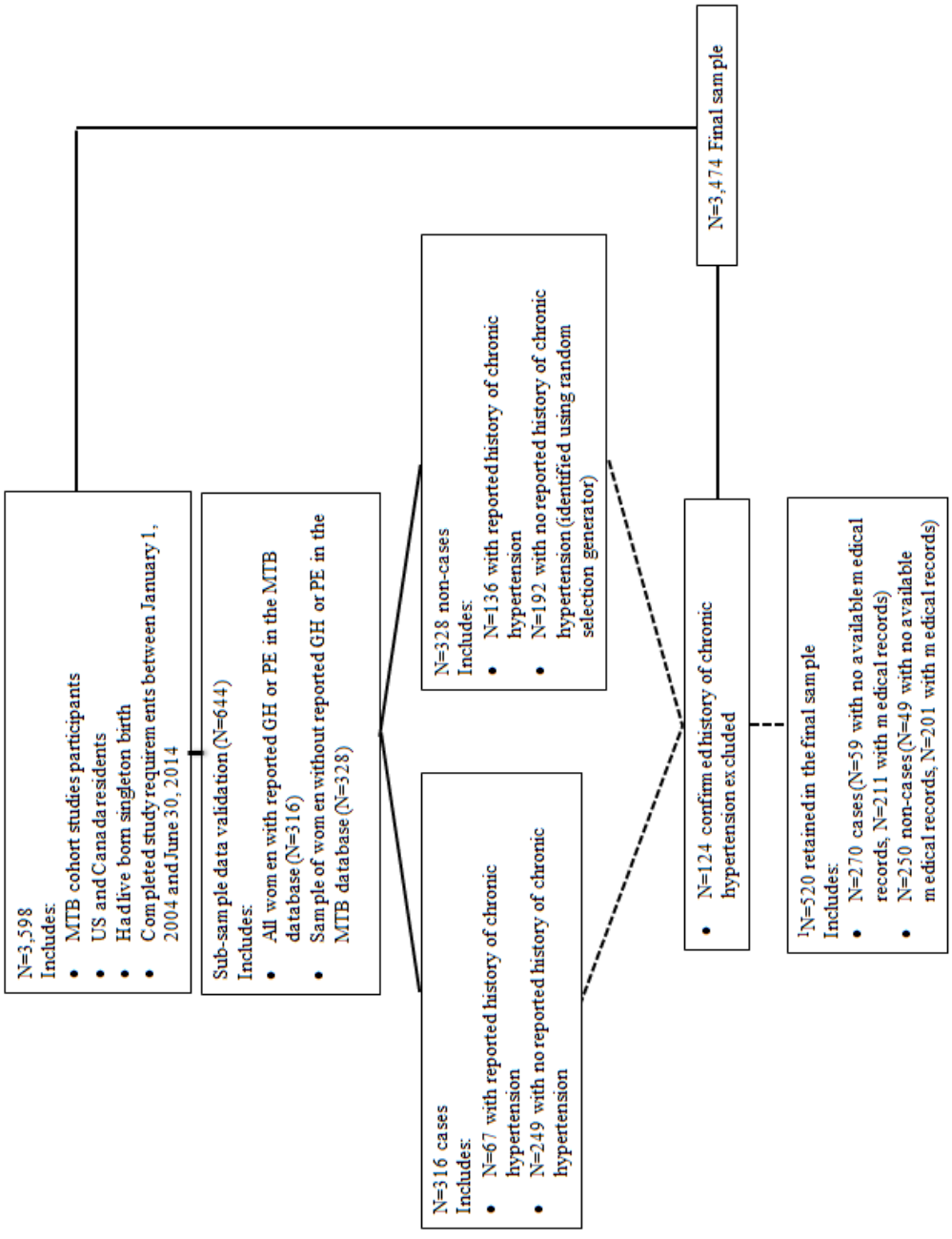
information on women's dietary intake to adequately examine the role of nutrition. Furthermore, because of folic acid fortification of the US and Canadian food supply which became mandatory in 1998, there was a high likelihood that women included in the study, including folic acid supplement late users and nonusers, were exposed to folic acid before and during pregnancy through their diet. Differences in dietary habits of women included in the study may have also impacted findings. For example, corn masa flour, which was excluded from the folic acid food fortification mandate, is a staple food in many Hispanic diets. It is possible that women in this group may have had lower folic acid intake from food compared to women in other ethnic groups. A study using data from the 2001-2002 National Health and Nutrition Examination Survey showed that a lower percentage of Hispanic women consumed the recommended dosage of folic acid from supplements and fortified foods compared to non-Hispanic White women.[70] However, it should also be noted that a majority of women included in the study had higher educational achievement, which have been shown to correlate with higher use of folic acid supplements.[71,72]

Further limitations, such as the high rates of comorbidities and possible selection bias, could limit generalizability of the study. Many women included in the study were enrolled in the MTB autoimmune disease and asthma studies. As a result, disease rates were predictably high in this cohort. Approximately 17.6% had asthma and 40.1% had autoimmune diseases in the overall sample. Furthermore, only women who called the MTB phone line were included in the study, which could have introduced a self-selection bias as women who did not call were excluded. Women who called the MTB toll-free

number generally called to seek advice and information on exposures at the time of their pregnancy. As shown in Table 2.1, these women were primarily Caucasian with higher education, were less likely to smoke, and had higher rates of chronic diseases compared to the general population.[14,73] Moreover, most of these women were referred by their healthcare provider, which could indicate that a higher proportion received some prenatal care early in pregnancy.

Although our study did not indicate significant protective effects of folic acid supplements for the prevention of GH and PE, we continue to recommend the use of folic acid supplements during pregnancy for other benefits it confers to both mother and child. Furthermore, as our non-significant findings may indicate an association between the timing of folic acid initiation and PE, we would also recommend that future studies examine how the timing of use of folic acid-containing supplements, duration of use, and dietary intake impact the risk for developing hypertensive disorders.

Chapter 2, in full, has been submitted for publication of the material as it may appear in the Journal of Women's Health, De Ocampo, Maria P.G.; Araneta Maria Rosario G.; Macera, Caroline A.; Alcaraz, John E.; Moore, Thomas R.; Chambers, Christina D., Mary Ann Liebert, Inc. The dissertation author was the primary investigator and author of this paper.




1. There were 27 instances in which mother's self-report of the outcomes did not agree with information in medical records.

Figure 2.1: Flowchart of data validation of study outcomes

Table 2.1: MotherToBaby cohort study participant characteristics by exposure to folic acid-containing supplements (N=3,474)

| Characteristics | Early Users N=2,901 N (%) | Late Users N=488 N (%) | Nonusers N=85 N (%) | P-value |
|---|---|---|---|---|
| Outcomes | | | | |
| None | 2,685 (92.6) | 443 (90.8) | 76 (89.4) | <0.01 |
| Gestational Hypertension | 110 (3.8) | 16 (3.3) | 3 (3.5) | |
| Preeclampsia | 106 (3.7) | 29 (5.9) | 6 (7.1) | |
| Education | | | | |
| ≤HS grad | 239 (8.2) | 147 (30.1) | 8 (9.4) | <0.01 |
| Some college | 520 (17.9) | 143 (29.3) | 30 (35.3) | |
| College grad | 1,141 (39.3) | 132 (27.0) | 37 (43.5) | |
| Post grad | 998 (34.4) | 65 (13.3) | 10 (11.8) | |
| Missing | 3 (0.1) | 1 (0.2) | 0 (0.0) | |
| Race/Ethnicity | | | | |
| Caucasian | 2,305 (79.5) | 261 (53.5) | 63 (74.1) | <0.01 |
| Black | 81 (2.8) | 41 (8.4) | 6 (7.1) | |
| Hispanic | 278 (9.6) | 143 (29.3) | 11 (12.9) | |
| Asian/Pacific Islander | 162 (5.6) | 24 (4.9) | 4 (4.7) | |
| Native American/AK Native | 18 (0.6) | 4 (0.8) | 1 (1.2) | |
| Other | 56 (1.9) | 15 (3.1) | 0 (0.0) | |
| Missing | 1 (0.0) | 0 (0.0) | 0 (0.0) | |
| Parity | | | | |
| Nulliparous | 1,549 (53.4) | 224 (45.9) | 33 (38.8) | <0.01 |
| Primiparous | 925 (31.9) | 132 (27.0) | 23 (27.1) | |
| Multiparous | 426 (14.7) | 132 (27.0) | 29 (34.1) | |
| Missing | 1 (0.0) | 0 (0.0) | 0 (0.0) | |
| Gravidity | | | | |
| 1 | 1,128 (38.9) | 183 (37.5) | 20 (23.5) | <0.01 |
| 2 | 913 (31.5) | 123 (25.2) | 26 (30.6) | |
| ≥3 | 860 (29.6) | 182 (37.3) | 39 (45.9) | |
| Previous spontaneous abortion or stillbirth[1] | | | | |
| Yes | 775 (43.7) | 88 (28.9) | 15 (23.1) | <0.01 |
| No | 998 (56.3) | 217 (71.1) | 50 (76.9) | |
| Asthma | | | | |
| Yes | 510 (17.6) | 96 (19.7) | 7 (8.2) | 0.04 |
| No | 2,391 (82.4) | 392 (80.3) | 78 (91.8) | |
| Diabetes (Type 1 and 2) | | | | |
| Yes | 19 (0.7) | 15 (3.1) | 1 (1.2) | <0.01 |
| No | 2,882 (99.3) | 473 (96.9) | 84 (98.8) | |
| Autoimmune diseases[2] | | | | |
| Yes | 1,171 (40.4) | 189 (38.7) | 33 (38.8) | 0.77 |
| No | 1,730 (59.6) | 299 (61.3) | 52 (61.2) | |
| Aspirin use | | | | |
| Yes | 204 (7.0) | 17 (3.5) | 6 (7.1) | 0.01 |
| No | 2,697 (93.0) | 471 (96.5) | 79 (92.9) | |

1. Excluded primigravid women
2. Autoimmune diseases include rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, psoriasis, Crohn's disease, multiple sclerosis, lupus, antiphospholipid syndrome, celiac disease, connective tissue disease, fibromyalgia, Sjorgen's syndrome, ulcerative colitis, inflammatory bowel disease, autoimmune liver disease, autoimmune thyroid dysfunction, and iritis

Table 2.1 (continued): MotherToBaby cohort study participant characteristics by exposure to folic acid-containing supplements (N=3,474)

| Characteristics | Early Users N=2,901 N (%) | Late Users N=488 N (%) | Nonusers N=85 N (%) | P-value |
|---|---|---|---|---|
| Number of cigarettes per day at conception | | | | |
| 0 | 2,724 (93.9) | 419 (85.9) | 79 (92.9) | <0.01 |
| 1-9 | 106 (3.6) | 40 (8.2) | 3 (3.5) | |
| ≥10 | 71 (2.5) | 29 (5.9) | 3 (3.5) | |
| Alcohol use | | | | |
| No alcohol | 1,534 (52.9) | 253 (51.8) | 42 (49.4) | 0.90 |
| Occasionally | 1,281 (44.2) | 218 (44.7) | 41 (48.2) | |
| Once a week or more | 86 (3.0) | 17 (3.5) | 2 (2.4) | |
| Caffeine use | | | | 0.34 |
| Yes | 2,431 (83.8) | 420 (86.1) | 69 (81.2) | |
| No | 470 (16.2) | 68 (13.9) | 16 (18.8) | |
| Antidepressant use[3] | | | | |
| Nonuser | 2,605 (89.8) | 437 (89.5) | 77 (90.6) | <0.01 |
| Discontinuer | 71 (2.4) | 28 (5.7) | 3 (3.5) | |
| Continuer | 222 (7.7) | 23 (4.7) | 5 (5.9) | |
| Missing | 3 (0.1) | 0 (0.0) | 0 (0.0) | |
| Partner change | | | | |
| Yes | 188 (6.5) | 50 (10.2) | 12 (14.1) | <0.01 |
| No | 2,669 (92.0) | 424 (86.9) | 71 (83.5) | |
| Missing | 44 (1.5) | 14 (2.9) | 2 (2.4) | |
| Fetal sex | | | | |
| Male | 1,473 (50.8) | 252 (51.6) | 44 (51.8) | 0.97 |
| Female | 1,424 (49.1) | 236 (48.4) | 41 (48.2) | |
| Missing | 4 (0.1) | 0 (0.0) | 0 (0.0) | |
| Cohort study | | | | |
| MTB California | 332 (11.4) | 95 (19.5) | 11 (12.9) | <0.01 |
| MTB US and Canada | 2,569 (88.6) | 393 (80.5) | 74 (87.1) | |
| | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | **P-value** |
| Age (years)[4] | 32.4 (4.7) | 30.5 (6.0) | 32.9 (5.6) | <0.01 |
| Pre-pregnancy BMI[5] | 24.5 (5.3) | 25.9 (6.1) | 25.9 (7.5) | <0.01 |

3. Discontinuers were women who discontinued antidepressant use before 20 weeks of gestation. Continuers were women who continued antidepressant use past 20 weeks of gestation.
4. Missing = 3
5. Missing = 25

Table 2.2: Risk for gestational hypertension and preeclampsia by folic acid-containing supplement use among women in the MotherToBaby cohort studies (2004-2014)

| Outcomes | N | N (%) with outcome | Model 1 OR[1] (95% CI) | Model 2 OR[2] (95% CI) | Model 3 OR[3] (95% CI) | Model 4 OR[4] (95% CI) |
|---|---|---|---|---|---|---|
| **Gestational Hypertension** | | | | | | |
| Nonusers | 85 | 3 (3.5) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Early users | 2,901 | 110 (3.8) | 1.04 (0.32, 3.34) | 1.22 (0.37, 4.01) | 1.09 (0.32, 3.64) | 1.08 (0.32, 3.63) |
| Late users | 488 | 16 (3.3) | 0.92 (0.26, 3.22) | 1.02 (0.28, 3.65) | 0.95 (0.26, 3.50) | 0.95 (0.26, 3.48) |
| **Preeclampsia** | | | | | | |
| Nonusers | 85 | 6 (7.1) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Early users | 2,901 | 106 (3.7) | 0.50 (0.21, 1.17) | 0.46 (0.19, 1.11) | 0.48 (0.19, 1.19) | 0.48 (0.19, 1.20) |
| Late users | 488 | 29 (5.9) | 0.83 (0.33, 2.06) | 0.60 (0.23, 1.57) | 0.61 (0.23, 1.63) | 0.63 (0.24, 1.68) |

1. Model 1: unadjusted
2. Model 2: adjusted for education, race/ethnicity, parity, and pre-pregnancy BMI
3. Model 3: adjusted for education, race/ethnicity, parity, pre-pregnancy BMI, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, previous spontaneous abortion or stillbirth, and antidepressant use
4. Model 4: adjusted for education, race/ethnicity, parity, pre-pregnancy BMI, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, previous spontaneous abortion or stillbirth, antidepressant use, cohort study and enrollment year

# CHAPTER 3

## Association of Maternal Cigarette Use with Gestational Hypertension and Preeclampsia

ABSTRACT

Objective: To assess the effects of maternal cigarette smoking on the development of gestational hypertension (GH) and preeclampsia (PE) in pregnancy and secondarily, to determine if these associations differed by folic acid-containing supplement use.

Methods: Exposure and outcome data were obtained through interviews and review of participant's medical records from the MotherToBaby (MTB) cohort studies. Outcomes were self-reported and validated using participant's medical records. GH was defined as blood pressure ≥140/90 mm Hg on two of more occasions after 20 weeks of gestation. PE was defined as GH with proteinuria. Demographics, maternal medical history, lifestyle factors, substance use, and fetal sex were assessed as potential confounders. Unadjusted and adjusted risks for GH and PE were examined using odds ratios and 95% confidence intervals.

Results: There were 3,474 women included in the final sample, 251 of whom smoked during pregnancy. Among smokers, 12 developed GH and 11 developed PE. Among nonsmokers, 227 developed GH and 130 developed PE. After adjustment in the final model, smokers had significantly lower odds (aOR: 0.41; 95% CI: 0.21, 0.79) of developing GH compared to nonsmokers. Among women who used folic acid-containing supplements after recognition of pregnancy, smokers showed greater reduction in risk for GH compared to nonsmokers (aOR: 0.31; 95% CI: 0.13, 0.75). PE risk for smokers was also reduced, but not significant (aOR: 0.54, 95% CI 0.21, 1.38).

Conclusion: Cigarette smoking was associated with a 59% decreased risk of GH. This risk reduction was most evident among smokers who began using folic acid-containing

supplements after recognition of pregnancy. No significant association was found between smoking and PE. Limitations of the study included low power and low external validity.

## INTRODUCTION

Smoking during pregnancy has been shown to contribute to pregnancy complications and adverse birth outcomes, such as preterm delivery, reduced birth weight, and infant death.[74,75] Despite this fact, between 14.8-17.2% of women of reproductive age smoke cigarettes, and an average of 8.4% smoke during pregnancy.[76,77]

Previous studies have shown that smoking has a protective effect against gestational hypertension and preeclampsia.[9,23,26,78–87] A population-based study of 10,666 women found that those who smoked had 0.48 (95% CI: 0.29, 0.77) and 0.55 (95% CI: 0.37, 0.84) decreased odds of developing GH and PE, respectively.[9] Some studies have also suggested a dose-response association in that women who smoked a greater number of cigarettes had a greater reduction in risk.[23,24,79,85] Studies have not reported significant risk reductions for women who stopped smoking before or during early pregnancy.[23,26]

Although studies of smoking during pregnancy and risk for adverse pregnancy and neonatal outcomes have been examined previously in the literature, we are not aware of any study that has investigated the impact of smoking in the context of use of folic acid-containing supplements on hypertensive disorders of pregnancy. The use of folic acid-containing supplements during pregnancy has known beneficial effects for both mother and child, including reducing the risk for GH and PE.[22,50,51,53,27] One study of the Ottawa and Kingston Birth Cohort, which enrolled women between 12-20 weeks of gestation, found that women who used folic acid-containing supplements had 0.37 reduced odds of developing PE compared to nonusers.[27] Another birth cohort from the Gansu Provincial Maternity & Child Care Hospital conducted in 2010-2012 in Lanzhou,

China had similar reduced risk for PE with the use of folic acid (OR: 0.50; 95% CI: 0.30, 0.81).[22] Additionally, the study indicated that timing of supplement use may also impact risk for PE.[22] Although many of these previous studies addressed smoking somewhat, they have not fully examined whether the effects of cigarette smoking differed by use of folic acid-containing supplements. Smoking during pregnancy has been shown to promote oxidation and disrupt absorption and metabolism of essential nutrients, which in turn could potentially diminish the benefits of folic acid for pregnant women. A cross-sectional study of pregnant women indicated that those who smoked had significantly lower levels of serum folate and red blood cell folate compared to women who did not smoke despite the fact that dietary folate intake and homocysteine levels were not significantly different.[88] Other studies using different populations of women found similar results.[89,90] In these studies, differences in dietary habits between smokers and nonsmokers cannot be ruled out and may be a contributing factor in the lower folate levels found among smokers.[89–92]

The purpose of this study was to examine the effects of cigarette smoking during pregnancy on the risk for GH and PE. A secondary purpose was to examine the impact of smoking on hypertensive disorders in women who used folic acid-containing supplements before and after recognition of pregnancy.

# METHODS

## Data Sources and Study Population

The MotherToBaby (MTB) network, including MTB United States and Canada and MTB California, is a program of the Organization of Teratology Information Specialists (OTIS). It is comprised of 13 sites and serves approximately 80,000 women and healthcare providers across North America each year. MTB US and Canada pregnancy studies have ongoing research on asthma, autoimmune diseases, and vaccines. Mothers concerned about exposures during pregnancy called the OTIS toll-free number for free counseling and risk assessment. These women were simultaneously screened for eligibility in the cohort studies. Women who resided in the US or Canada, were no more than 20 weeks in gestation, and had no prior diagnosis of any major birth defects in the current pregnancy were recruited for these pregnancy studies.

Once enrolled, women completed a comprehensive intake interview that included data collection on demographics, previous pregnancy history and outcomes, last menstrual period, prenatal tests, lifestyle factors, family health history, mother's medical history, and other pregnancy exposures. Follow-up interviews were conducted approximately every three months until the end of the pregnancy, and information on exposures was updated including changes in medication use, substance use and supplement use. Information on new prenatal tests and pregnancy complications was also collected. An outcome interview was completed at the end of pregnancy, and information on pregnancy complications and pregnancy outcomes was collected. Information from medical records was also collected from the obstetrician, birth hospital and the child's

pediatrician. Medical records were used to validate maternal reports of study outcomes, supplement use, medication use, medical history, ultrasounds, pregnancy complications, prenatal tests, delivery outcomes, and newborn complications.

The final sample this study was restricted to women who were enrolled and completed an outcome interview for the MTB cohort studies between January 1, 2004 and June 30, 2014, had a live singleton birth, had no pre-existing chronic hypertension, resided in the United States or Canada, and had available data on study outcomes and the exposures of interest.

This study was approved by the Human Research Protections Program of the University of California, San Diego.

## Exposures

Data on smoking was collected at multiple time points during pregnancy, including at intake and subsequent follow-up interviews. Information on the start and stop times, number of cigarettes smoked, and frequency of smoking was collected. Women who reported using cigarettes at any time during pregnancy were classified as smokers and women who did not report using cigarettes at any time during pregnancy were classified as nonsmokers. Women who reported quitting smoking before pregnancy were categorized as nonsmokers in analysis. Data on the number of cigarettes smoked at the time of conception was categorized (0, <10, and ≥10 cigarettes per day). The time of conception was defined as 4 weeks before until 4 weeks after the last menstrual period. Smoking habits was also categorized to never smokers, former smokers (women who

only smoked before pregnancy and women who stopped smoking before 4 weeks of gestation), and current smokers (women who smoked past 4 weeks of gestation). Folic acid-containing supplement use was classified by timing of initiation of use (prior to pregnancy or after recognition of pregnancy and nonuse).

## Outcomes

Study outcomes were self-reported by participants at any interview after 20 weeks of gestation. Maternal reports were later validated using medical records from the obstetrician or delivery hospital. GH was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two or more occasions after 20 weeks of gestation without the presence of proteinuria, and PE was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on two or more consecutive readings 4 or more hours apart with proteinuria of 0.3 g or more after 20 weeks of gestation. Women with GH that later progressed to PE were included in the GH and PE analyses.

## Data Validation

The validation of data for the MTB cohort studies and this study is described in chapter 2. Additionally, results of further analysis examining GH and PH risk stratified by smoking and available medical records are provided in the supplementary tables.

## Statistical Analysis

Analyses were conducted using SAS v.9.3 (SAS Institute Inc., Cary, NC). Comparisons of the participant characteristics were analyzed using ANOVA for continuous variables and chi-square test for categorical variables ($p < 0.05$ was considered to be statistically significant). Multivariable binary logistic regression was used to estimate the unadjusted and adjusted odds ratios and their 95% CIs in assessing the risk for GH and PE. Model 1 examined the unadjusted association between the exposure and the outcomes. Model 2 adjusted for confounders identified in the study. The following potential confounders were considered in the study: maternal age and pre-pregnancy body mass index (BMI) (both continuous); enrollment year, study cohort (MTB US and Canada or MTB California), maternal education, race/ethnicity, nulliparity, previous spontaneous abortion or stillbirth, primigravidity, current asthma status, diabetes status, autoimmune disease status, alcohol use during pregnancy, aspirin use at any time during pregnancy, antidepressant use, caffeine use, change in paternity since the last pregnancy, and fetal sex (all categorical). A confounder was added in the regression models if the adjusted odds ratio changed by 10% or greater from the unadjusted model. In addition to covariates included in Model 2, Model 3 adjusted for significant risk factors for hypertensive disorders of pregnancy that were identified from previous studies.[9,11,23,53,27,66,93–95] Model 4 adjusted for all covariates included in Models 2 and 3, plus enrollment year and the specific cohort study (MTB US and Canada vs. MTB California). Further analysis stratified by timing of folic acid-containing supplement use

initiation (before pregnancy vs. after recognition of pregnancy or nonuse) was also examined. Analyses were conducted separately for GH and PE.

## RESULTS

There were 3,474 women included in the study. Women who were enrolled in the MTB California pregnancy studies made up 12.6% (N=438) of the total study participants. Of the women who were enrolled in MTB US and Canada pregnancy studies, 43.0% (N=1,493) were enrolled in autoimmune disease studies, 11.8% (N=410) were enrolled in asthma studies, and 57.3% (N=1,983) were enrolled in influenza vaccine studies.

There were 251 women who smoked during pregnancy. Smokers were younger, had higher pre-pregnancy BMI, were more likely to have asthma and autoimmune diseases, and were less likely to begin folic-acid containing supplement use before pregnancy compared to nonsmokers (Table 3.1). Smokers were also more likely to have had lower education, to have experienced change in paternity, and more likely to use caffeine, alcohol, and antidepressants during pregnancy compared to nonsmokers. They were also more likely to have been enrolled in MTB California compared to nonsmokers. Most smokers used less than 10 cigarettes per day at the time of conception and continued to smoke past 4 weeks of gestation.

Results of the overall model indicated that smokers had 0.41 decreased odds for GH compared to nonsmokers (95% CI: 0.21, 0.79), after adjusting for maternal age, education, pre-pregnancy BMI, race/ethnicity, nulliparity, primigravidity, previous

spontaneous abortion or stillbirth, use of antidepressants, diabetes, asthma, autoimmune disease, change in paternity, cohort study, and enrollment year. Analysis did not indicate a significant association between smoking and PE before or after adjustment. Although not significant, the point estimate for the overall model in Table 3.2 suggested a decreased risk for PE among smokers (Model 4 aOR: 0.71; 95% CI: 0.35, 1.44).

Number of cigarettes used per day and smoking habits were also examined in Table 3.2. No significant association was found between the number of cigarettes used per day at the time of conception and risk for PE. However, women who smoked less than 10 cigarettes per day had decreased odds for GH (Model 4 aOR: 0.34; 95% CI: 0.13, 0.87). In terms of smoking habits, women who were current smokers had lower risk for GH (Model 4 aOR: 0.31; 95% CI: 0.13, 0.74), but not PE, compared to never smokers. Participants who were former smokers, which included women who smoked before pregnancy and women who stopped smoking less than 4 weeks of gestation, did not have significantly lower risk for GH or PE compared to never smokers.

This study also examined the risk for smokers stratified by timing of initiation of folic acid-containing supplement use. Overall, 97.6% of women in the study reported using folic-acid containing supplements. Non-supplement users were grouped with women who initiated supplementation after recognition of pregnancy. Among women who began using folic acid-containing supplements before pregnancy, no significant association was found between smoking and GH and PE. Among women who began folic acid-containing supplements after recognition of pregnancy, smokers had significantly

lower risk for GH (Model 4 aOR: 0.31; 95% CI: 0.31, 0.75) compared to nonsmokers. No association was found between smoking and PE in this same group.

## DISCUSSION

Findings in this study did not indicate a significant association between cigarette use and PE. An important contributing factor to this non-significant finding was the low power of the study. The estimated power for detecting an association between cigarette use and PE was approximately 13.1%. Furthermore, although the direction of the association between cigarette use and PE was consistent with other studies, the risk reduction observed was greater compared to previous reports.[9,23,78,79,82–87] Additional analyses conducted to examine the number of cigarettes used and smoking habits showed similar non-significant risk reduction.

Another factor that may account for the non-significant results was the potential misclassification of the exposure and study outcomes. Since data on cigarette use was primarily collected through maternal self-reports, smoking rates may have been affected by underreporting. Outcome data was also collected through maternal self-reports; however, this data was validated using medical records by study staff for the MTB US and Canada and MTB California research studies. For about 19% of women included in the study, maternal self-reports were the only source of data used for determining the outcomes. A secondary validation of the outcomes was completed for 15% of women (N=520; see Figure 2.1) included in our study. This subsample included all women with reported GH or PE and some with no reports of the outcomes. Predictive values resulting from these chart reviews were quite high, which provided some assurance that the

likelihood of misclassification of outcomes would be low. Furthermore, the overall incidences of GH (6.9%) and PE (4.1%) in our study were similar to previously published reports.[5,9,24,79]

Findings in this study were in agreement with previously published studies in that cigarette smoking had an inverse association with GH.[9,23,24,26,84] Our study showed 0.41 decreased odds for developing GH in pregnancy, which was slightly greater reduction in risk compared to previous reports.[9,23,24,26,84] The mechanism driving the protective effect of smoking on the incidence of hypertensive disorders of pregnancy is not well understood. Wikstrom et al. have suggested that the carbon monoxide exposure in smoking may play a role.[24] Their study comparing the effects of the use of cigarettes and snuff, a smokeless tobacco product, reported a significant inverse association between tobacco use and risk for GH and PE only among cigarette users and not snuff users.[24] It has been proposed that exposure to carbon monoxide in cigarettes reduces maternal risk for hypertensive disorders of pregnancy by inhibiting inflammation, apoptosis, and production of antiangiogenic proteins.[24,96,97]

To our knowledge, no other study has yet examined the impact of both smoking and use of folic acid-containing supplements on hypertensive disorders of pregnancies. Previous studies have indicated that both factors have protective effect.[9,22,23,26,50,51,53,27] Wen et al. found 0.37 lower odds (95% CI: 0.18, 0.75) for PE in women who took folic-acid containing supplements during the second trimester of pregnancy.[27] Bodnar et al. found similar results (aOR: 0.55 95% CI: 0.32, 0.95) in women who took supplements during the periconceptional period.[51] However, it has also been demonstrated that folate

levels in women who smoke were significantly lower compared to nonsmokers, which could indicate that smoking may lower the protective impact of folic acid by potentially influencing metabolism and dietary habits of smokers.[88–92] Non-significant findings between smoking and PE among women who began folic acid-containing supplement use before or after recognition of pregnancy could potentially indicate the adverse impact of smoking on the beneficial effects of folic acid; however, a more probable cause may be the lack of power of the study. In contrast, our study also found lower risk for GH among smokers who began supplement use after recognition of pregnancy. Because of sample size limitation, women categorized in this group included those who did not use folic acid at any time during pregnancy, which comprised of about 2.4% of the total sample. As these women did not use supplements, the significant findings could have been potentially driven by smoking exclusively for these women and thus seeming to contribute to the combined effects of smoking and use of folic acid-containing supplements. Differences in smoking practice could have also influenced study results. There was a lower proportion of smokers among those who began supplement use before pregnancy (3.4%) compared to women who began supplement use after recognition of pregnancy (12.8%). There were also proportionately fewer smokers who indicated using 10 or more cigarettes per day at the time of conception in women who began supplement use before pregnancy compared to women who began after recognition of pregnancy (38.0%; N=27 and 42.2%; N=76, respectively).

Additional limitations to this study should be considered. Because of study methodology limitations, we cannot rule out reverse causation. Although most smokers

were active smokers at conception and throughout pregnancy, there were six women who only smoked after conception, including two who reported only smoking after 20 weeks of gestation. Along with the low number of outcomes included in the study, this could have contributed in producing spurious findings. Selection bias also cannot be ruled out. All women included in this study were identified from the population of pregnant women who contacted the MTB network and were later enrolled in the MTB pregnancy studies. Women who called the MTB network were more likely to be Caucasian and had higher education. Subsequently, as women were enrolled in pregnancy studies in asthma, autoimmune diseases, and vaccine trials, a greater proportion had comorbidities compared to the general population, which may limit the external validity of the study.

In conclusion, cigarette use during pregnancy in this study significantly decreased risk for GH, but not PE. Smokers who began using folic acid-containing supplements after recognition of pregnancy had greater reduced risk for GH than smokers who began using supplements before conception. Further efforts should be made to confirm these findings and to better understand the biological mechanism of the interaction between smoking and folic acid on hypertensive disorders of pregnancy.

Chapter 3, in full, has been submitted for publication of the material as it may appear in the Women & Health, De Ocampo, Maria P.G.; Araneta Maria Rosario G.; Macera, Caroline A.; Alcaraz, John E.; Moore, Thomas R.; Chambers, Christina D., Taylor & Francis. The dissertation author was the primary investigator and author of this paper.

Table 3.1: Participant characteristics in the MotherToBaby cohort studies by smoking status, 2004-2014 (N=3,474)

| Characteristics | Smokers N=251 N (%) | Nonsmokers N=3,223 N (%) | P-value |
|---|---|---|---|
| Gestational Hypertension | | | |
| Yes | 12 (4.9) | 227 (7.1) | 0.18 |
| No | 235 (95.1) | 2,969 (92.9) | |
| Preeclampsia | | | |
| Yes | 11 (4.5) | 130 (4.2) | 0.84 |
| No | 235 (95.5) | 2,969 (95.8) | |
| Cigarettes per day at conception | | | |
| 0 | 6 (2.4) | 3216 (99.8) | <0.01 |
| <10 | 142 (56.6) | 7 (0.2) | |
| ≥10 | 103 (41.0) | 0 (0.0) | |
| Smoking habits | | | |
| Never smokers | 0 (0.0) | 3216 (99.57) | <0.01 |
| Former smokers | 89 (35.5) | 7 (0.2) | |
| Current smokers | 162 (64.5) | 0 (0.0) | |
| Education | | | |
| ≤HS grad | 75 (29.9) | 319 (9.9) | <0.01 |
| Some college | 99 (39.4) | 594 (18.4) | |
| College grad | 54 (21.5) | 1,256 (39.0) | |
| Post grad | 23 (9.2) | 1,050 (32.6) | |
| Missing | 0 (0.0) | 4 (0.1) | |
| Race/Ethnicity | | | |
| Caucasian | 197 (78.5) | 2,432 (75.5) | <0.01 |
| Black | 15 (6.0) | 113 (3.5) | |
| Hispanic | 22 (8.8) | 410 (12.7) | |
| Asian/Pacific Islander | 7 (2.8) | 183 (5.7) | |
| Native American/AK Native | 5 (2.0) | 18 (0.6) | |
| Other | 5 (2.0) | 66 (2.1) | |
| Missing | 0 (0.0) | 1 (0.0) | |
| Parity | | | |
| Nulliparous | 131 (52.2) | 1,675 (52.0) | 0.22 |
| Primiparous | 69 (27.5) | 1,011 (31.4) | |
| Multiparous | 51 (20.3) | 536 (16.6) | |
| Missing | 0 (0.0) | 1 (0.0) | |
| Gravidity | | | |
| 1 | 97 (38.7) | 1,234 (38.3) | 0.08 |
| 2 | 63 (25.1) | 999 (31.0) | |
| ≥3 | 91 (36.3) | 990 (30.7) | |
| Previous spontaneous abortion or stillbirth[1] | | | |
| Yes | 58 (37.7) | 820 (41.2) | 0.39 |
| No | 96 (62.3) | 1,169 (58.8) | |
| Asthma | | | |
| Yes | 59 (23.5) | 554 (17.2) | 0.01 |
| No | 192 (76.5) | 2,669 (82.8) | |

1. Excluded primigravid women

Table 3.1 (continued): Participant characteristics in the MotherToBaby cohort studies by smoking status, 2004-2014 (N=3,474)

| Characteristics | Smokers N=251 N (%) | Nonsmokers N=3,223 N (%) | P-value |
|---|---|---|---|
| Diabetes (Type 1 and 2) | | | |
| Yes | 5 (2.0) | 30 (0.9) | 0.10 |
| No | 246 (98.0) | 3,193 (99.1) | |
| Autoimmune diseases[2] | | | |
| Yes | 123 (49.0) | 1,270 (39.4) | <0.01 |
| No | 128 (51.0) | 1,953 (60.6) | |
| Aspirin use | | | |
| Yes | 15 (6.0) | 212 (6.6) | 0.71 |
| No | 236 (94.0) | 3,011 (93.4) | |
| Initiation of folic acid-containing supplement use | | | |
| Began before pregnancy | 71 (28.3) | 1,993 (61.8) | <0.01 |
| Began after recognition of pregnancy and nonusers | 180 (71.7) | 1,230 (38.2) | |
| Alcohol use | | | |
| No alcohol | 92 (36.7) | 1,737 (53.9) | <0.01 |
| Occasionally | 149 (59.4) | 1,391 (43.2) | |
| Once a week or more | 10 (4.0) | 95 (3.0) | |
| Caffeine use | | | |
| Yes | 241 (96.0) | 2,679 (83.1) | <0.01 |
| No | 10 (4.0) | 544 (16.9) | |
| Antidepressant use[3] | | | |
| Nonuser | 208 (82.9) | 2,910 (90.3) | <0.01 |
| Discontinuer | 12 (4.8) | 90 (2.8) | |
| Continuer | 31 (12.4) | 219 (6.8) | |
| Missing | 0 (0.0) | 3 (0.1) | |
| Change in paternity | | | |
| Yes | 45 (17.9) | 205 (6.4) | <0.01 |
| No | 200 (79.7) | 2,964 (92.0) | |
| Missing | 6 (2.3) | 54 (1.7) | |
| Fetal sex | | | |
| Male | 124 (49.4) | 1,645 (51.0) | 0.81 |
| Female | 126 (50.2) | 1,575 (48.9) | |
| Missing | 1 (0.4) | 3 (0.1) | |
| Cohort study | | | |
| MTB California | 62 (24.7) | 376 (11.7) | <0.01 |
| MTB US and Canada | 189 (75.3) | 2,847 (88.3) | |
| | **Mean (SD)** | **Mean (SD)** | **P-value** |
| Age (years)[4] | 29.9 (5.4) | 32.3 (4.9) | <0.01 |
| Pre-pregnancy BMI[5] | 26.3 (6.5) | 24.7 (5.4) | <0.01 |

2. Autoimmune diseases included rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, psoriasis, Crohn's disease, multiple sclerosis, lupus, antiphospholipid syndrome, celiac disease, connective tissue disease, fibromyalgia, Sjorgen's syndrome, ulcerative colitis, inflammatory bowel disease, autoimmune liver disease, autoimmune thyroid dysfunction, and iritis
3. Discontinuers were women who discontinued antidepressant use before 20 weeks of gestation. Continuers were women who continued antidepressant use past 20 weeks of gestation.
4. Missing = 3
5. Missing = 25

Table 3.2: Gestational hypertension and preeclampsia risk by smoking status of pregnant women in the MotherToBaby cohort studies (N=3,474)

| Outcomes | N | N (%) with outcome | Model 1 OR[1] (95% CI) | Model 2 OR[2] (95% CI) | Model 3 OR[3] (95% CI) | Model 4 OR[4] (95% CI) |
|---|---|---|---|---|---|---|
| **GESTATIONAL HYPERTENSION** | | | | | | |
| **Smoking during pregnancy** | | | | | | |
| Nonsmoker | 3,196 | 227 (7.1) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 247 | 12 (4.9) | 0.67 (0.37, 1.21) | 0.49 (0.26, 0.93) | 0.41 (0.21, 0.79) | 0.41 (0.21, 0.79) |
| **Number of cigarettes smoked per day** | | | | | | |
| 0 | 3,195 | 228 (7.1) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| <10 | 148 | 6 (4.1) | 0.55 (0.24, 1.26) | 0.41 (0.17, 1.02) | 0.34 (0.13, 0.86) | 0.34 (0.13, 0.87) |
| ≥10 | 100 | 5 (5.0) | 0.69 (0.28, 1.70) | 0.49 (0.19, 1.24) | 0.39 (0.15, 1.02) | 0.39 (0.15, 1.03) |
| **Smoking habits** | | | | | | |
| Never smokers | 3,189 | 227 (7.1) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Former smokers | 95 | 6 (6.3) | 0.88 (0.38, 2.03) | 0.68 (0.27, 1.70) | 0.56 (0.22, 1.45) | 0.57 (0.22, 1.46) |
| Current smokers | 159 | 6 (3.8) | 0.51 (0.22, 1.17) | 0.38 (0.27, 0.89) | 0.31 (0.13, 0.74) | 0.31 (0.31, 0.74) |
| **PREECLAMPSIA** | | | | | | |
| **Smoking during pregnancy** | | | | | | |
| Nonsmoker | 3,099 | 130 (4.2) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 246 | 11 (4.5) | 1.07 (0.57, 2.01) | 0.78 (0.40, 1.55) | 0.71 (0.35, 1.43) | 0.71 (0.35, 1.44) |
| **Number of cigarettes smoked per day** | | | | | | |
| 0 | 3,098 | 131 (4.2) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| <10 | 146 | 4 (2.7) | 0.64 (0.23, 1.75) | 0.43 (0.14, 1.39) | 0.38 (0.12, 1.24) | 0.39 (0.12, 1.26) |
| ≥10 | 101 | 6 (5.9) | 1.43 (0.62, 3.33) | 1.03 (0.43, 2.47) | 0.92 (0.37, 2.28) | 0.92 (0.37, 2.30) |
| **Smoking habits** | | | | | | |
| Never smokers | 3,092 | 130 (4.2) | 1.0 (---) | 1.0 (---) | 1.0 (---) | 1.0 (---) |
| Former smokers | 94 | 5 (5.3) | 1.28 (0.51, 3.21) | 0.96 (0.35, 2.69) | 0.84 (0.30, 2.40) | 0.85 (0.30, 2.43) |
| Current smokers | 159 | 6 (3.8) | 0.89 (0.39, 2.06) | 0.67 (0.28, 1.59) | 0.60 (0.25, 1.46) | 0.61 (0.25, 1.48) |

1. Model 1: unadjusted
2. Model 2: adjusted for education and pre-pregnancy BMI
3. Model 3: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity and antidepressant use
4. Model 4: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity, antidepressant use, cohort study and enrollment year

Table 3.3: Gestational hypertension and preeclampsia risk by smoking status and initiation of folic acid-containing supplement use in the MotherToBaby cohort studies (N=3,474)

| Outcomes | N | N (%) with outcome | Model 1 OR[1] (95% CI) | Model 2 OR[2] (95% CI) | Model 3 OR[3] (95% CI) | Model 4 OR[4] (95% CI) |
|---|---|---|---|---|---|---|
| **BEFORE PREGNANCY** | | | | | | |
| **Gestational Hypertension** | | | | | | |
| Nonsmoker | 1,977 | 131 (6.6) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 70 | 5 (7.1) | 1.08 (0.43, 2.74) | 0.76 (0.28, 2.04) | 0.65 (0.24, 1.82) | 0.66 (0.24, 1.83) |
| **Preeclampsia** | | | | | | |
| Nonsmoker | 1,920 | 74 (3.9) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 69 | 4 (5.8) | 1.54 (0.55, 4.33) | 1.18 (0.39, 3.54) | 1.21 (0.40, 3.66) | 1.27 (0.42, 3.84) |
| **AFTER RECOGNITION OF PREGNANCY** | | | | | | |
| **Gestational Hypertension** | | | | | | |
| Nonsmoker | 1,219 | 96 (7.9) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 177 | 7 (4.0) | 0.48 (0.22, 1.06) | 0.38 (0.16, 0.88) | 0.31 (0.13, 0.74) | 0.31 (0.13, 0.75) |
| **Preeclampsia** | | | | | | |
| Nonsmoker | 1,179 | 56 (4.7) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 177 | 7 (4.0) | 0.83 (0.37, 1.84) | 0.63 (0.27, 1.52) | 0.54 (0.21, 1.37) | 0.54 (0.21, 1.38) |

1. Model 1: unadjusted
2. Model 2: adjusted for education and pre-pregnancy BMI
3. Model 3: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity and antidepressant use
4. Model 4: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity, antidepressant use, cohort study and enrollment year

SUPPLEMENTARY TABLES

Table S1: Gestational hypertension and preeclampsia risk for pregnant women in the MotherToBaby cohort studies by smoking and available medical records (N=3,474)

| Outcomes | N | N (%) with outcome | Model 1 OR[1] (95% CI) | Model 2 OR[2] (95% CI) | Model 3 OR[3] (95% CI) | Model 4 OR[4] (95% CI) |
|---|---|---|---|---|---|---|
| **GESTATIONAL HYPERTENSION** | | | | | | |
| **Women with available medical records** | | | | | | |
| Nonsmoker | 2,620 | 186 (7.1) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 175 | 10 (5.7) | 0.79 (0.41, 1.53) | 0.55 (0.27, 1.11) | 0.46 (0.22, 0.96) | 0.46 (0.22, 0.95) |
| **Women without available medical records** | | | | | | |
| Nonsmoker | 576 | 41 (7.1) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 72 | 2 (2.8) | 0.37 (0.09, 1.58) | 0.36 (0.08, 1.56) | cannot estimate | cannot estimate |
| **PREECLAMPSIA** | | | | | | |
| **Women with available medical records** | | | | | | |
| Nonsmoker | 2,536 | 102 (4.0) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 175 | 10 (5.7) | 1.45 (0.74, 2.82) | 1.03 (0.50, 2.14) | 0.95 (0.45, 2.01) | 0.95 (0.45, 2.02) |
| **Women without available medical records** | | | | | | |
| Nonsmoker | 563 | 28 (5.0) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 71 | 1 (1.4) | 0.27 (0.04, 2.04) | 0.23 (0.03, 1.79) | cannot estimate | cannot estimate |

1. Model 1: unadjusted
2. Model 2: adjusted for education and pre-pregnancy BMI
3. Model 3: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity and antidepressant use
4. Model 4: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity, antidepressant use, cohort study and enrollment year

Table S2: Gestational hypertension and preeclampsia risk for pregnant women in the MotherToBaby cohort studies by smoking and available obstetrician medical records (N=3,474)

| Outcomes | N | N (%) with outcome | Model 1 OR[1] (95% CI) | Model 2 OR[2] (95% CI) | Model 3 OR[3] (95% CI) | Model 4 OR[4] (95% CI) |
|---|---|---|---|---|---|---|
| **GESTATIONAL HYPERTENSION** | | | | | | |
| **Women with available obstetrician medical records** | | | | | | |
| Nonsmoker | 2,513 | 179 (7.1) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 165 | 10 (6.1) | 0.84 (0.44, 1.62) | 0.57 (0.28, 1.16) | 0.47 (0.23, 0.98) | 0.47 (0.23, 0.98) |
| **Women without available obstetrician medical records** | | | | | | |
| Nonsmoker | 683 | 48 (7.0) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 82 | 2 (2.4) | 0.33 (0.08, 1.39) | 0.33 (0.08, 1.43) | cannot estimate | cannot estimate |
| **PREECLAMPSIA** | | | | | | |
| **Women with available obstetrician medical records** | | | | | | |
| Nonsmoker | 2,432 | 98 (4.0) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 165 | 10 (6.1) | 1.54 (0.79, 3.01) | 1.09 (0.52, 2.26) | 0.99 (0.47, 2.11) | 0.99 (0.47, 2.10) |
| **Women without available obstetrician medical records** | | | | | | |
| Nonsmoker | 667 | 32 (4.8) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 81 | 1 (1.2) | 0.25 (0.03, 1.84) | 0.22 (0.03, 1.64) | cannot estimate | cannot estimate |

1. Model 1: unadjusted
2. Model 2: adjusted for education and pre-pregnancy BMI
3. Model 3: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity and antidepressant use
4. Model 4: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity, antidepressant use, cohort study and enrollment year

Table S3: Gestational hypertension and preeclampsia risk for pregnant women in the MotherToBaby cohort studies by smoking and available delivery hospital medical records (N=3,474)

| Outcomes | N | N (%) with outcome | Model 1 OR[1] (95% CI) | Model 2 OR[2] (95% CI) | Model 3 OR[3] (95% CI) | Model 4 OR[4] (95% CI) |
|---|---|---|---|---|---|---|
| **GESTATIONAL HYPERTENSION** | | | | | | |
| **Women with available delivery hospital medical records** | | | | | | |
| Nonsmoker | 2,536 | 178 (7.0) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 165 | 10 (6.1) | 0.86 (0.44, 1.65) | 0.60 (0.29, 1.21) | 0.50 (0.24, 1.03) | 0.49 (0.24, 1.02) |
| **Women without available delivery hospital medical records** | | | | | | |
| Nonsmoker | 660 | 49 (7.4) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 82 | 2 (2.4) | 0.31 (0.07, 1.31) | 0.29 (0.07, 1.26) | Cannot estimate | Cannot estimate |
| **PREECLAMPSIA** | | | | | | |
| **Women with available delivery hospital medical records** | | | | | | |
| Nonsmoker | 2,456 | 98 (4.0) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 165 | 10 (6.1) | 1.55 (0.79, 3.04) | 1.12 (0.54, 2.32) | 1.02 (0.48, 2.17) | 1.02 (0.48, 2.17) |
| **Women without available delivery hospital medical records** | | | | | | |
| Nonsmoker | 643 | 32 (5.0) | 1.0 (----) | 1.0 (----) | 1.0 (----) | 1.0 (----) |
| Smoker | 81 | 1 (1.2) | 0.24 (0.03, 1.77) | 0.21 (0.03, 1.58) | Cannot estimate | Cannot estimate |

1. Model 1: unadjusted
2. Model 2: adjusted for education and pre-pregnancy BMI
3. Model 3: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity and antidepressant use
4. Model 4: adjusted for education, pre-pregnancy BMI, race/ethnicity, diabetes status, asthma status, autoimmune disease status, maternal age, nulliparity, primigravidity, previous spontaneous abortion or stillbirth, change in paternity, antidepressant use, cohort study and enrollment year

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