KIDNEYS CONDITIONS ASSOCIATED WITH HYPERTENSION IN PREGNANCY

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By

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Abstract

We defined hypertension in pregnancy as a composite of gestational hypertension, preeclampsia and eclampsia. The etiology of hypertension in pregnancy remains controversial. The three chapters of this thesis explore the risk of hypertension in pregnancy from various kidney conditions. Chapter 1 introduces the reader to the thesis. Chapter 2 is a systematic review that studied the risk of developing hypertension in pregnant women with chronic kidney disease but not on dialysis. We found that women with chronic kidney disease had at least a twofold higher relative risk of developing hypertension during pregnancy compared with women having no chronic kidney disease. Chapter 3 is a retrospective study looking at the risk of developing gestational hypertension and preeclampsia in women who had symptomatic gastroenteritis after drinking water infected with *E. coli* O157:H7 during the Walkerton outbreak in May 2000. We conducted this study using linked datasets at the Institute of Evaluative Sciences (ICES) Toronto, Ontario. We observed that there was no increased risk of developing gestational hypertension or preeclampsia among the symptomatic women compared with women from the neighbouring towns who were asymptomatic or did not drink the water. Chapter 4 is a protocol of a prospective cohort study recruiting female kidney donors and healthy non-donors as the comparative group to study pregnancy outcomes in these individuals. This is a multicentre study involving 12 transplant centres throughout Canada. There are 59 participants in this study to date (Feb 28, 2013) of which seven have been pregnant so far. Data collection for this study is ongoing.

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I like to thank Dr. Amit Garg my supervisor, Dr. Sarah McDonald and Dr. Lehana Thabane my doctoral committee members for their support and guidance during my doctoral studies.

I also take this opportunity to thank my loved ones. This would not have been possible without their love and support.

Contributions by Others

Chapter 1: My own work

Chapter 2: I was responsible for the supervision, concept and design of the review. I coordinated acquisition of data, analyzed and interpreted data, drafted manuscript and incorporated co-author's comments during revisions. All statistical analysis was performed by me. Angela Reitsma and Arunmozhi Dominic were co-reviewers for full text inclusion in the review. All co-authors approved the final version of manuscript before submission to journal.

Chapter 3: I was primarily responsible for the study supervision, concept and design. I drafted the data creation plan (DCP) and made revisions to it as we progressed through the analysis. Data collection for the Walkerton Health Study was made possible by the efforts of an entire investigative team. Investigators involved in primary data collection for the Walkerton Health Study included: Dr. William Clark, Dr. Amit Garg, Dr. Jennifer Macnab, Dr. Louise Moist, and Dr. Rita Suri. Dr. Jessica Sontrop had access to the Walkerton Health Study data and helped with linking this data to the Institute of Clinical Evaluative Sciences (ICES), Toronto. Anjie Huang from ICES had access to data and performed the statistical analysis according to the DCP. In addition, this chapter of my thesis was funded by the Canadian Institutes of Health Research (CIHR) operating grant. I was a co-investigator on this grant and was primarily responsible for drafting the grant

protocol and submitted manuscript. All co-authors approved the final version of manuscript before submission to journal.

Chapter 4: I was primarily responsible for developing the study protocol and case report forms. I worked with Ms Jennifer Arnold an experienced research co-ordinator to launch the study in 12 transplant centres. I participated in team meetings for the past 3 years updating the group on the progress of the study. I have reviewed the data that has been acquired to date.

Chapter 5: My own work

Table of contents

ABSTRACT III

ACKNOWLEDGEMENTS IIV

CONTRIBUTIONS BY OTHERS V

TABLE OF CONTENTS VII

LIST OF ABBREVIATIONS IIX

PREFACE X

CHAPTER 1 1

INTRODUCTION 1

FIGURE 1: PHYSIOLOGIC CHANGES DURING NORMAL PREGNANCY 2

CHAPTER 2 13

PREGNANCY OUTCOMES IN WOMEN WITH CHRONIC KIDNEY DISEASE: A SYSTEMATIC REVIEW. 13

Table 1: Study characteristics 30

Table 2a: Baseline characteristics (comparator group healthy women with normal kidney function) 32

Table 2b: Baseline characteristics (comparator group women with normal kidney function but other co-morbidities) 33

Table 3: Adverse maternal and fetal outcomes 34

CHAPTER 3 38

HYPERTENSION IN PREGNANCY AFTER ESCHERICHIA COLI O157:H7 GASTROENTERITIS .. 39

FIGURE 1: PARTICIPANT SELECTION 57
Table 1. Participant characteristics 58
Table 2. Hypertension in pregnancy after *E.coli* O157:H7 gastroenteritis 60

CHAPTER 4 107

HYPERTENSION IN PREGNANCY FOLLOWING LIVE KIDNEY DONATION: A PROSPECTIVE COHORT STUDY 108
Table 1. Eligibility criteria for the LKD pregnancy study (donors and non-donors) 122
Table 2. Biomarkers 122
Table 3. Composite outcome: Hypertension in pregnancy 122
Table 4. Data collected so far 123

CHAPTER 5 146

CONCLUSIONS AND FUTURE DIRECTIONS 146

List of abbreviations

E.*coli*: Escherichia *coli*

CKD: Chronic kidney disease

LKD: Living kidney donation

IQR: Interquartile Range

CCI: Canadian Classification of Health Interventions

WHO: World Health Organization

ICES: Institute of Clinical Evaluative Sciences

ODD: Ontario Diabetes Database

CIHI: Canadian Institute for Health Information

OHIP: Ontario Health Insurance Plan

RPDB: Registered Persons Database

DCP: Data Creation Plan

Preface

This doctoral thesis is a 'sandwich' thesis consisting of an introductory chapter 1, three 'core' thesis chapters 2, 3 and 4 and a conclusion chapter 5. The introductory chapter introduces the three core thesis chapters of my PhD thesis. The three core chapters of the thesis include three different study designs/methods involved in data collection and analysis. This consists of a systematic review, a retrospective cohort study and a prospective cohort study protocol. I am the first author on all submitted or acceptable for submission manuscript and played a major role in study design, grant writing, data collection, data analysis and manuscript writing.

Chapter 1 is introduction where I give precise information of the core concepts involved in each of the study design. This is my own work.

Chapter 2 is published in the Clinical Journal of the American Society of Nephrology in Nov 2011.

Chapter 3 is resubmitted with minor revisions at Hypertension in pregnancy Journal and Chapter 4 will be submitted to a journal for peer review very soon. I have recognized contributions to these manuscripts in the "contributions by others" section on page 6 and 7 of the thesis.

Chapter 5 is the conclusion of my thesis. In this chapter, I summarize the findings in each of the core thesis chapters, recognize the major methodological limitations and wrap up with future directions. This is my own work.

Chapter 1

Introduction

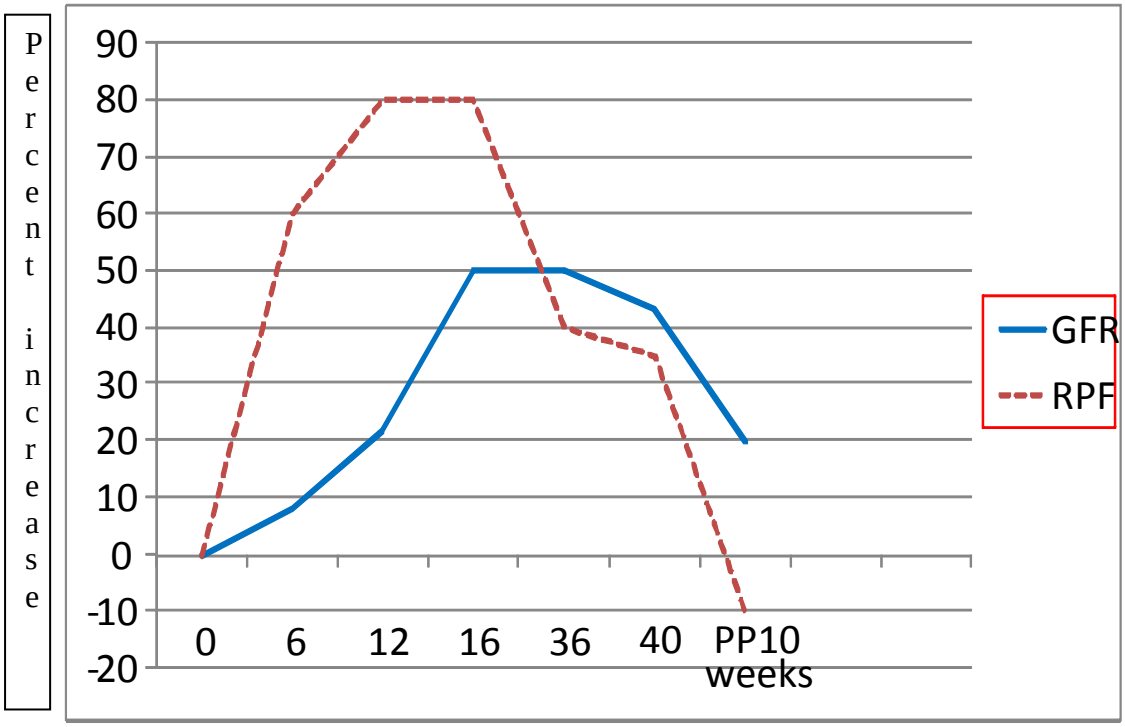
Hypertension in pregnancy is an important health risk for pregnant women but yet remains under explored. This is partly because it is considered a spectrum of disease rather than a single disease entity.[1] It is usually addressed as a spectrum of disease because it begins as new onset high blood pressure after 20 weeks of pregnancy called gestational hypertension followed by more severe forms called preeclampsia and eclampsia.[2] One of the many etiologies involved in the risk of developing hypertension during pregnancy include women with compromised kidney function.[3] Many of the physiological changes of pregnancy involves increased blood volume, renal plasma flow and glomerular filtration with decrease in blood pressure.[4] Even a subtle or unnoticed decline in renal function prior to pregnancy is exacerbated during pregnancy. This can in turn manifest as high blood pressure or spilling of protein in the urine. A major challenge in these women is that kidney disease is silent and can go undiagnosed until it manifests during pregnancy as high blood pressure or preeclampsia. Three renal conditions that can affect pregnancy outcomes due to hypertension in pregnancy include kidney disease, E. *coli* infections and kidney donation. As mentioned earlier, subclinical evidence of vascular injury that is not evident otherwise may be exacerbated during pregnancy. This doctoral thesis examines the risk of developing hypertension in pregnancy in women with such kidney conditions.

1.1 DEFINITIONS

Hypertension in pregnancy is considered as a composite of gestational hypertension, preeclampsia and eclampsia. Gestational hypertension is defined as new onset of hypertension (blood pressure ≥140/90 mmHg) after 20 weeks of pregnancy, whereas with proteinuria it is called preeclampsia.[5] In addition to new onset high blood pressure (≥140/90 mmHg) and protein in the urine after 20 weeks pregnancy, when women have general tonic-clonic seizures it is defined as eclampsia.[5]

1.2 PHYSIOLOGY OF NORMAL PREGNANCY

Figure 1: Physiologic changes during normal pregnancy



GFR: Glomerular filtration rate, RPF: Renal plasma flow, PP: Postpartum pregnancy

During normal pregnancy there is an increase in the renal plasma flow and glomerular filtration rate by 30-50% (**Figure 1**).[6] This parallels the increase in blood volume and cardiac output during pregnancy. There is also a profound increase in 24 hour creatinine clearance due to the dilatation of renal calyces, renal pelvis and ureters starting from the second trimester of pregnancy. The kidneys are key regulators of salt and water, and pregnancy is a time of net salt and water retention. Over the whole period of gestation, there is retention of about 7.5L of water and 900 mmol of sodium.[4] This would overburden women with a diseased kidney or those with one kidney, leading to perinatal complications. This led me to think about the association between the three kidney conditions and hypertension in pregnancy included in the core chapters of my thesis.

1.3 POPULATIONS AT POTENTIAL RISK OF DEVELOPING HYPERTENSION IN PREGNANCY

1.3.1 **Pregnancy Outcomes in Women with Chronic Kidney Disease**

Pregnancy with renal disease is relatively uncommon. The diagnosis of renal insufficiency before or during pregnancy is only 0.03% to 0.2%.[7, 8]However, pregnancy in this population is considered high risk.[9]

There have been a few case reports and case series that claim successful pregnancy outcomes with minimal perinatal complications in women with renal disease or on dialysis.[10-13] On the other hand, studies have shown that there has been increased risk of developing preeclampsia and preterm births in those with moderate to severe renal dysfunction.[14, 15] The variation in results may be attributable to the difference in

management and the underlying pathology. Though many experts conclude that it is best to avoid pregnancy in such a population, if pregnancy does occur, the precise risks involved are still obscure.

Conclusively, there is a paucity of scientific data regarding pregnancy outcomes in women with renal disease on which to base clinical management and counseling recommendations.[16] Though there have been a number of observational studies, no systematic review was done on this topic to date. While a number of observational studies have shown that women with CKD have an increased risk of developing adverse maternal and fetal outcomes, a robust synthesis of this information was lacking. I conducted a systematic review as Chapter 2 of my thesis to determine: 1) the risk of adverse maternal outcomes in women with CKD compared with women without CKD and 2) the risk of adverse fetal outcomes comparing the two groups of women.

1.3.2 **Walkerton Health Study: Pregnancy Outcomes**

In May of 2000, the municipal water supply of Walkerton, (Canada) became contaminated with *E. coli* O157:H7 from livestock fecal matter. Thousands of individuals who drank the contaminated water became severely ill; 27 developed hemolytic uremic syndrome and seven died. Although tragic, the unique circumstances of this outbreak provided a rare opportunity to study the natural history and long-term outcomes following exposure to this pathogen within a single large cohort. The Walkerton Health Study was launched in 2002 to follow the long-term outcomes following exposure to *E. coli*

O157:H7. Seven years after the Walkerton outbreak, adults with symptoms of severe acute gastroenteritis at the time of the outbreak were at higher risk of newly diagnosed hypertension and chronic kidney disease compared with those who were asymptomatic during the outbreak. A trend towards increased gestational hypertension was observed in the Walkerton Health Study; however, conclusions were weakened by the small number of events and large loss to follow-up.

Chapter 3 of this thesis was designed to conduct a more thorough investigation of hypertension in pregnancy in the decade following *E. coli* O157:H7 bacterial gastroenteritis. Specifically, the primary objective was to test the hypothesis that acute *E. coli* O157:H7 infection is associated with a composite outcome of gestational hypertension or preeclampsia in subsequent pregnancies over the following ten years. As a secondary objective the association between *E. coli* O157:H7 infection and the individual components of the composite outcome, gestational hypertension and preeclampsia were assessed separately. To do this, data from the Walkerton Health Study to Ontario healthcare databases were linked, which allowed a near-complete follow-up of study participants and allowed comparison to a group of female residents in surrounding rural communities that were unaffected by the outbreak.

Health care database is defined as electronic information collected for financing or record keeping purposes by the administrator of a health service, typically a government or a health insurance provider.[17, 18] Health care in Canada is funded and delivered through

publicly funded health care system. Canadians have universal access to health care. In this study, existing administrative and provincial government payer datasets routinely collected for publicly funded health services were used.

The Institute of Clinical Evaluative Sciences (ICES) is an independent, non-profit research organization and is located in Sunnybrook Health Sciences Centre in Toronto and Queen's University in Kingston. Since its inception in 1992, ICES has played a key role in providing scientific insights to the research community. Health information at ICES is not examined on an individual basis and thus solely used for research and statistical purposes. All data are kept confidential to protect the privacy of the individuals.

Diagnoses and procedures are coded within administrative databases to facilitate record retrieval and synthesis of information. The most commonly used coding system worldwide is the International Classification of Diseases (ICD), which is published and maintained by the World Health Organization (WHO). There are two ICD platforms in use: the International Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10), and the International Classification of Disease, Ninth Revision, (ICD-9). The Canadian Classification of Health Interventions (CCI) is a procedural coding scheme developed and maintained by the Canadian Institutes of Health Information Discharge Abstract Database (CIHI-DAD). Other codes frequently used in Ontario, Canada are the OHIP diagnoses and fee codes.

I used the following datasets housed at the Institute of Clinical Evaluative Sciences (ICES) for Chapter 3 of my thesis study: 1) Ontario Diabetes Database (ODD) is a validated administrative data registry of Ontario residents for whom a diagnosis of diabetes is recorded in hospital discharge information or in claims for outpatient physician services (through the Ontario Health Insurance Plan); 2) The Canadian Institute for Health Information (CIHI) collects and analyzes information on health and health care in Canada and makes it publicly available. Canada's federal, provincial and territorial governments created CIHI as a not-for-profit, independent organization dedicated to forging a common approach to Canadian health information. CIHI's data and reports inform health policies, support the effective delivery of health services and raise awareness among Canadians of the factors that contribute to good health. CIHI DAD receives data directly from participating hospitals and is a national database for information on all separations from acute care institutions, including discharges, deaths, sign-outs and transfers. Following its inception in 1963, when it was developed to collect data on separations from institutions in Ontario, it has expanded to provide coverage in all provinces except Quebec; 3) A resident of Ontario is entitled to health care services paid for by the Ontario Health Insurance Plan (OHIP). The Ministry of Health and Long-Term Care pays for a wide range of services covered by OHIP; 4) The Registered Persons Database (RPDB) is used in various ministry-processing systems to verify eligibility for services. A significant use of the data is in the fee-for-service medical claims system where claims can be paid to the provider if the patient has eligibility and a valid health

card; 5) The MOMBABY dataset is an ICES-derived dataset that links the CIHI/DAD inpatient admission records of delivering mothers and their newborns.

I created the Data Creation Plan (DCP) which is a form used to specify the variables required from the various datasets along with other information such as time frame, inclusion/exclusion criteria, type of statistical analysis to be used etc. The DCP submitted to ICES for the purpose of this study is attached to Chapter 3 Appendices of my thesis.

1.3.3 **Hypertension in Pregnancy after Kidney Donation**

Pregnancy outcomes after unilateral nephrectomy have been studied using animal models. Studies have demonstrated that reduced nephron mass is related to an increase in blood pressure and protein excretion in late pregnancy.[19, 20] An imbalance between the production of vasoconstrictor and vasodilatory products has long been deemed important in the development of hypertension in pregnancy.[21] In mice, uninephrectomy and pregnancy served as additive stimuli for renal hypertrophy which was dependent on Vascular Endothelin Growth Factor (VEGF).[22, 23] In rats, an increased risk of gestational hypertension and preeclampsia after unilateral nephrectomy was due to increased renal reactivity to angiotensin II involving 20-Hydroxyeicosatetraenoic Acid (20-HETE).[24]

In addition, human studies of living kidney donors depict higher serum uric acid and homocysteine levels after donation.[25] These factors may also increase the risk of hypertension during pregnancy. [26-28]

On this basis, is it possible that donating a kidney increases a woman's risk of developing hypertension in pregnancy?

Chapter 4 of my thesis is a study protocol that examines whether becoming pregnant with one kidney after donation increases the risk of hypertension in pregnancy. This study partners with the Living Kidney Donor (LKD) Study, a prospective cohort study designed to evaluate the long-term outcomes of living kidney donors. The LKD Study is actively recruiting live donors and healthy non-donors from 12 major transplant centres in Canada, with 391 female participants (259 donors and 132 non-donors) enrolled to date. We are inviting eligible female participants of the LKD Study to participate in the LKD Pregnancy Study.

This doctoral thesis studies a few pre-pregnancy renal conditions that can lead to complications during pregnancy. In order to identify complications in women with such kidney conditions, an estimate of the risk involved in each of these conditions should be readily available. I have attempted to provide such an estimate and/or a method to estimate risk in the following three chapters of my thesis.

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Chapter 2

Published Manuscript attached

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Pregnancy outcomes in women with chronic kidney disease: a systematic review.

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Pregnancy outcomes in women with chronic kidney disease: A systematic review

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ABSTRACT

Background and objectives: Pregnant women with chronic kidney disease (CKD) are at risk of adverse maternal and fetal outcomes. We conducted a systematic review of observational studies that described this risk.

Design, setting, participants, and measurements: We searched several databases from their date of inception through June 2010 for eligible articles published in any language. We included any study that reported maternal or fetal outcomes in at least five pregnant women in each group with or without CKD. We excluded pregnant women with a history of transplantation or maintenance dialysis.

Results: We identified 13 studies. Adverse maternal events including gestational hypertension, preeclampsia, eclampsia and maternal mortality were reported in 12 studies. There were 312 adverse maternal events among 2,682 pregnancies in women with CKD (weighted average of 11.5%) compared to 500 events in 26,149 pregnancies in normal healthy women (weighted average of 2%). One or more adverse fetal outcomes such as premature births, intrauterine growth restriction, small for gestational age, neonatal mortality, stillbirths and low birth weight were reported in nine of the included studies. Overall, the risk of developing an adverse fetal outcome was at least two times higher among women with CKD compared to those without.

Conclusions: This review summarizes current available evidence to guide physicians in their decision-making, advice and care for pregnant women with CKD. Additional studies are needed to better characterize the risks.

INTRODUCTION

Recent guidelines stage chronic kidney disease (CKD) according to levels of kidney function irrespective of the type of kidney disease [1]. Pregnancy in women with CKD, is considered high risk [2;3]. Diseased kidneys may be unable to adapt to the normal physiological changes of pregnancy leading to perinatal complications [4;5]. Knowledge of this risk guides patient counselling and follow-up. While a number of observational studies have shown that women with CKD have an increased risk of developing adverse maternal and fetal outcomes, a robust synthesis of this information is lacking [2;6-13]. We conducted this systematic review to determine: 1) the risk of adverse maternal outcomes in women with CKD compared to women without CKD (comparator group), and 2) the risk of adverse fetal outcomes comparing the two groups of women.

STUDY POPULATION AND METHODS

We conducted and reported this systematic review according to published guidelines using a pre-specified protocol [14;15].

Eligibility criteria

We included any observational study that reported maternal or fetal outcomes in five or more pregnant women with CKD and five or more pregnant women without CKD as a comparator group. Primary studies defined CKD as any of the following: abnormal serum creatinine / abnormal glomerular filtration rate (GFR) and / or proteinuria with a specific primary or secondary kidney disease. The comparator group consisted of women without

CKD and these women may or may not have had other co-morbidities (such as diabetes mellitus or systemic lupus erythematosus). Adverse maternal outcomes were as defined by the primary study authors, and included gestational hypertension, preeclampsia, eclampsia and maternal mortality. Adverse fetal outcomes included premature births, intrauterine growth restriction (IUGR), small for gestational age (SGA), neonatal mortality, stillbirths and low birth weight (LBW). We included full text papers and abstracts published in any language that reported at least one outcome of interest. We excluded studies of women with CKD with a history of kidney transplantation or maintenance dialysis, as well as studies of women with acute kidney injury or a single kidney.

Information sources

We designed and implemented a systematic literature search with the help of an experienced librarian. We searched the following electronic databases from the date of inception up to June 2010: MEDLINE and PreMedline (OVID, 1966 to 2010), EMBASE (OVID, 1980 to 2010), BIOSIS Previews (1969 to 2010), the ISI Science Citation Index Expanded (1981 to 2010), Cochrane Controlled Trials Register (Wiley InterScience, all years); SCOPUS (1966 to 2010), and specialty search engines Google Scholar and Elsevier's SCIRUS. The search strategy included a combination of keywords and MeSH terms and was adapted for each database to account for differences in indexing. We imposed no language restrictions or other limits in the search process. We also searched grey literature sources (nephrology conference proceedings and Web of Science database) and conference abstracts. We conducted citation tracking using SCOPUS and the ISI

Science Citation Index, and used related articles features in PubMed, OVID, Elsevier's Scirus and Google Scholar.

Study selection

Two reviewers (IN, AR) independently screened titles and abstracts. We retrieved the full text for any article considered potentially relevant by at least one reviewer. To ensure accuracy two reviewers then independently screened full texts articles for inclusion in this review. We resolved disagreements by discussion or with the help of a third reviewer (AD). We reviewed all non-English citations with the help of translators.

Data abstraction and analysis

Two reviewers (IN, AR) independently abstracted data using a standardized form that proved robust in pilot testing. This was done in duplicate to increase accuracy and reduce measurement bias [16]. We resolved any disagreements with the help of a third reviewer (AD). We abstracted the following data: a) study characteristics such as year of publication, country where study was conducted, study design, sample size, year of study and funding sources; b) methodological characteristics such as definitions of CKD and outcomes used, whether confounding variables were accounted for in the study, and whether the studies reported loss to follow-up; c) patient characteristics including the number of women and pregnancies in each group, mean age, race, whether the control group was normal healthy women or women with other co-morbidities but normal kidney function; d) the number of adverse events (both individual and as a composite) and any adjusted measures of association. Finally, we contacted the authors of the studies included

in the review for any missing data. We assessed agreement between two reviewers using the κ statistic. Kappa was calculated for full text eligibility [17]. We entered all data into Review Manager Version 5 [18].

RESULTS

Study selection

We screened and evaluated 4,917 citations, and assessed 156 full text articles for eligibility. The chance-corrected agreement for full text eligibility was good (estimated κ = 0.88). We excluded 104 studies because they had no comparator group, 30 were reviews, five had no outcomes of interest, two had no useable data, and two studies included women with acute kidney injury (Figure 1). Thirteen studies were eligible for review [2;7;8;12;19-27]. Twelve of the 13 studies described maternal outcomes and all 13 reported at least one fetal outcome of interest. We contacted six primary authors and two confirmed or provided additional data [20;25].

Description of studies, methods, and participants

Thirteen studies from seven countries reported at least one outcome of interest and followed a total of 28,917 pregnancies of whom 26,192 (range 8 to 20,034 across studies) had normal kidney function and 2,725 (range 7 to 1257 across studies) had CKD [2;7;8;12;19-24;26;27] (Table 1). Most studies were done in North America (n=7), followed by Europe (n=3), Japan (n=2) and South America (n=1). Of the 13 studies, eight provided a definition for CKD [8;12;20;22;24-27], two identified CKD through medical coding [2;19], and two

defined their CKD population through kidney biopsy [21;23]. One study did not report their definition of CKD [7]. Many of the studies did not provide a clear definition of the maternal outcomes studied [2;7;19-21;26;27]. Some women with CKD had a history of hypertension or proteinuria prior to pregnancy which may have influenced the ascertainment of outcomes such as preeclampsia [2;7;19;21;26;27]. Seven studies had a control group of normal healthy women [2;7;19;22;23;25;27] and the remaining six studies had a control group of women with other co-morbidities but with normal kidney function [8;12;20;21;24;26]. These co-morbidities included diabetes mellitus, hypertension in patients with IgA nephropathy and lupus nephritis (all with normal kidney function as defined by the primary authors). Two large retrospective studies included over 25,000 pregnancies [2;7]. The first of these studies included approximately 21,000 pregnancies but did not define CKD nor did it account for other variables which may have confounded the relationship between CKD and outcome [7]. The other large retrospective cohort study used an administrative database [2]. Of the thirteen studies, seven collected data prospectively [8;12;20;23-26] and six used pre-existing data from health records [2;7;19;21;22;27]. Seven studies accounted for potential confounding factors such as maternal age, parity, race, socioeconomic status, diabetes status, trimester of first antenatal visit, smoking, year of delivery, marital status, place of childbirth, hospital, attending clinician, maternal education, alcohol use, medication used during study period and early antenatal referral [2;8;19;20;22;25;27]. Of the seven studies, three studies used matching to control for confounding [19;22;27] whereas the remaining adjusted for potential confounders in multivariable analysis [2;8;20;25] (Table 1). Loss to follow up was reported in only one study and was less than five percent [25].

The mean age of women included in the studies was 28 years. In studies where race was reported, more than half of women were white [2;8;19;22;25;27] (Table 2 a, b). Serum creatinine levels were reported in five studies and ranged from 0.8 to 4.61 mg/dL (70 to 407 µmol/L) in women with CKD [8;20;22;24;27]. All studies except four reported outcomes on singleton pregnancies [7;21;23;26]. One study included a small percentage of women (5%) who had acute glomerulonephritis / acute renal failure [19]. Another study included a small percentage of renal transplant patients (<1%) [25]. We included these studies in the review as we deemed these numbers of ineligible women to be too small to have a significant influence on the results obtained.

Adverse maternal outcomes

Twelve studies reported adverse maternal outcomes including gestational hypertension, preeclampsia, eclampsia and maternal mortality (Figure 2, Table 3). Most studies demonstrated at least a two-fold increase in the risk of adverse maternal outcomes in women with CKD compared to those without (Figure 2). The overall adverse maternal events were five fold higher in women with CKD compared to women without CKD. There were 312 adverse events among 2,682 pregnancies in women with CKD (weighted average 11.5%) compared to 500 events in 26,149 pregnancies in women with no CKD (weighted average 2%). Two studies each followed over 5000 women with CKD and non-CKD groups combined [2;7]. All studies except one reported hypertensive disorders of pregnancy [7].

Adverse fetal outcomes

Fetal outcomes of interest such as premature births, IUGR, SGA, neonatal mortality, stillbirths and LBW were variably defined and reported in the studies [2;8;12;19;20;22-27] (Table 3). Nine studies reported premature births [7;8;12;19;21;22;24;26]. Premature birth was defined as birth <34 weeks [12;24], <36 weeks [8], <37 weeks gestation [19;20;22] , and was not defined in three of the studies [7;21;26]. In one study premature birth was defined as birth <37 weeks as a component in a composite outcome [2]. All nine studies reported 229 premature births among 1,760 pregnancies (weighted average 13%) in women with CKD, and 1,290 among 21,195 pregnancies (weighted average 6%) of women with no kidney disease. Compared to controls, the incidence of premature birth in women with CKD was consistently higher across all nine studies (and was statistically different in most studies). Compared to controls, the risk of IUGR in women with CKD was observed to be five times higher in one study [8] and the risk of SGA at least three-fold higher in two studies [12;26]. The risk of neonatal mortality was five fold higher in two studies [8;26], the risk of still-birth was nine fold higher in one study [27], and the risk of low birth weight was five fold higher in one study [26]. However, the total number of events in many studies was small, and the results across studies were not consistent (Table 3).

DISCUSSION

Over the past four decades, 13 studies have described the association between CKD and adverse maternal and fetal outcomes with the use of an internal comparator group. Women with CKD appear to have at least a two-fold higher risk of developing adverse

maternal outcomes compared to women without CKD. Similarly, premature births occurred at least twice as often in women with CKD compared to women without CKD. However, these data are derived mostly from small studies performed in a single-center and the studies were overall of low methodological quality. Definitions of CKD were quite variable and many studies did not report the most meaningful outcome such as maternal mortality. While there is consensus that women with CKD have a higher risk of adverse maternal and fetal outcomes compared to women without CKD, the magnitude of this risk is not clear. Nor is there clear evidence as to the degree of risk at various stages of CKD, information needed for informed consent in women considering the risk benefits of pregnancy. These results provide an impetus for future high quality, large multi-centre studies of pregnancy outcomes to better quantify risks in women with CKD (recognizing the time, effort and funding involved in such studies). There is a need to characterize the risk associated with low renal function separately from the comorbid conditions that can occur with CKD which also modify risk.

Our review has a number of strengths. It is the first systematic review and complements previous narrative reviews on this topic [28;29]. Many of the previous narrative reviews did not include studies with an internal comparator group [6]. We also identified two additional studies not included in previous reviews [20;25]. We did a comprehensive search to identify relevant literature in accordance to published guidelines and a pre-specified protocol. Two reviewers independently identified, selected, and abstracted data from articles to avoid potential biases. We considered studies published in any language. In addition, we were able to confirm data from some of the primary authors.

The quality of the primary studies inherently limits the conclusions that can be drawn from this review. Thus, the review serves to efficiently summarize past studies, but is not definitive as to what risks should be quoted to women with CKD. Confounding factors that may distort the association between CKD and adverse pregnancy outcomes were not addressed in six studies [7;12;21;23;24;26] and inadequately addressed in the remaining studies. Studies that included multiple pregnancies within the same women did not describe statistical techniques to account for correlated observations [8;12;21;22;24].

Often studies were retrospective and used administrative data where there was a possibility of misclassification, surveillance, selection and ascertainment bias [2;19]. Only one of the included studies reported loss to follow-up [25]. Also, while one would expect a dose-response relationship with worse pregnancy outcomes in more advanced CKD [30;31] this was not considered in most prior studies. CKD was defined using different criteria in the included studies. Only one study used the modern classification of CKD [25]. Other studies used serum creatinine and / or proteinuria, 24 hour creatinine clearance and kidney biopsy to define their population of interest [8;12;20-27] and two studies used medical coding [2;19]. One of the studies did not explicitly define their criteria for inclusion [7].

Another limitation of the included studies is that women were not completely free of the outcomes of interest prior to becoming pregnant. According to the International Society for the Study of Hypertension in Pregnancy (ISSHP), a research definition of preeclampsia is newly diagnosed hypertension after 20 weeks of pregnancy with well documented proteinuria [32]. Many of the women included in the studies had hypertension or proteinuria before pregnancy [2;8;12;19;22;24;26;27]. Finally, in addition to comparing

maternal and fetal outcomes between women with and without CKD, there is a clear need to compare maternal outcomes of CKD progression in women with CKD who do and do not become pregnant. The latter information is also central to informing the pregnancy choices of women with CKD.

Given the potential for risk, women with CKD who wish to become pregnant should have preconception counselling and antenatal care with a multidisciplinary "high-risk pregnancy" team. This review summarizes key published information on the estimated risk of adverse maternal and fetal outcomes in women with CKD compared to women without CKD. This review is an efficient way for clinicians to become aware of the current published literature and to understand the limitations of available literature. They can integrate the information with their clinical expertise when counselling women with CKD about pregnancy. The results of this review provide a foundation for future studies to better characterize the risks.

CONCLUSIONS

This systematic review of observational studies highlights a higher risk of adverse maternal and fetal outcomes in women with CKD compared to women without CKD. However, well-designed and methodologically rigorous studies are needed to better estimate the magnitude of this risk. Such studies could provide important insights into ways of counselling women with CKD. In the meantime, it would be rational to use the findings of our review to design such robust studies.

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Table 1: Study characteristics¥

Study (year)	Country of study	Study design	Total # of pregnancies studied, n‡	Year of study	Definition of Chronic kidney disease	Defined maternal outcomes	Defined fetal outcomes	Accounted for potential confounding factors
Gazarek F, 1966 [7]	Czechoslovakia	Retrospective cohort	21291	Not reported	Not reported	Maternal mortality not defined	Premature births not defined	No
Houser MT, 1979 *[21]	United States	Retrospective cohort	16	Not reported	Biopsy	Toxemia not defined	Premature births not defined	No
Leppert P, 1979 [22]	United States	Retrospective cohort	145	1974-1976	Biopsy, sr.cr>1.2 mg/dl, persistent proteinuria +1	Gestational hypertension defined as BP≥140/85 mmHg at the 3rd trimester; preeclampsia defined as BP≥ 140/90 mmHg or ≥ 2+proteinuria and edema occurring beyond the 20th week of pregnancy	Preterm defined as <37 weeks gestation at child birth, stillbirths defined as fetal death occurring beyond 28 weeks gestation; SGA, neonatal mortality, low birth weight not defined	Matched on age ±5 years, race, SES, hospital
Nagai Y, 1989 [24]	Japan	Prospective cohort	19	Not reported	Biopsy, sr.cr, 24 hour cr.cl, proteinuria	Hypertension defined as BP of ≥140/90 mmHg at the time of delivery	Low birth weight defined as <2500g birth weight	No
Kimmerle R, 1995 [12]	Germany	Prospective cohort	146	1982-1992	Cr. cl <80 mg/ml, proteinuria >400mg/day or ≥1+ dipstick in absence of urinary tract infection and other causes of kidney disease	Preeclampsia defined as acute worsening of hypertension (>15% diastolic pressure) in the presence of proteinuria >3 g/day and generalised edema	Preterm birth defined as <34 weeks gestation, Small for gestational age defined as birth weight less than the 10th percentile; stillbirth not defined	No
Holley JL, 1996 [8]	United States	Prospective cohort	86	1991-1993	Sr.cr≥0.8 mg/dl in the first trimester, or proteinuria≥300 mg/24 hours, with known kidney disease	Not reported	Premature births <36 weeks gestation; intrauterine growth restriction and neonatal mortality not defined	Age, race, parity, diabetes status
Rosenn B, 1997 *[26]	United States	Prospective cohort	408	1978-1993	Diabetic nephropathy defined as proteinuria >500 mg in 24 hours prior to 16 weeks gestation with no bacteriuria	Preeclampsia not defined	Premature births, IUGR/SGA, neonatal mortality, low birth weight not defined	No
Fink J, 1998 [19]	United States	Retrospective cohort	675	1987-1993	ICD -9 codes including diabetic and hypertensive nephropathy, acute and chronic	Preeclampsia and eclampsia not defined	Premature births <37 weeks gestation; SGA defined using William et al [33]method; neonatal death defined as infant death within 28 days of birth	Adjusted for maternal age, trimester of first prenatal visit, parity and smoking. Year of delivery was matched.

					glomerulonephritis, nephrotic syndrome, acute and chronic renal failure, disorders with impaired renal function, small kidneys of unknown cause, renal agenesis and cystic diseases			
Murakami S, 2000 [23]	Japan	Prospective cohort study	86	1980-1999	Biopsy	Preeclampsia defined as proteinuria >300mg in 24 hour urine collection and BP>160/110mmHg	SGA not defined	No
Fischer M, 2004 [2]	United States	Retrospective cohort	5517	1989-2001	Medical coding	Preeclampsia, eclampsia or abruptio placenta not defined	Prematurity <37 weeks gestation, Neonatal mortality death of new born <28 days, low birth weight <2500 grams	Maternal age, parity, race, marital status, place of birth, attending clinician, maternal education, cigarette and alcohol use
Trevisan G, 2004 [27]	Brazil	Retrospective cohort	75	1989-1999	Sr.cr ≥1.5mg/dl	Preeclampsia not defined	Stillbirth defined as dead fetus	Maternal age, gestational age, time of delivery were matched between the comparison groups.
Gladman D, 2010 [20]	Canada	Prospective study	120	1970-2003	Sr.cr>120mmol/l 6 months prior to pregnancy until outcomes, proteinuria>500mg/24 hours, nephrotic syndrome	Gestational hypertension , preeclampsia and maternal mortality not defined	Low birth weight defined as below the 10th percentile for sex and gestational age; Stillbirth as death of fetus in utero past 20 weeks gestation; perinatal death defined as neonate death within 7 days of birth	Adjusted for medication used in the study period (if sample size is large enough), otherwise adjusted only for repeated measures
Piccoli G, 2010 [25]	Italy	Prospective study	333	1999-2007	As defined by the KDOQI guidelines	Preeclampsia defined as the appearance of hypertension (≥140/90 mmHg) with proteinuria ≥300mg/24 hrs after 20 weeks gestation in a previously normotensive women; maternal mortality not defined	Small for gestational age defined as birth weight below the 10th percentile according to Italian birth weight references; Neonatal mortality not defined	Maternal age, parity, race, early antenatal referral

¥ Studies ordered as per year of publication. Loss to follow-up was reported in one study as less than 5% [25]. Only two studies reported funding of which one was funded by the US public health service grant, NIH Clinical research grant, Ciba pharmaceutical company [22] and the other by NIH, clinical research training in kidney disease [19]

‡ number of pregnancies studied in women with and without chronic kidney disease

* published abstracts

Abbreviations: ICD-9: international classification of diseases-9; Sr.cr: serum creatinine; Cr.cl: creatinine clearance; SES: socioeconomic status; IUGR: intrauterine growth restriction; SGA: small for gestational age

Conversion factors for units: serum creatinine in mg/dL to µmol/L, x88.4.

Table 2a: Baseline characteristics (comparator group healthy women with normal kidney function)

Study, year	Women with chronic kidney disease				Healthy women with normal kidney function			
	# of women studied, n	# of pregnancies studied, N	Age (years), mean (SD)	White race, (%)	#of women studied, n	# of pregnancies studied, N	Age (years), mean (SD)	White race, (%)
Gazarek F, 1966 [7]	1257	1257	(...)	(...)	20034	20034	(...)	(...)
Leppert P, 1979 [22]*	7	9	(...)	54	39	80	(...)	54
Fink J, 1998 [19]*	169	169	(...)	73	506	506	(...)	83
Murakami S, 2000 [23]	19	19	(...)	(...)	67	67	(...)	(...)
Fischer M, 2004 [2]*	911	911	(...)	93	4606	4606	(...)	92
Trevisan G, 2004 [27]*	25	25	29 (5)	92	50	50	29 (6)	87
Piccoli G, 2010 [25]*	91	91	31 (5)	89	267	267	29 (5)	77

*Multiple gestations (twins, triplets etc) were excluded from the study

(...) not reported or reported in a way from which data cannot be extracted

Abbreviations used:

SD: standard deviation

%: percentage

N, n =number

Table 2b: Baseline characteristics (comparator group women with normal kidney function but other co-morbidities)

Study, year	Women with chronic kidney disease				Women with normal kidney function but with other co-morbidities				
	# of women studied, n	# of pregnancies studied, N	Age (years), mean (SD)	White race, (%)	Co-morbidity	# of women studied, n	# of pregnancies studied, N	Age (years), mean (SD)	White race, (%)
Houser MT, 1979 [21]	6	7	(...)	(...)	lupus nephritis	5	9	(...)	(...)
Nagai Y, 1989 [24]*	10	11	(...)	(...)	IgA nephropathy	7	8	(...)	(...)
Kimmerle R, 1995 [12]*	33	36	29 (5)	(...)	diabetes	91	110	28 (4)	(...)
Holley JL, 1996 [8]*	40	43	29 (6)	84	diabetes	43	43	28 (5)	84
Rosenn B, 1997 [26]	73	73	27 (5)	(...)	diabetes	335	335	25 (5)	(...)
Gladman D, 2010 [20]*	81	81	28 (5)	(...)	lupus nephritis	112	112	31 (5)	(...)

*Multiple gestations (twins, triplets etc) were excluded from the study

(...) not reported or reported in a way from which data cannot be extracted

Abbreviations used:

SD: standard deviation

%: percentage

N, n =number

Table 3: Adverse maternal and fetal outcomes

Study	Maternal hypertension[a]		Maternal mortality		Premature births[§]		IUGR		SGA[+]		Neonatal mortality[¥]		Stillbirths[‡]		Low birth weight[†]	
	CKD	No CKD	CKD	No CKD	CKD	No CKD	CKD	No CKD	CKD	No CKD	CKD	No CKD	CKD	No CKD	CKD	No CKD
Gazarek F, 1966 [7]	-	-	44/1257 (4) *	181/20034 (1) *	111/1257 (8) *	1165/20034 (6) *	-	-	-	-	-	-	-	-	-	-
Houser MT, 1979 [21]	2/7 (29) *	2/8 (25) *	-	-	2/7 (29) *	0/9 (0) *	-	-	-	-	-	-	-	-	-	-
Leppert P, 1979 [22]	6/88 (7)	2/57 (4)	-	-	2/114 (2)	1/80 (1)			1/114 (1) *	0/80 (0) *	0/114 (0) *	0/80 (0) *	0/114 (0)	2/80 (3)	3/114 (3) *	0/80 (0) *
Nagai Y, 1989 [24]	5/11 (45)	4/8 (50)	-	-	1/11 (9)	0/8 (0)	-	-	-	-	-	-	-	-	1/11 (9)	1/8 (13)
Kimmerle R, 1995 [12]	7/36 (19)	3/110 (3)	-	-	11/36 (30)	3/110 (3)	-	-	8/36 (22)	2/110 (2)	-	-	0/36 (0) *	0/110 (0) *	-	-
Holley JL, 1996 [8]	-	-	-	-	7/43 (16)	4/43 (9)	2/43 (5) *	0/43 (0) *	-	-	3/43 (7) *	0/43 (0) *	-	-	-	-
Rosenn B, 1997 [26]	32/73 (44) *	54/335 (16) *	-	-	38/73 (52) *	74/335 (22) *	-	-	8/73 (11) *	13/335 (4) *	4/73 (5) *	3/335 (1) *	-	-	31/73 (42) *	27/335 (8) *
Fink J, 1998 [19]	55/169 (33)	31/506 (6)	-	-	37/169 (22)	27/506 (5)	-	-	34/169 (20) *	25/506 (5) *	5/169 (3)	1/506 (0)	-	-	-	-
Murakami S, 2000 [23]	15/19 (79)	18/67 (27)	-	-	-	-	-	-	4/19 (21) *	34/67 (51) *	-	-	-	-	-	-
Fischer M, 2004 [2]	125/911 (14)	197/4606 (4)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trevisan G, 2004 [27]	10/25 (40) *	3/50 (6) *	-	-	-	-	-	-	-	-	-	-	9/25 (36)	2/50 (4)	-	-
Gladman D, 2010 [20]	10/70 (14) *	2/50 (4) *	0/70 (0) *	0/50 (0) *	20/50 (40)	16/70 (23)	-	-	-	-	1/47 (2)	2/67 (3)	3/50 (6)	3/70 (4)	18/47 (38)	13/67 (19)
Piccoli G, 2010 [25]	1/36 (3)	3/97 (3)	0/36 (0) *	0/97 (0) *	-	-	-	-	15/91 (16)	28/267 (10)	0/91 (0) *	0/267 (0) *	-	-	-	-

All numbers in Table 3 are reported as: Number of events/Number of pregnancies (percentage), Percentages are rounded to the nearest whole number
(-) data not reported in primary article or not extractable
*outcomes not defined in primary article
[a] Maternal hypertension defined as ≥140/85 mmHg in the third trimester [22], ≥140/90 mmHg at the time of delivery [24], accelerated hypertension, edema or hypoalbuminemia [12], ICD 9 medical coding [19], onset of preeclampsia before 30 weeks gestation and proteinuria at 6 weeks postpartum [23], a composite of preeclampsia, eclampsia and abruptio placenta [2], preeclampsia defined as appearance of hypertension with systolic blood pressure ≥140 mmHg or diastole≥90 mmHg with proteinuria ≥300 mg/24 hours after 20 weeks gestation [25]
[§] Premature births defined as <34 weeks [12;24], <36 weeks [8], <37 weeks [19;20;22] gestation at the time of childbirth

+ Small for gestational age (SGA) defined as birth weight lower than the 10th percentile [12], and birth weight below 10th percentile according to Italian birth weight references [25]
¥ Neonatal mortality defined as neonate death within 7 days of birth [20], infant death within 28 days of birth [19]
‡ Stillbirths defined as fetal death [27], fetal death occurring beyond 28 weeks gestation [22], death of fetus in utero past 20 weeks gestation [20]
ǂ Low birth weight defined as <2500 grams birth weight [24], defined as below the 10th percentile for sex and gestational age [20]

Figure 1: Study Selection

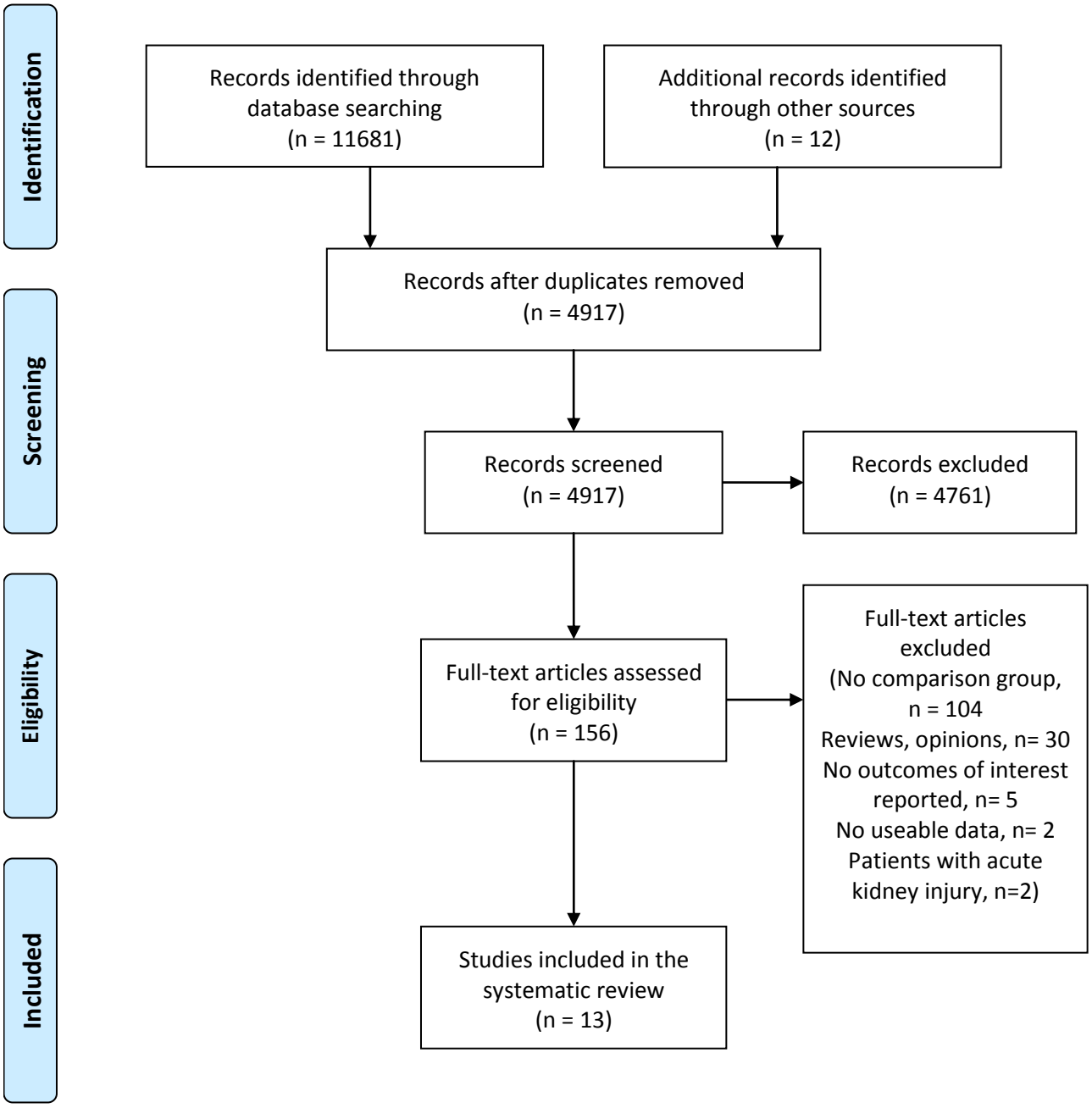
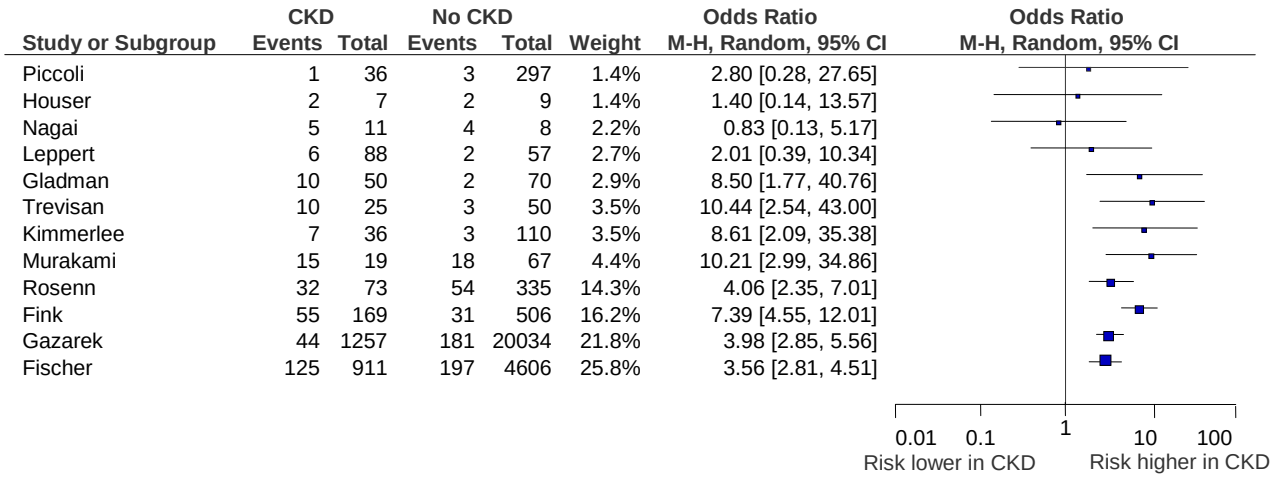


Figure 2: Adverse Maternal Outcomes (gestational hypertension, preeclampsia, eclampsia and maternal mortality)



Chapter 3

Manuscript resubmitted to Hypertension in Pregnancy Journal attached.

Hypertension in Pregnancy after Escherichia coli O157:H7 Gastroenteritis: A Cohort Study

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Running Title: Nevis et al: *Hypertension in pregnancy after E. coli* O157:H7 *gastroenteritis*

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Abstract

Background: *Escherichia coli* O157:H7 is a common cause of bacterial gastroenteritis and may increase the risk of hypertension. We studied the risk of hypertension in pregnancy following a large *E. coli* O157:H7 outbreak that occurred in Walkerton, Canada in the year 2000.

Methods: We linked data collected from Walkerton residents to provincial healthcare databases. We studied the pregnancies of three groups of women: two groups from Walkerton (those with and without acute gastroenteritis during outbreak) and a third group from neighbouring rural communities unaffected by the outbreak. The primary outcome was a composite of gestational hypertension or preeclampsia. Secondary outcomes were gestational hypertension and preeclampsia examined separately.

Results: The median time to pregnancy after cohort entry was five years. The composite outcome was not significantly higher among women with gastroenteritis during the outbreak compared with residents of neighbouring communities [8 of 117 (6·8%) vs. 96 of 2166 (4·4%) pregnancies respectively; adjusted relative risk 1·5 (95% confidence interval (CI) 0·8 to 3·2)]. When examined separately the risk of preeclampsia was significantly higher among women with gastroenteritis [4 of 117 (3·4%) vs. 17 of 2166 (0·8%) pregnancies; adjusted relative risk 3·8 (95% CI 1·3 to 11·6)]. However, the risk of preeclampsia was lower than expected in the referent group and overall there were a small number of events in all the groups.

Conclusion: There was no significant association between *E. coli* O157:H7 gastroenteritis and our primary assessment of hypertension in pregnancy.

INTRODUCTION

For the past three decades, *E. coli* O157:H7 outbreaks have occurred with increasing frequency worldwide.[1] The Centers for Disease Control and Prevention estimate that *E. coli* O157:H7 infections cause approximately 73,000 gastro-enteric illnesses annually in the US, resulting in over 2000 hospitalizations and 60 deaths with 13 major outbreaks occurring since 2006.[2;3] A similar toxigenic strain, *E. coli* O104:H4, was responsible for the recent outbreak in Germany (May, 2011), causing 3792 cases of acute gastroenteritis and 43 deaths.[4] These Shiga-toxin-producing bacteria can cause both renal and vascular injury.[5] While the short-term effects of *E. coli* O157:H7 infection are well described, the long-term sequelae are largely unknown due to the difficulty of studying a wide geographic dispersal of cases.

In May of 2000, the municipal water supply of Walkerton, (Canada) became contaminated with *E. coli* O157:H7 from livestock manure.[6] Thousands of individuals who drank the contaminated water became severely ill; 27 developed hemolytic uremic syndrome and 7 died. The Walkerton outbreak attracted international media attention as the most serious water-contamination outbreak in recent history. Although tragic, the unique circumstances of this outbreak provided a rare opportunity to study the natural history and long-term outcomes following exposure to this pathogen within a single large cohort.[7] We launched the Walkerton Health Study in 2002 to follow the long-term outcomes following exposure to *E. coli* O157:H7.[8] We have previously demonstrated that

seven years after the Walkerton outbreak, adults with symptoms of severe acute gastroenteritis during the outbreak were at higher risk of newly diagnosed hypertension and chronic kidney disease compared with those who were asymptomatic during the outbreak.[7;9] We also observed a trend towards increased gestational hypertension among female participants of the Walkerton Health Study; however, conclusions were weakened by the small number of events and large loss to follow-up.[10] We designed the present study to conduct a more thorough investigation of hypertension in pregnancy in the decade following *E. coli* O157:H7 gastroenteritis. To do this, we linked data from the Walkerton Health Study to Ontario healthcare databases, which allowed a complete follow-up of study participants and a comparison to a group of female residents in surrounding rural communities that were unaffected by the outbreak. Specifically, the primary objective was to test the hypothesis that acute *E. coli* O157:H7 infection is associated with a composite outcome of gestational hypertension or preeclampsia in subsequent pregnancies over the following ten years. As a secondary objective we assessed the association between *E. coli* O157:H7 gastroenteritis and the individual components of the composite outcome, gestational hypertension and preeclampsia, separately.

METHODS

Design, Setting, Participants and Data Sources

In this community-based cohort study we linked multiple data sources to identify comparison groups, ascertain baseline characteristics, and obtain outcomes: 1) The *Walkerton Health Study* (2002) contains information on acute gastrointestinal illness at the time of the outbreak,[11] 2) The Canadian Institute for Health Information *Mom-Baby Linked Dataset* and *Discharge Abstract Database* contains records of childbirth and diagnostic and procedural information for all hospitalizations in Ontario, 3) The *Ontario Health Insurance Plan* (OHIP) database contains all health claims for inpatient and outpatient physician services, and 4) The *Ontario Registered Persons Database* contains demographic and vital status information for all Ontario residents.[12] The databases and the linked dataset were held securely in a de-identified form and were analyzed at the Institute for Clinical Evaluative Sciences. These databases have been used extensively in population-based health outcomes research and are essentially complete for the study variables. We used the 9^{th} revision of the International Classification of Diseases (ICD 9) codes to identify health conditions and outcomes until 2002 followed by the 10^{th} revision of the International Classification of Diseases (ICD 10) codes until end of follow-up.[13] The design and reporting of this study follows recommended guidelines for observational studies (Appendix S1- A).[14]

We compared pregnancy outcomes across three groups of women. Two groups were from the Walkerton cohort: females with and without acute gastroenteritis at the time of the outbreak. As Walkerton is a small rural town in southwestern Ontario (population 4851 in 2001), the third group was selected to contain female residents of neighbouring rural

towns that were unaffected by the outbreak (referent group). These rural towns were similar to Walkerton in terms of demographics, employment, and healthcare services (Exeter, Listowel, Mount Forest, Penetanguishene, Shelburne, and St. Mary's: Appendix S1-B).

Cohort entry dates in the Walkerton Health Study were from January 3, 2002 to December 11, 2002. Residents of neighbouring towns were randomly assigned a cohort entry date according to the distribution of such dates in Walkerton participants. The same inclusion and exclusion criteria were applied to all groups (Figure 1). We included women who were between the ages of 10 and 42 at cohort entry, and who carried at least one singleton pregnancy until at least 20 weeks gestation after cohort entry. Women who were pregnant at cohort entry were not eligible to participate. We excluded women with history of gastrointestinal disease before the outbreak because the assessment of new acute gastrointestinal symptoms may not necessarily be attributed to the outbreak. To reduce the potential for confounding, we excluded women with a pre-pregnancy history of hypertension, diabetes, or chronic kidney disease, or a past history of hypertension during pregnancy using database codes. We included only singleton pregnancies that occurred during follow-up (i.e. we excluded < 0.1% of participants in each group with multiple-birth pregnancies). We restricted our observations to the first pregnancy after cohort entry in sensitivity analyses. Follow-up continued until March 31, 2011.

Outcomes

The primary outcome was a composite of any gestational hypertension *(systolic/diastolic blood pressure of >140/90 mmHg at or after 20 weeks of pregnancy)* or preeclampsia *(gestational hypertension with proteinuria)* as assessed with validated database codes (Appendix S1- C). Given an incidence of 0·06%, no events of eclampsia were expected nor observed.[15] The secondary outcomes were gestational hypertension and preeclampsia examined separately. Other outcomes included prematurity, low birth weight, maternal death, still birth *(fetal death after 22 weeks of gestation)*, neonatal death *(death of a newborn within 1 to 28 days of birth)*, and perinatal deaths *(defined in this study as stillbirths and neonatal deaths)* (detailed in Appendix S1-D).

Statistical Analysis

We used SAS version 9.1.3 (SAS Institute Inc., Cary NC) for all analyses. In addition to providing descriptive baseline characteristics for the three groups—reported as mean (standard deviation [SD]) or count (percent), we compared baseline characteristics using analysis of variance (ANOVA) or Kruskal-Wallis for continuous variables depending on the distribution, and χ^2 for categorical variables. The p values for the characteristics of pregnancies were calculated using generalized estimated equations. We estimated the relative risks, corresponding 95% confidence intervals and associated p-values for study outcomes from log-binomial models using generalized estimating equations to account for the potential within-woman clustering from additional pregnancies during follow-up. We set the level of significance at alpha = 0·05 and adjusted for the following risk factors for hypertension in pregnancy: age at the time of pregnancy (per year), parity, a measure

of comorbidity (Johns Hopkins Aggregated Diagnosis Groups) [16] and socioeconomic status (assessed using neighbourhood income).[17;18]

RESULTS

Participant selection is presented in **Figure 1**. Of 2220 Walkerton females who joined the study in 2002, 964 were between the ages of 10 and 42 at the time of the cohort entry. From 2002 to 2011, we identified 171 eligible pregnancies in 113 Walkerton females; 117 pregnancies in women with acute gastroenteritis during the outbreak and 54 in those without gastroenteritis. We identified 2166 eligible pregnancies in 1,416 female residents of neighbouring towns (referent group). The median time to pregnancy after cohort entry was five years. Characteristics of women and pregnancies at the time of cohort entry and pregnancy are presented in **Table 1**. The average age at study entry (24 years) and at childbirth (28 years) was similar across groups. Compared with the referent group, women with gastroenteritis were more likely to be in the middle neighbourhood income category and have more comorbidity. The number of previous pregnancies and time since last childbirth did not differ across groups. Health surveillance during pregnancy is presented in the second part of Table 1. Women affected with gastroenteritis during the outbreak had on average one more prenatal visit compared with the referent group (10·5 vs. 9·4; *P=0·01*). However, the number of antenatal (abdominal and pelvic) ultrasounds did not differ across groups. Also, when restricted to the first pregnancy after study entry,

the number of prenatal visits did not differ statistically among the two groups **(Appendix S1-E)**.

Hypertension in Pregnancy

The composite outcome (gestational hypertension or preeclampsia) was not significantly higher among women with a history of gastroenteritis compared with the referent group [8 of 117 (6·8%) vs. 96 of 2166 (4·4%) pregnancies, respectively; adjusted relative risk 1·5 (95% confidence interval (CI) 0·8 to 3·2)] (**Table 2**). When considered separately, gestational hypertension was not significantly higher [6 of 117 (5·1%) vs. 79 of 2166 (3·6%) pregnancies; adjusted relative risk 1·5 (95% CI, 0·6 to 3·3)]; however, the risk of preeclampsia was significantly higher [4 of 117 (3·4%) vs. 17 of 2166 (0·8%) pregnancies; adjusted relative risk 3·8 (95% CI 1·3 to 11·6)]. We found similar results when we considered only the first pregnancy after study entry (**Appendix S1-F)**.

Other Perinatal Outcomes

Other outcomes did not differ significantly among women with a history of gastroenteritis and residents of neighbouring towns. The number of events observed among the two groups and their percentages were as follows: prematurity 9 (7·7%) vs. 114 (5·3%) *(p value: 0·22)*; low birth weight 6 (5·1%) vs. 71 (3·3%) *(p value: 0·20)*; maternal death 0 (0·0%) vs. 0 (0·0%); still birth 0 (0·0%) vs. 8 (0·4%); neonatal death 0 (0·0%) vs. 7 (0·3%); and perinatal deaths 0 (0·0%) vs. 14 (0·6%).

DISCUSSION

We studied pregnancy outcomes during the decade following an *E. coli* O157:H7 water-contamination outbreak in Walkerton, Ontario (Canada). There was no significant association between *E. coli* O157:H7 gastroenteritis and the composite risk of gestational hypertension and preeclampsia in subsequent pregnancies. Although the association with preeclampsia was statistically significant, the interpretation must be cautious for reasons described below.

E. coli O157:H7 can cause both renal and systemic vascular injury and endothelial dysfunction.[19] Pregnancy places additional demands on the kidney, and a sub-clinical renal injury from *E. coli* O157:H7 infection could be exacerbated by the physiologic demands of pregnancy.[20] During pregnancy, kidney blood flow increases by 50-80%, and the glomerular filtration rate increases by 50%.[20;21] Although the pathogenesis of preeclampsia is multi-factorial with complex interactions among the placental, immunologic, and vascular systems, the kidneys play an important role.[20] Chronic kidney disease increases a woman's risk of developing the disorder by two to four fold, and endothelial dysfunction is increasingly recognized as a causal risk factor for preeclampsia.[22;23] Most agree that preeclampsia represents an abnormal vascular response to placentation, however recent research suggests that preeclampsia is the result of heterogeneous causes.[24]

Despite intensive study, hypertension in pregnancy remains a life-threatening condition and accounts for 16% of maternal mortality in developed countries and also increases the risk of premature delivery and fetal death.[25;26] The current standard of care for pregnant women includes judicious blood pressure assessment throughout pregnancy.[27] Some serum biomarkers show promise for predicting preeclampsia, and a recent study supports the role of measuring urinary podocytes in early pregnancy.[28] If infection from toxigenic *E. coli* such as O157:H7 were to increase the risk of preeclampsia years later, then women with a history of such infection may benefit from additional screening if such tests were to become a standard of care.

The unique circumstances of the Walkerton outbreak provided a rare opportunity to study the natural history following exposure to *E. coli* O157:H7 within a single cohort. Because this study originated in the context of an environmental disaster, there are several associated limitations. As in other outbreak situations, the contaminated water contained multiple bacteria, including *Campylobacter jejuni*, which may have contributed to exposure misclassification. However, the infectious dose of *E. coli* O157:H7 is much lower than *C. jejuni* (10-100 cells vs. 500-10,000 cells), and it is unlikely that those with gastroenteritis were not exposed to *E. coli* O157:H7.[29] Unlike *E. coli* O157:H7, *C. jejuni* is only rarely associated with renal sequelae, and therefore the effect of any exposure misclassification may be in the direction of the null hypothesis.

Our study has limitations. The low number of pregnancies in follow-up and low number of events means the confidence intervals of our estimates are wide, leading to uncertainty about true associations. Similar studies in other cohorts with a larger number of events will be useful; however, such studies will be difficult to conduct due to the unpredictability of outbreak occurrences and geographic dispersal of cases. The rates of preeclampsia and other maternal and fetal outcomes in the referent group were lower than expected.[30;31] However, we specifically excluded women with a history of hypertension, diabetes, or chronic kidney disease, and therefore our cohort was selected to be healthier than the general population. Differences in outcome incidence between this study and others may also be explained by differences in the methods, and diagnostic codes used to identify the outcomes.[32] In particular, ICD-10 diagnostic codes have low sensitivity to identify gestational hypertension. Conversely, it may be argued that the low rate of preeclampsia among the referent group may reflect the changing incidence rates of preeclampsia in developed nations [33] and the higher rate of preeclampsia observed among the Walkerton group may be an effect of small sample size. Adjustment for potential confounders such as age, parity, comorbidities and socio-economic status did not substantively affect the measures of association in the current study; however, we were unable to compare risk factors such as family history of preeclampsia, pre-pregnancy body mass index, and pre-pregnancy renal function because they were unavailable in our data sources. Rather, to minimize the effect of confounding we excluded women with pre-pregnancy hypertension, diabetes, chronic kidney disease or past history of hypertension during pregnancy. The number of antenatal visits, though significantly

different between groups, was not added to the multivariable model because it may be an intermediate variable on the causal pathway. Compared with female residents of neighbouring rural towns that were unaffected by the outbreak, Walkerton females with acute gastroenteritis had statistically non-significant higher rates of gestational hypertension, premature delivery, and delivery of a low birth weight infant. Although these outcomes may relate to endothelial dysfunction, we lacked the statistical power to rule out clinically important differences on these outcomes.

Despite these limitations, we were able to track pregnancy outcomes within a large, well-defined community-based cohort for nine years. We defined exposure using a previously validated definition of acute gastroenteritis that incorporated information from both public health records and medical records.[11] We assessed the study outcomes using validated healthcare codes with high specificities and positive predictive values.[34]

CONCLUSIONS

In summary, we observed no significant association between *E. coli* O157:H7 gastroenteritis and the composite risk of gestational hypertension and preeclampsia in subsequent pregnancies. Outbreaks of toxigenic *E. coli* continue to occur worldwide, and our analysis of the Walkerton Health Study provides new information on the risk for hypertension in pregnancy after *E. coli* O157:H7 gastroenteritis.

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Financial Disclosures: None

Ethics approval: We obtained ethics approval from the Western University's Research Ethics Board, and obtained written consent from Walkerton participants for the linked analyses.

Role of the Sponsor: The opinions, results and conclusions reported in this paper are those of the authors and are independent of the funding sources.

Declaration of interest: The authors report no conflict of interest.

Number of words in main body: 2549

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Figure 1: Participant Selection

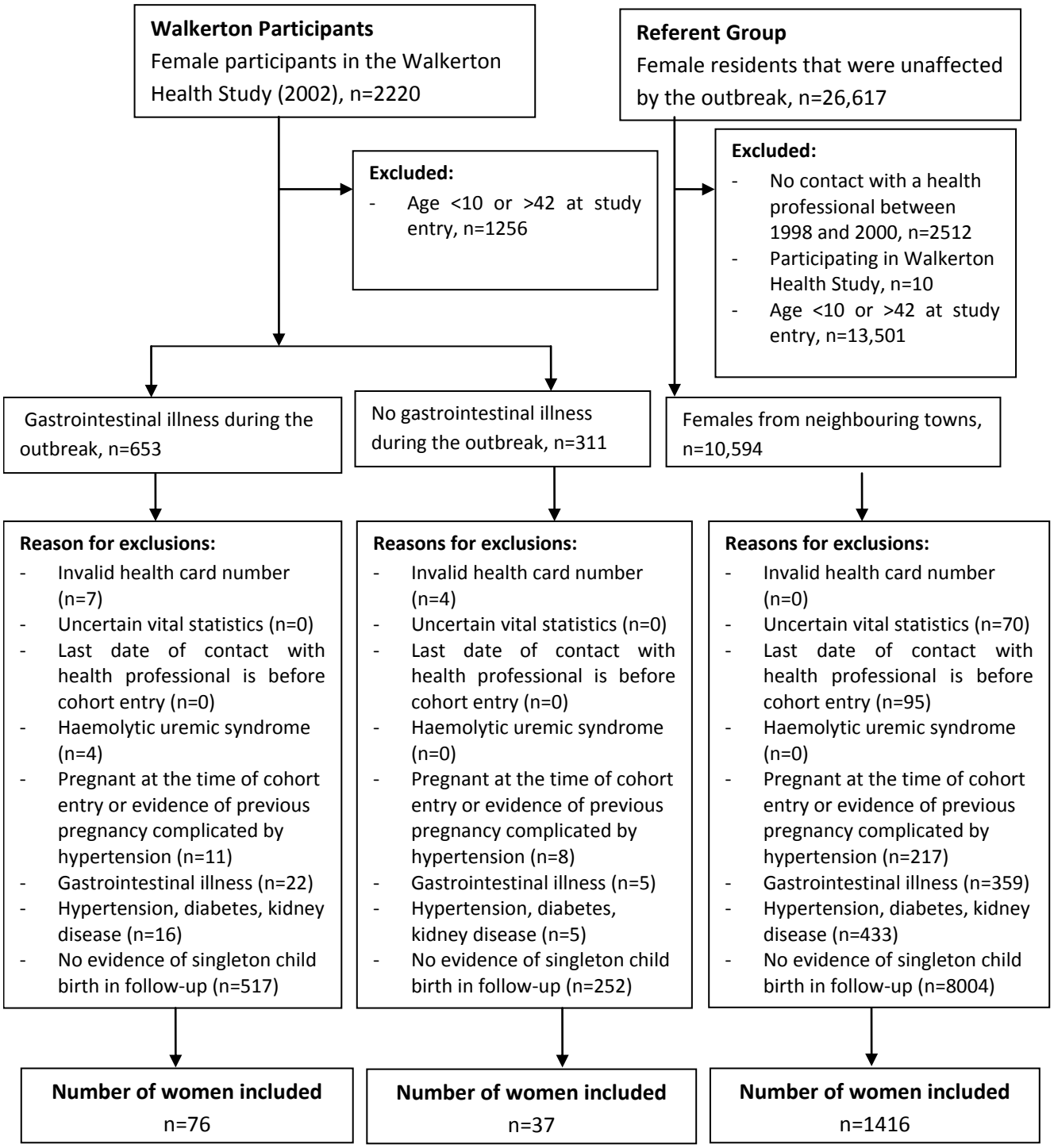

Table 1. Participant characteristics

	Walkerton participants		Residents of neighbouring towns	P Value‡
	With gastroenteritis	Without gastroenteritis		
Characteristics at cohort entry				
No. of women	76	37	1416	
Age — yr	23·6±6.3	22·9±7.5	23·5±5·8	0·80
Age category — no. (%)				0·56
<20	20 (26)	13 (35)	358 (25)	
20-29	42 (55)	17 (46)	832 (59)	
≥30	14 (18)	7 (19)	226 (16)	
Neighbourhood income quintile — no. (%)				<0·001
1-2 (lowest)	22 (29)	13 (35)	571 (40)	
3 (middle)	39 (51)	15 (41)	309 (22)	
4-5 (highest)	15 (20)	9 (24)	536 (38)	
Comorbidity index	3·8±2·6	3·1±2·1	3·2±2·4	0·06
ADG† category — no. (%)				0·12
0	7 (9)	4 (11)	146 (10)	
1-2	19 (25)	10 (27)	497 (35)	
3-5	29 (38)	17 (46)	556 (39)	
≥ 6	21 (28)	6 (16)	217 (15)	
Childbirths, 1991 to cohort entry — no. (%)				0·58
0	57 (75)	27 (73)	988 (70)	
≥1	19 (25)	10 (27)	428 (30)	
Characteristics at pregnancy				
No. of pregnancies	117	54	2166	
Age at childbirth— yr	28·1±5·4	27·9±6·5	27·9±5·1	0·73
<20	4 (3)	6 (11)	106 (5)	
20-29	71 (61)	26 (48)	1241 (57)	
≥30	42 (36)	22 (41)	798 (38)	
Year of childbirth — no. (%)				0·04
2002 - 2003	8 (7)	8 (15)	305 (14)	
2004 - 2005	28 (24)	8 (15)	508 (24)	
2006 - 2007	28 (24)	11 (20)	542 (25)	
2008 - 2009	35 (30)	18 (33)	550 (25)	
2010 - 2011	18 (15)	9 (17)	261 (12)	
Years since cohort entry**	5.3 (3-7)	5.4 (3-7)	4.7 (3-7)	0·09
Years since last pregnancy§	1·6±2·4	2·0±3·0	1·8±2·3	0·51
Surveillance during pregnancy				
Number of prenatal/antenatal visits	10·5±3·3	9·2±3·8	9·4±3·7	0·01
Number of abdominal/pelvic ultrasounds	3·1±1·8	3·8±2·7	3·0±1·9	0·16

*Plus-minus values are means ±SD. SD: Standard Deviation.

** Median (IQR)

†Estimated using the Johns Hopkins Aggregated Diagnosis Group Scoring System.[16]

§Restricted to 19, 10, 428 women with previous pregnancies among the three groups respectively.

‡ P values were calculated using ANOVA or Kruskal-Wallis for continuous variables and χ^2 tests for categorical variables. P values for characteristics at pregnancy were calculated using generalized estimated equations.

Table 2. Hypertension in pregnancy after *E.coli* O157:H7 gastroenteritis

	Number of Events / **Number of Pregnancies** (%)	**Relative Risk (95% confidence interval)**	
		Unadjusted	**Adjusted***
Gestational hypertension or preeclampsia			
Walkerton participants with gastroenteritis	8/117 (6·8)	1·6 (0·8–3·2)	1·5 (0·8–3·2)
Walkerton participants without gastroenteritis	0/54 (0·0)	-	-
Residents from neighbouring towns	96/2166 (4·4)	1·00 (reference)	1·00 (reference)
Gestational hypertension			
Walkerton participants with gastroenteritis	6/117 (5·1)	1·4 (0·6–3·3)	1·5 (0·7–3·4)
Walkerton participants without gastroenteritis	0/54 (0·0)	-	-
Residents from neighbouring towns	79/2166 (3·7)	1·00 (reference)	1·00 (reference)
Preeclampsia			
Walkerton participants with gastroenteritis	4/117 (3·4)	4·5 (1·5–13·4)	3·8 (1·3–11·6)
Walkerton participants without gastroenteritis	0/54 (0·0)	-	-
Residents from neighbouring towns	17/2166 (0·8)	1·00 (reference)	1·00 (reference)

*Relative risks were adjusted for age at the time of pregnancy (per year), parity, a measure of comorbidity (Johns Hopkins Aggregated Diagnosis Groups) [16] and and socioeconomic status (assessed using neighbourhood income).

Appendix S1-A. Checklist of recommendations for reporting of observational studies using the Strobe guidelines

	Item No	Recommendation	Reported
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	abstract
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	abstract
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	introduction
Objectives	3	State specific objectives, including any prespecified hypotheses	introduction
Methods			
Study design	4	Present key elements of study design early in the paper	methods
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	methods
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	methods
		(b) For matched studies, give matching criteria and number of exposed and unexposed	not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	methods
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	appendix
Bias	9	Describe any efforts to address potential sources of bias	discussion
Study size	10	Explain how the study size was arrived at	methods, based on availability of the data
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	methods
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	methods
		(b) Describe any methods used to examine subgroups and interactions	methods
		(c) Explain how missing data were addressed	not applicable
		(d) If applicable, explain how loss to follow-up was addressed	not applicable
		(e) Describe any sensitivity analyses	methods
Results			

Participants	13	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	methods, results, figure 1
		(b) Give reasons for non-participation at each stage	methods, figure 1
		(c) Consider use of a flow diagram	figure 1
Descriptive data	14	(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders	table 1
		(b) Indicate number of participants with missing data for each variable of interest	essentially complete
		(c) Summarise follow-up time (e.g. average and total amount)	results, table 2
Outcome data	15	Report numbers of outcome events or summary measures over time	results, table 2
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included	results, table 2
		(b) Report category boundaries when continuous variables were categorized	table 1
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	not applicable
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	results
Discussion			
Key results	18	Summarise key results with reference to study objectives	discussion
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	discussion
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	discussion
Generalisability	21	Discuss the generalisability (external validity) of the study results	discussion
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	cover page

Appendix S1-B. Comparison of Walkerton and neighbouring towns for community-level analyses

	Walkerton	**Listowel**	**Shelburne**	**St. Marys**	**Exeter**	**Mount Forest**	**Penetang-uishene**
Town Demographics*							
Population	**4851**	5905	4122	6293	4452	4584	8316
Population 15 years and over	**3955**	4835	3220	5030	3675	3765	6775
Female population	**2530**	3130	2150	3265	2415	2390	4265
Median age	**39·6**	38·8	36·6	39·3	41·2	41·7	40·6
Number of visible minority	**85**	115	110	75	85	105	100
% Living at same address 1 yr prior	**91·7**	86·3	85·6	88·5	88·7	87·8	82·7
Median total income	**21 265**	24 683	21 034	25 332	24 748	20 247	20 351
Employment rate	**62·8**	64·1	64·9	65·7	63·7	57·2	58·8
Availability of Healthcare							
Hospital in town	**Yes**	Yes	Yes	Yes	Yes	Yes	Yes
Number of hospital beds	**38**	51	33	21	19	37	51
Emergency room in hospital	**Yes**	Yes	No	Yes	Yes	Yes	No
Number of primary care physicians	**6**	12	4	13	10	5	12

*Data from 2002 Statistics Canada. 2001 Community Profiles.

Appendix S1-C. Codes used to identify maternal outcomes

Condition	Database	Codes	Validity
Gestational Hypertension	CIHI (ICD-9)	6429	
	CIHI (ICD-10)	O1300	Sensitivity: 68·2%[1] Specificity: 99·6%[1] Positive predictive value: 94·4%[1]
Preeclampsia	CIHI (ICD-9)	6424, 6425	Sensitivity: 69·7-100%[2,3] Specificity: 98·9-100%[2,3] Positive predictive value: 77-100%[2,3]
	CIHI (ICD-10)	O1400	

1. Hadfield RM, Lain SJ, Cameron CA, Bell JC, Morris JM, Roberts CL. The prevalence of maternal medical conditions during pregnancy and validation of their reporting in hospital discharge data. *Aust N Z J Obstet Gynaecol* 2008; 48(1):78-82.
2. Korst LM, Gregory KD, Gornbein JA. Elective primary caesarean delivery: accuracy of administrative data. *Paediatr Perinat Epidemiol* 2004; 18(2):112-119.
3. Yasmeen S, Romano PS, Schembri ME, Keyzer JM, Gilbert WM. Accuracy of obstetric diagnoses and procedures in hospital discharge data. *Am J Obstet Gynecol* 2006; 194(4):992-1001.

Appendix S1-D. Codes used to identify other outcomes

Condition	Database	Definitions	Variables used
Still birth	Mom-Baby dataset	Dead born fetus with at least 22 completed weeks of gestation	(b_stillbirth)
Neonatal death	Mom- Baby dataset	Newborn death in 28 days following childbirth	Record for death in CIHI/RPDB
Premature birth	Mom- Baby dataset	Newborn's gestation week < 37 weeks	(b_gestwks_del) & (m_gestwks_del)
Low birth weight	Mom- Baby dataset	Birth weight <2500 grams	(b_weight_del)

Appendix S1-E. Characteristics at time of first pregnancy in cohort study

	Walkerton participants		Residents of neighbouring towns	P Value‡
	With gastroenteritis	Without gastroenteritis		
Characteristics at pregnancy				
No. of pregnancies	76	37	1416	
Age — yr	27·9±5.4	27·2±6·6	27·4±5·2	0·62
Age category — no. (%)				0·08
<20	2 (3)	6 (16)	94 (7)	
20-29	48 (63)	17 (46)	832 (59)	
≥30	26 (34)	14 (38)	490 (35)	
Year of childbirth — no. (%)				0·05
2002 - 2003	8 (11)	8 (22)	305 (22)	
2004 - 2005	22 (29)	7 (19)	419 (30)	
2006 - 2007	16 (22)	8 (22)	325 (23)	
2008 - 2011	30 (39)	14 (38)	367 (26)	
Years since cohort entry **	4·6 (3-6)	4·3(2-6)	3·5 (2-6)	0·06
Years since last pregnancy§	1·2±2·8	1·6±3·4	1·4±2·7	0·73
Surveillance during pregnancy				
Number of prenatal/antenatal visits	10·6±3·4	9·5±3·6	9·6±3·6	0·07
Number of abdominal/pelvic ultrasounds	3·1±1·9	3·2±1·8	2·9±1·7	0·50

*Plus-minus values are means ± SD. SD: Standard Deviation

**Median (IQR)

§ Restricted to 19, 10, 428 women with previous pregnancies among the three groups respectively

‡ P values were calculated using ANOVA or Kruskal-Wallis for continuous variables and χ^2 tests for categorical variables.

Appendix S1-F. Hypertension in first pregnancy after *E. coli* O157:H7gastroenteritis

	Number of Events /Number of Pregnancies (%)	**Relative Risk (95% confidence interval)**	
		Unadjusted	**Adjusted***
Gestational hypertension or preeclampsia			
Walkerton participants with gastroenteritis	7/76 (9·2)	1·7 (0·8–3·5)	1·6 (0·8–3·3)
Walkerton participants without gastroenteritis	0/37 (0·0)	-	-
Residents from neighbouring towns	78/1416 (5·5)	1·00 (reference)	1·00 (reference)
Gestational hypertension			
Walkerton participants with gastroenteritis	5/76 (6·6)	1·5 (0·6–3·5)	1·4 (0·4–3·6)
Walkerton participants without gastroenteritis	0/37 (0·0)	-	-
Residents from neighbouring towns	64/1416 (4·5)	1·00 (reference)	1·00 (reference)
Preeclampsia			
Walkerton participants with gastroenteritis	4/76 (5·3)	5·3 (1·8–15·8)	4·9 (1·6–14·5)
Walkerton participants without gastroenteritis	0/37 (0·0)	-	-
Residents from neighbouring towns	14/1416 (1·0)	1·00 (reference)	1·00 (reference)

*Relative risks were adjusted for age at the time of pregnancy (per year), parity, a measure of comorbidity (Johns Hopkins Aggregated Diagnosis Groups) (13) and and socioeconomic status (assessed using neighbourhood income).

Hypertension in Pregnancy after Bacterial Gastroenteritis : A cohort study (DATA CREATION PLAN)

Number of Study	**2012 0900 269 000**
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PIA Approved	Yes
Date Last Modified and by Whom	Version 14, November 1, 2011 (IN, after teleconference) Version 13, October 26, 2011 (IN, after comments from AH) Version 12, October 20, 2011 (IN) Version 11, October 19, 2011 (IN, after comments from AH) Version 10, October 17, 2011 (Teleconference: IN, AG, AH, JS) Version 9, October 11, 2011 (IN, after comments from AH) Version 8, October 3, 2011 (IN, after comments from AH) Version 7, September 23, 2011 (Teleconference: IN, AG, AH, JS) Version 6, August 7, 2011 (IN) Version 5, August 5, 2011 (JS) Version 4, August 4, 2011 (IN) Version 3, August 2, 2011 (JS) Version 2,August 1, 2011(IN, AG)

	Version 1, July 23, 2011 (IN)
Short Description of Research Question	To determine whether women who experienced gastrointestinal illness during an *Escherichia coli* 0157:H7water outbreak have a higher risk of developing hypertensive disorders of pregnancy (gestational hypertension, preeclampsia, eclampsia)over a subsequent decade compared to those without such a history.
Expectation of Cohort Size	Anticipate at least 90 pregnancies in women who were symptomatic (exposed) and 778 pregnancies in women who were asymptomatic (unexposed).
List of Datasets Used	1. CIHI-DAD(July 1991-March 2011) 2. OHIP (July 1991-March 2011) 3. RPDB 4. MOMBABY dataset (July 1991 to March 2011) [which uses the CIHI DAD] 5. ODD 6. Hypertension 7. Walkerton Health Study data set (external) *Note:* A Data Sharing Agreement between Walkerton and ICES was established on August 25, 2005.
Anticipated Cohort Size from WHS dataset and Statistics Canada	Total number of female participants between ages 10 and 42 years enrolled in 2002: 964 Exposed group: 653 *(Severe gastroenteritis: 248, Mild-moderate gastroenteritis: 405)* Unexposed group '1': Asymptomatic Walkerton Health Study participants: 311 Unexposed group '2': Female residents from surrounding communities approximately: 23,135

Defining the Cohorts

Definitions of Exposed and Unexposed

Groups	Description	Data Source
Exposed	Female participants who had symptomatic gastroenteritis at the time of the Walkerton outbreak	Walkerton Health Study
Unexposed group '1'	Female participants who were asymptomatic at the time of the Walkerton outbreak	Walkerton Health Study
Unexposed group '2'	Female residents of small, rural towns in Ontario similar to Walkerton	Ontario healthcare databases

Index Date '1'

1. Date of first interview for Walkerton Health Study *(ENTRY=1; IPDATE=Jan 3 2002-Dec 11 2002)*for exposed and unexposed group '1'
2. For unexposed group '2', randomly assign index entry dates with the following distribution:
   Minimum: Jan 3, 2002
   25th percentile: April 7, 2002
   Median: May 29, 2002
   75th percentile:July29, 2002
   Maximum: Dec 11, 2002

Primary Outcome	Hypertensive disorders of pregnancy (gestational hypertension, preeclampsia and eclampsia)

Index Date '2'

For all 3 groups, **date of childbirth** (evidence of at least one childbirth in MOMBABY dataset) ≥ 6 months after index date '1'.

Any childbirth that is dated within 140 days of the date of childbirth will be considered the same pregnancy.

Note: We chose to include women who delivered a child ≥6 months after index date '1' [date of study enrolment] because we require a date of childbirth to identify women who carried their pregnancy to at least 20 weeks gestation, as our primary outcome cannot develop until after 20 weeks gestation. We understand that we might lose very few women who were pregnant over 20 weeks at the time of first interview and delivered preterm.

Inclusion Criteria

***See Table 1 for flow diagram for inclusion in the study**

For participants in the Walkerton Health Study (WHS) (exposed group and unexposed group '1')

1. All female participants between 10 and 42 years of age at index date '1'
2. Enrolled in the study in 2002 *(ENTRY=1; IPDATE=Jan 3 2002-Dec 11 2002)*

Exposedgroup (symptomatic gastroenteritis)

WHS participants with symptomatic gastroenteritis (deterministic linkage through OHIP number)*[diohip; EXPCASE3 = 2 (severe gastroenteritis), EXPCASE3 = 1 (mild to moderate gastroenteritis)]*

Please note that there may be participants in the exposure group who were not residents of Walkerton.

Unexposedgroup '1'

Asymptomatic WHS participants (deterministic linkage through OHIP number)*[(EXPCASE3 = 0) (not ill during outbreak)]*

Inclusion Criteria

Unexposed group '2'

1. Residents of surrounding communities. Select individuals from RPDB based on postal codes. Individuals must reside in one of the surrounding communities which is determined by postal code in RPDB : Exeter, Goderich, Listowel, Mt. Forest, Penetanguishene, Shelburne, St. Mary's, Wingham (see **Appendix A** for postal codes)
2. Must have had at least one encounter with a health professional (e.g. physician, dentist, chiropractor, as determined by ICES) between Q2 1998 to Q2 2000 (2 years prior to outbreak) in a specified postal code (any postal code in Appendix A other than Walkerton postal code).

   Note: This is done to make sure that they lived in the region around the time of the outbreak).

Exclusion Criteria

Exclusion criteria

For all groups: exclude if have any of the following (see **Appendix B** for exclusion codes). Apply each of these criteria sequentially

1. Invalid IKN
2. Missing age in RPDB(expect this will be close to 0)
3. Missing gender in RPDB(expect this will be close to 0)

4. Date of death in RPDB < index date '1' (expect this will be close to 0)
5. Exclude if date of last contact (DOLC) is before index date '1'.

Exclusion criteria for exposed and unexposed group '1' (WHS participants):

1. Age <10 years and >42 years at index date'1'
2. If diagnosed with hemolytic uremic syndrome during outbreak (CFThus=1)
3. No evidence of ≥ 1 childbirth between≥6 months AFTER index date '1' and Jan 6, 2011 using MOM BABY dataset

   Note: We are using Jan 6, 2011as this can be the last possible date for index date '2' (childbirth).

4. Evidence of childbirth in MOMBABY dataset <6 months after index date '1'.

   Note: As mentioned in index date '2' column above, women who were pregnant at index date '1' [time of study enrolment] or delivered soon after study enrolment are excluded to have a cleaner cohort of women with pre-pregnancy baseline characteristics. We also do not expect this number to be large (<5). If the numbers are large then we may reconsider this exclusion criterion.

5. Evidence of a date of death BEFORE last childbirth in MOM BABY dataset prior to Jan 6, 2011(data cleaning step)

   Note: For exclusion criteria #6 to #9 split into periods
   a) Jan 1, 1991 to May 17, 2000
   b) May 18, 2000 to index date '1'

6. Evidence of hypertension between Jan 1, 1991 andindex date '1'. Using hypertension dataset.

   Note: Chronic hypertension is already a known risk factor for pre-eclampsia. Using this exclusion controls for confounding. Therefore, in this study we will exclude any woman with a history of hypertension prior to index date '1', as we expect the numbers will be too few to allow for meaningful assessment. Reassess if it turns out more women are excluded from the analysis based on this criteria than we would like.

7. Evidence of previous pregnancy complicated by a hypertensive disorderbetween Jan 1, 1991) and index date '1'. This is defined by evidence of any of the following codes: Gestational Hypertension: **ICD-9**: 6429 [Hypertension during pregnancy]; **ICD-10**: O13001-O13004, O13009 [Gestational hypertension without proteinuria]; Preeclampsia: **ICD-9**: 6424 [Mild preeclampsia], 6425 [Severe preeclampsia]; **ICD-10**: O14001-O14004, O14009 [Gestational hypertension with significant proteinuria]; Eclampsia: **ICD-9**: 6426 [Eclampsia]; **ICD-10**: O15001, O15003 [Eclampsia in pregnancy], O15101, O15103 [Eclampsia in labour], O15201, O15203 [Eclampsia in the puerperium], O15209 [Eclampsia unspecified]; [Use CIHI-DAD, NACRS and CIHI-SDS datasources for this assessment].

8. Evidence of diabetes between Jan 1, 1991 and index date '1'. Diabetes mellitus: Listed as having diabetes in **ODD**.

   Note: Diabetes is a known risk factor for pre-eclampsia. Using this exclusion makes the analysis cleaner, and if it is a small number of patients this should not impact the results. Reassess if we lose >5 women based on this criteria.

9. Evidence of kidney disease or dialysis code between Jan 1, 1991 and index date '1'

10. Evidence of chronic gastrointestinal disease between Jan 1, 1991 and May 16, 2000
11. Evidence of any multiple gestation (twins, triplets etc.) between index date '1'and Jan 6, 2011, using variables in MOM BABY dataset.

    Note: Multiple gestation is a risk factor for pre-eclampsia. Again, as in previous criteria, reassess if it turns out > 5 women are excluded from the analysis based on this criteria than we would like. Excluding these women from the analysis simplifies the interpretation of the results. There are a few variables to determine multiple births in MOM BABY dataset. We are flagging multiple birth if any ONE of the following happens:

    B_MULTIBIRTH = T, M_MULTIBIRTH = T, or A M_KEY links to multiple B_KEYs. In theory there should be 100% agreement between these three variables, but in reality there are slight differences – a coder may miss a multiple birth code in mother or newborn record but missing in both should be rare.

12. Date of death in RPDB < 4 months AFTER index date '1'
13. Date of last contact is < 4 months AFTER index date '1'
14. Diabetes from index date '1' to last birth code in follow-up prior to Jan 6, 2011
15. Hypertension from index date '1' to last birth code in follow-up prior to Jan 6, 2011
16. Evidence of kidney disease or dialysis code from index date '1' to last birth code in follow-up prior to Jan 6, 2011

Note: We realize that for exclusions # 14 to #16 we might exclude women who had a pregnancy before the condition developed. However, we expect these numbers to be very low.

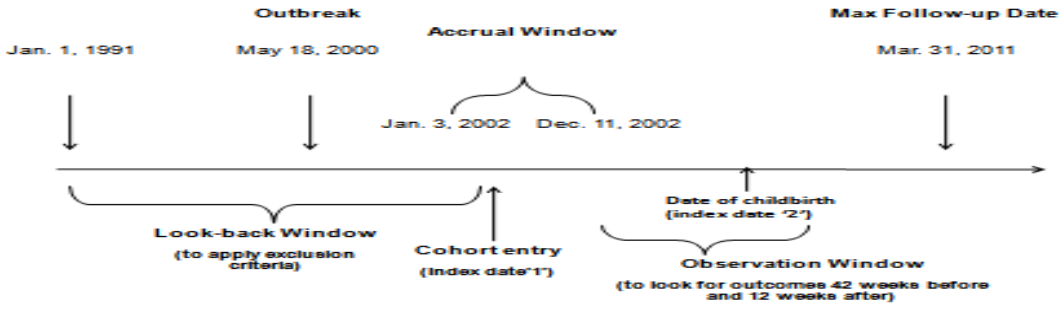
Exclusion for unexposed group '2'

Apply same exclusion criteria (#1 to #16 above)

AND

1. Exclude from group if participating in Walkerton health study (OHIP number also identified in Walkerton data set (diohip).These participants will be included as part of their appropriate Walkerton exposure group)

Time Frame Definitions



Cohort entry (index date '1')	Date of first interview/ date of enrollment (IPDATE=Jan 3 2002-Dec 11 2002)
Pregnancy identification (index date '2')	A woman is identified as being pregnant for at least 20 weeks by the date of birth in the MOM BABY dataset. This date of childbirth is referred to as index date '2'.
Possible last date of Index date '2'	Jan 6, 2011 (date of childbirth)
Unit of analysis	For the primary analysis pregnancy is the unit of analysis (rather than individual woman)
How to deal with subsequent pregnancies within the same women?	As pregnancies (and not women) are the unit of analysis, a woman can have more than one index date '2' (i.e. if they have had more than one pregnancy after index date '1'). To identify unique pregnancies after index date '1', follow each woman after index date '1' until end of follow-up period (Jan 6, 2011) for evidence of births in MOM BABY dataset; for women with more than one birth in MOM BABY dataset, it is a unique, separate birth if it occurs > 140 days (20 weeks) after most recent previous birth in MOM BABY dataset. *Note: for some of the baseline characteristics for each pregnancy, look 42 weeks PRIOR to index date '2'. In few instances, where a woman has 2 pregnancies and the index date '2' values are 20 to 42 weeks apart, there may be some overlap in the period of observation. We are ignoring this consideration – the 42 weeks prior to each index date '2' are considered for each pregnancy, disregarding any overlap, recognizing some visits etc. may be counted twice.*
Accrual start/end dates	Start of Accrual Period: **January 3, 2002** End of Accrual Period: **December 11, 2002**
Max follow-up date	Until March 31, 2011 (12 weeks after last possible pregnancy)
Period of outcome assessment	Look between 42 weeks before index date '2' to 12 weeks after index date '2' for outcomes of interest (unless stated otherwise). If index date '2' is within 42 weeks AFTER index date '1'; just look back to index date '1'
When does observation window terminate for any given pregnancy?	Terminates at the first of the following: • **Outcome observed (see Variable Definitions):** • Until12 weeks after date of childbirth OR index date '2'

	• March 31, 2011 (end of study)
Look-back window(s)	For all groups and all variables, look back to January 1, 1991 (or earliest available data in ICES for a given database, unless otherwise specified).

Variable Definitions

Cohort Characteristics

See **Table 2**

At the time of index date '1' (Table 2A)

- Age in years
- Socio-economic status (income quintile)
  (We expect many missing variables as we are including ages between 10 and 42 years. In such situations, substitute income quintile=3)

- Number of Aggregated Diagnosis Groups (ADG's) 1 year before index date '1'
  Use 1-year fixed look back window prior to 'index date 1' to calculate ADGs, use ICES algorithm
- Number of childbirths (using MOM BABY dataset) between Jan 1, 1991 and May 17, 2000
- Number of childbirths (using MOM BABY dataset) between May18, 2000 and index date '1'
- Number of years between last childbirth and index date '1'
  (for those who had pregnancies before index date '1')

At the time of index date '2' (Table 2B)

Note: pregnancies are unit of analysis and not women

- Maternal age at index date '2'
- Year of index date '2' (child birth)
- Number of previous pregnancies prior to first pregnancy after index date '1' (in other words, the number of prior index date '2s' in a woman prior to current index date '2'; do not count the current index date '2' pregnancy)
- Number of years (time) since last pregnancy [using MOM BABY dataset].
- Number of years (time) since index date '1'.
- Birth weight: (Use MOMBABY dataset) A mombaby record has M_IKN, B_IKN, M_KEY, B_KEY etc. Link mother IKN with data element M_IKN in mombaby dataset. The linked mombaby record will have the linked baby CIHI DAD identifier B_KEY and mother's CIHI DAD identifier M_KEY. In the case of non-multiple births this means 2 separate records. When find baby CIHI DAD birth

record (i.e. B_KEY from mombaby dataset, look at variable WEIGHT which is supposed to be the weight at birth. Run frequency before use, as there are sometimes 9999 values in this field which should be interpreted as missing. The field is a character variable and will need to be converted to a numeric before use.

Surveillance characteristic

1. Number of prenatal or antenatal visits in the 42 weeks (294 days) before index date '2' – Each unique prenatal or antenatal visit is defined as the presence of one or more of the following codes on **a single day** (i.e. only count one visit when there are multiple codes on a single day; can use CIHI-DAD, OHIP, NACRS and CIHI-SDS for the data sources): **ICD-9**: V220, V221 [Supervision normal pregnancy], V230 [Pregnancy with history of infertility], V231 [Pregnancy with history of trophoblastic disease], V232 [Pregnancy with history of abortion], V233 [Grand multiparity], V234 [Pregnancy with poor obstetric history], V235 [Pregnancy with poor reproductive history], V238, V239 [Supervision of high risk pregnancy],V288, V289 [Antenatal screening]; **CCP**: 02.88 [Diagnostic ultrasound of gravid uterus]; **ICD10**: Z34 [Supervision of normal pregnancy], Z35 [Supervision of high risk pregnancy] **CCI**: 5AB01 [Antepartum care], 5AB03 [Obstetrical ultrasound investigations]; **OHIP DxCode**: 970 [Prenatal care]; **OHIP fee code**: P003 [Prenatal care, general assessment, major visit], P004 [Minor assessment, all other prenatal visits], P005 [Prenatal care, Antenatal preventative health assessment];

   This is done to show that exposed and unexposed women had average number of health care visits during their pregnancy. Visits for pregnancy care either to her family practitioner or obstetrician will be counted as an antenatal/prenatal visit during pregnancy.

2. Number of abdominal / pelvic ultrasounds in the 42 weeks (294 days) before index date '2'. Each unique ultrasound is defined by as the presence of one or more of these codes on a single day (i.e. only count one ultrasound if there are multiple codes on a single day).

J128	DIAG. US. Abdomen/Retroperitoneum - Abdom. scan ltd. study
J135	DIAG. US. Abdomen/Retroperitoneum - abdom. scan, complete
J138	DIAG. US. Pelvic Intracavit-e.x. transrectal transvag vulation induct.
J157	DIAG. US. Gestational age for Maternal Serum screening
J158	DIAG. US. Limited for high risk pregnancy
J159	DIAG. US. Complete on or after 16 weeks one /normal pregnancy
J160	DIAG. US. Complete for high risk pregnancy or complications
J162	DIAG. US. Pelvis - Pelvic, complete
J428	DIAG. US. Abdomen/Retroperitoneum - Abdom. scan ltd. study
J435	DIAG. US. Abdomen/Retroperitoneum - abdom. scan, complete
J438	DIAG. US. Intracavitary - e.x. transrectal transvaginal
J457	DIAG. US. Gestational age for Maternal Serum screening
J458	DIAG. US. Limited for high risk pregnancy
J459	DIAG. US. Complete on or after 16 weeks one /normal delivery
J460	DIAG. US. Complete for high risk pregnancy
J462	DIAG. US. Pelvis - pelvic, complete

Note: Additional characteristics in table 2C is done restricting to first pregnancy after index date '1' ***(Table 2C)***

Here the unit of analysis = woman = first pregnancy after index date '1'

Additional characteristics for WHS participants only (exposed and unexposed group '1' at the time of index date '1' as ascertained by Walkerton Dataset)**: (Table 2D)**

- Smoking, at least 100 cigarettes in lifetime and smoked in the year before the outbreak (PHNSMOKE = 1)
- Self reported hypertension prior to outbreak (PHNHTN= 1)
- Self reported family history of hypertension prior to outbreak (FHNHTN= 1)
- Self reported kidney failure or poor kidney function prior to outbreak (PHNKIHD=1OR PHNKI= 1)
- Self reported cardiovascular/hypertension medications prior to outbreak (HTN_CVDmeds= 1)
- Taking Aspirin >1 time/week prior to outbreak (PHMDASA=1)
- Obesity at index date (FIRSTBMI≥ 30)
- Self-reported low sodium diet one year prior to outbreak (PHNsalt = 1)
- Creatinine, umol/L (CRS_1)
- Urine protein, g/d (PROTU_1)
- Urine albumin creatinine ratio, mg/mmol – or cut-points (MICR_1)

Outcome Definitions

Primary Outcome: Composite of hypertensive disorders of pregnancy (see **Appendix D** for codes)
(Explanation: with respect to significance testing, this outcome will be considered the primary test used in hypothesis testing, with a two-tailed p value <0.05 considered significant).

1. Hypertensive disorders of pregnancy(evidence of the first of any of the following events between 42 weeks before and 12 weeks after index date '2')
    a. Gestational Hypertension: **ICD-9**: 6429 [Hypertension during pregnancy]; **ICD-10**: O13001-O13004, O13009 [Gestational hypertension without proteinuria]
    b. Preeclampsia: **ICD-9**: 6424 [Mild preeclampsia], 6425 [Severe preeclampsia]; **ICD-10**: O14001-O14004, O14009 [Gestational hypertension with significant proteinuria]
    c. Eclampsia: **ICD-9**: 6426 [Eclampsia]; **ICD-10**: O15001, O15003 [Eclampsia in pregnancy], O15101, O15103 [Eclampsia in labour], O15201, O15203 [Eclampsia in the puerperium], O15209 [Eclampsia unspecified].

Secondary Outcomes:

Maternal outcomes
(every component of hypertensive disorder is considered separately, looking for codes in the mother's record)
evidence of any of the following codes between 42 weeks before or 12 weeks after index date '2'

1. Gestational Hypertension: **ICD-9**: 6429 [Hypertension during pregnancy]; **ICD-10**: O13001-O13004, O13009 [Gestational hypertension without proteinuria];
2. Preeclampsia: **ICD-9**: 6424 [Mild preeclampsia], 6425 [Severe preeclampsia]; **ICD-10**: O14001-O14004, O14009 [Gestational hypertension with significant proteinuria]
3. Eclampsia: **ICD-9**: 6426 [Eclampsia]; **ICD-10**: O15001, O15003 [Eclampsia in pregnancy],

O15101, O15103 [Eclampsia in labour], O15201, O15203 [Eclampsia in the puerperium], O15209 [Eclampsia unspecified];

4. Maternal death: Evidence of RPDB death 1 day prior to or 42 days after index date '2' (first birth code, MOM BABY dataset).
   [Maternal death is defined as death while pregnant or within 42 days of termination of pregnancy irrespective of the duration or site of pregnancy [1]]

Fetal outcomes

1. Single stillbirth:
   a. Stillbirths on date of index date '2'. Use variable "m_stillbirth" to detect stillbirth from MOM BABY dataset.
      [Still birth is defined as birth of a baby showing no signs of life (fetal death) with a gestational age of at least 22 completed weeks[2]. Stillbirth occurs in nearly 1% of all births in North America, and is one of the most common adverse pregnancy outcomes[3]]
2. Neonatal death:
   a. Evidence of fetal death in 28 days following index date '2'. Use MOM BABY dataset to determine baby IKN, look in baby record for death in CIHI or RPDB within 28 days following index date '2'.
      Note: if there is a live birth who died shortly after birth, the CIHI DAD discharge disposition of the newborn should indicate there was a death
      [Neonatal death is defined as death of a newborn within 1 to 28 days of birth[4]]
3. Perinatal mortality:
   Single stillbirth or evidence of death in the first week of life (one week after childbirth)
   For single stillbirth use MOMBABY dataset as in #1 fetal outcomes. For fetal death in 7 days following index date '2' use MOMBABY dataset to determine baby IKN, look in baby record for death in CIHI or RPDB within 7 days following index date '2'
   [Perinatal mortality is defined as deaths in the first week of life and fetal deaths (stillbirths)[5]]
4. Premature birth:
   a. Determine premature birth from MOM BABY dataset. Use variable mother's gestational week at delivery (m_gestwks_del) AND newborn's gestation weeks at delivery (b_gestwks_del)

   Note: Both variables only available after fiscal year 2002/03 mother's gestational week or baby's gestational week < 37 weeks.
   [Premature birth is defined as birth before 37 weeks of gestation. The frequency of premature birth in the United States increased from 10.7% in 1992 to 12.3% in 2003[6]. Women with hypertensive disorders of pregnancy delivered prematurely more often than healthy controls (30% vs. 14%)[7]. This may be partly due to the induction of labour[8]]
   * Report mother and baby gestational age separate depending on the data source; then do a combined source analysis were preferably use baby data source unless it is missing, in which case use mother data source.

5. Low birth weight (using MOM BABY dataset):
   Low birth weight is defined as a weight of less than 2500 g (up to and including 2499 g), irrespective of gestational age.

   [WHO definition, this is irrespective of gestational age[9]]

6. Small for gestational age (SGA), intrauterine growth restriction (IUGR): Evidence of any of the following codes between 4 weeks before and 4 weeks after index date '2'.

ICD-9: 6565 [Poor fetal growth], 7649 [Fetal growth retardation], 7640-7641 [Light for dates], **ICD-10**: P0590 [Symmetric Intra-uterine growth retardation[IUGR]], P0591 [Asymmetric intra-uterine growth retardation [IUGR]], P0599 [Unspecified intra-uterine growth retardation [IUGR]];

Additional Exploratory Outcomes

Look for evidence of any of the following codes in mother's record between 42 weeks before or 12 weeks after index date '2'.

1. Gestational diabetes mellitus:**ICD-9**: 6480 [Diabetes in pregnancy]; **ICD-10**: 024 [Diabetes mellitus in pregnancy]
   AND
   use an algorithm of those who have more than one claim for blood glucose test on the same day 18 weeks before index date '2'

2. Caesarean section:**ICD-9**: 6697 [Caesarean delivery]; **CCP**:86.0 [Classical caesarean section], 86.1 [Cervical caesarean section], 86.2 [Extraperitoneal caesarean section], 86.8 [Caesarean section of other specified type], 86.9 [Caesarean section of unspecified type]; **ICD-10**: O82 [Single delivery by caesarean section]; **CCI**: 5MD60 [Caesarean section]; **OHIP fee code**: P018 [Caesarean section], P041 [Caesarean section incl. tubal interruption] , P042 [Caesarean section incl. hysterectomy]
   [Mode of delivery: Women with hypertensive disorders of pregnancy seem to have a higher risk of caesarean section compared to women with normotensive pregnancies. However, success with vaginal delivery among women with preeclampsia also depends on the level of expertise and staffing available at a hospital].

3. Placental abruption: **ICD-9**: 6412 [Premseparplacen], **ICD-10**: O45 [Premature separation of placenta –abruptio placentae]

Tracer Outcome

1. Placenta praevia: Look for evidence of any of the following codes in mother's record 12 weeks before index date '2'. **ICD-9**: 6410, 6411 [Placenta previa]; **ICD-10**: O44 [Placenta praevia];

2. Post-partum haemorrhage: Look for evidence of any of the following codes in mother's record 2 weeks after index date '2'. **ICD-9**: 666 [666.10 Postpartum Hem Nec, 666.12 Postpartum Hem-Del W P/P; 666.14 Postpart Hem Nec- Postpar]; **ICD-10**: 072 [Postpartum Hemorrhage]

Explanation: no biological reason why this should be associated with E. coli exposure, expect no difference between groups

Subgroup Analysis (Table 4 A, B)

i. Maternal age (of exposed) [≥ vs. <median] at time index date '2'
ii. Primigravida vs. multigravida at time of index date '2'
   (primigravida means current pregnancy is the first pregnancy and no prior pregnancy from Jan 1, 1991 to current pregnancy at time of index date '2' as assessed in MOM BABY dataset; vs. multigravida means ≥1 pregnancy prior to current pregnancy, this pregnancy could be before or after index date '1')
iii. Time of pregnancy since index date '1' [≥ vs. <median time since date of study enrolment]

Note: 1) For #1 to 3, sets defined by the exposed women vs. unexposed group '1' AND '2' (referent group). 2) Produce point estimate and 95% confidence interval for each stratum. 3) Calculate test of

interaction based on the output of point estimate, lower CI, and upper CI (Bland and Altman technique).4) For additional analysis restrict to first pregnancy (Table 4B)

Outline of Analysis Plan

Establishing the Cohort

- Apply exclusion criteria and identify number of participants in each group (see **Table 1**)

Exploratory and Descriptive Analyses

- Proc univariate on continuous variables (assess for normal distribution, implausible values and potential outliers)
- Report % of missing data for each variable. Method of imputation to be determined based on degree of missing data.
- Compare baseline characteristics [between three groups: chi square (categories) or one way ANNOVA (mean) or kruskal Wallis (median) as necessary] (see **Table 2 A,B,C,D)**
    - Exposed vs. Unexposed group '1' vs. Unexposed group '2' (three group comparison) at index date '1' (Table 2A)
    - Exposed vs. Unexposed group '1' vs. Unexposed group '2' (three group comparison) at index date '2' (Table 2B)

For Table 2B, since we have repeat measurement, following SAS code will be used to calculate P-value ie. GEE model was used to get the p-value. If baseline is continuous then using "normal", if it is a count then using "poisson" (like number of previous pregnancies, number of prenatal visits and number of abdomen/pelvic ultrasounds).

```
proc genmod data=cohort;
class group ikn;
model baseline_var=group/dist=normal (or poisson) cl;
repeated subject=ikn/type=cs;
run;
```

- Ensure that the characteristics for Table 2C are restricted to the first pregnancy after index date '1'. (Table 2C)
- Exposed vs. Unexposed group '1' (for additional comparisons of baseline characteristics ascertained from the Walkerton Health Study dataset) (Table 2D)

Covariates to be adjusted: compare exposed to unexposed groups '1' and '2' (Unexposed group '2' referent)

i. Age (continuous)
ii. Income quintiles [1 vs. 3-5 (referent), 2 vs. 3-5 (referent)]
iii. Number of ADG's prior to 1 year before index date '1' [(0-3 (referent) vs. ≥4].
iv. Number of births from Jan 1st, 1991 until index date '1' [0 vs. ≥1(referent)]; using births in MOM BABY dataset

Multivariable Analyses

- Models built for all eleven (11) outcomes (see **Table 3 A, B,C**)

Primary, secondary and tracer outcomes MV analysis: Exposed vs. Unexposed group '1' and '2' (Table 3 A, B)

- Number of pregnancies, number of pregnancies with event, event rate per 100 pregnancies, unadjusted Relative Risk (RR) and adjusted RR
- Use Generalized estimating equation (GEE) regression model to perform adjusted analyses
- For all outcomes, adjust for all four covariates as above
  The SAS code used for analysis is following:

```
proc genmod data=cohort descending;
class groups(param=ref ref="general pop") ikn
incquint_grp(param=ref ref="3-5")
ADG_grp(param=ref ref="0-3")
birth_grp(param=ref ref=">=1") ;

model outcome=age groups incquint_grp ADG_grp birth_grp/dist=bin link=log ;
repeated subject=ikn/type=AR;
estimate "RR walkerton exposed VS general pop" groups 1 0/exp;
estimate "RR walkerton unexposed VS general pop" groups 0 1/exp;

run;
```

Additional MV Analysis (Table 3 C):

- Number of pregnancies, number of pregnancies with event, event rate per 100 pregnancies, unadjusted Relative Risk (RR) and adjusted RR
- Use logistic regression model to perform adjusted analyses
- For all outcomes, adjust for all four covariates as above

Subgroup Analysis (Table 4 A,B) as above

LIST OF TABLES, FIGURES, AND APPENDICES

Appendix A:	Postal codes for Walkerton and surrounding communities
Appendix B:	Codes used for exclusions
Appendix C:	Codes used to identify maternal and fetal outcomes using diagnostic and procedural codes
Appendix D:	Codes used to identify surveillance, exploratory and tracer outcomes
Appendix E:	Data dictionary for pregnancy outcomes
Table 1 (A,B,C):	Flow diagram for inclusion in this study
Table 2 (A,B,C,D):	Characteristics of exposed and unexposed
Table 3 (A,B,C):	Outcomes
Table 4 (A,B):	Subgroup analysis

Appendix A: Postal codes for Walkerton and surrounding communities

Municipality	Postal Code
Walkerton	**N0G 2V0**
Exeter	N0M 1S-
Listowel	N4W -
Mount Forest	N0G 2L-
Penetanguishene	L9M -
Shelburne	L0N 1S-
St. Mary's	N4X -

Appendix B: Codes used for exclusions

Condition	Database	Codes
Chronic Kidney disease	ICD-9	5820-5829, 583-583.7, 586, 585, 5880-5889
	ICD-10	N01 N03 N18 N19 N25 N052 N053 N054 N055 N056 N072 N073 N074
	OHIP Dx	403, 581, 585, 590, 593, 788
	OHIP fee	R850, G324, G336, G325, G326, G327, G860, G862, G863, G865, G866, G099, R825, R826, R827, R833, R840, R841, R843, R848, R851, Z450, Z451, Z452, G332, G861, G864, R852, R853, R854, R885, G082, G083, G085, G090, G091, G092, G094, G096, G333, H540, H740
Chronic gastrointestinal diseases	ICD-9	531.4-531.7, 531.9, 532.4-532.7, 532.9, 533.4-533.7, 533.9, 534.4-534.7, 534.9, 535.1- 535.6, 536.0-536.2, 536.8-537.6, 537.0-537.8, 537.9, 555.0-555.2, 555.9, 556.0, 557.1, 557.9, 558.1, 558.2, 558.9, 560.0-560.2, 560.3, 560.8, 560.9, 562.0, 562.1, 564.1-564.4, 564.7-564.9, 569.5, 569.6, 569.8, 569.9
	ICD-10	K254-K259, K264-K269, K274-K279, K284-K289, K293, K295, K296, K297, K298, K299, K30, K311-K319, K50-K52, K55-K59, K63, K90, K91
	OHIP-Dx	555-557, 560, 562, 564, 531, 532, 534-537, 579

Dialysis codes

Dialysis - Any		
SOURCE	**CODE**	**DESCRIPTION**
ICD9	V451	Renal dialysis status
	V56.0	Admit for renal dialysis
	V56.8	Aftercare-dialysis NEC
	3610	Part detach-dialysis
ICD10	T824	Mechanical complication of vascular dialysis catheter
	Y602	Unintentional cut, puncture, perforation or haemorrhage during kidney dialysis or other perfusion
	Y612	Foreign object accidentally left in body during kidney dialysis or other perfusion
	Y622	Failure of sterile precautions during kidney dialysis or other perfusion
	Y841	Kidney dialysis as the cause of abnormal reaction or later complication, without mention of misadventure at the time of the procedure
	Z490	Preparatory care for dialysis
	Z491	Extracorporeal dialysis
	Z492	Other dialysis
	Z992	Dependence on renal dialysis
	E10220	Type 1 diabetes mellitus with end-stage renal disease [ESRD], adequately controlled with diet or oral agents
	E10221	Type 1 diabetes mellitus with end-stage renal disease [ESRD], adequately controlled with insulin
	E10222	Type 1 diabetes mellitus with end stage renal disease [ESRD], inadequately controlled with diet or oral agents (and insulin not used to stabilize)
	E10223	Type 1 diabetes mellitus with end stage renal disease [ESRD], inadequately controlled with diet or oral agents but adequately controlled with insulin
	E10224	Type 1 diabetes mellitus with end stage renal disease [ESRD], inadequately controlled with insulin
	E10229	Type 1 diabetes mellitus with end stage renal disease [ESRD], level of control unspecified
	N180	End-stage renal disease
	E11220	Type 2 diabetes mellitus with end-stage renal disease [ESRD], adequately controlled with diet or oral agents
	E11221	Type 2 diabetes mellitus with end-stage renal disease [ESRD], adequately controlled with insulin
	E11222	Type 2 diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with diet or oral agents (and insulin not used to stabilize)

	E11223	Type 2 diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with diet or oral agents but adequately controlled with insulin
	E11224	Type 2 diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with insulin
	E11229	Type 2 diabetes mellitus with end-stage renal disease [ESRD] ,level of control unspecified
	E13220	Other specified diabetes mellitus with end-stage renal disease [ESRD], adequately controlled with diet or oral agents
	E13221	Other specified diabetes mellitus with end-stage renal disease [ESRD], adequately controlled with insulin
	E13222	Other specified diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with diet or oral agents (and insulin not used to stabilize)
	E13223	Other specified diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with diet or oral agents but adequately controlled with insulin
	E13224	Other specified diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with insulin
	E13229	Other specified diabetes mellitus with end-stage renal disease [ESRD] ,level of control unspecified
	E14220	Unspecified diabetes mellitus with end-stage renal disease [ESRD], adequately controlled with diet or oral agents
	E14221	Unspecified diabetes mellitus with end-stage renal disease [ESRD], adequately controlled with insulin
	E14222	Unspecified diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with diet or oral agents (and insulin not used to stabilize)
	E14223	Unspecified diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with diet or oral agents but adequately controlled with insulin
	E14224	Unspecified diabetes mellitus with end-stage renal disease [ESRD], inadequately controlled with insulin
	E14229	Unspecified diabetes mellitus with end-stage renal disease [ESRD], level of control unspecified
CCP	5127	Arteriovenostomy for renal dialysis
	5142	Revision of arteriovenous shunt for renal dialysis
	5143	Removal or arteriovenous shunt for renal dialysis
	5195	Hemodialysis
	6698	Peritoneal dialysis
CCI	1OT53DATS	Implantation of internal device, abdominal cavity, of catheter (peritoneal dialysis) using endoscopic (laparoscopic) approach
	1OT53HATS	Implantation of internal device, abdominal cavity, of catheter (peritoneal dialysis) using percutaneous (incision) approach
	1OT53LATS	Implantation of internal device, abdominal cavity, of catheter (peritoneal dialysis) using open (laparotomy) approach
	1PZ21HPD4	Dialysis, urinary system NEC peritoneal dialysis using dialysate
	1PZ21HQBR	Dialysis, urinary system NEC hemodialysis
	1SY55LAFT	Removal of device, muscles of chest and abdomen of permanent catheter [peritoneal dialysis] using open approach

	7SC59QD	Instruction, personal care for dialysis
	1KY76LA	Bypass, artery with vein using arteriovenous fistula
	1KY76LASJ	Bypass, artery with vein using arteriovenous shunt (e.g. Quinton-Scribner)
	1KY76LAXXA	Bypass, artery with vein using interposition graft made of autologous tissue (e.g. saphenous vein)
	1KY76LAXXL	Bypass, artery with vein using xenograft (e.g. bovine carotid artery)
	1KY76LAXXN	Bypass, artery with vein using interposition graft made of synthetic material (e.g. Gore-Tex)
OHIP feecode	R849	Dialysis – Heamodialysis - Initial & acute
	R850	Dialysis – Haemodialysis - insert of Scribner shunt
	G323	Dialysis – Haemodialysis - Acute, repeat (max 3)
	G324	Dialysis – Haemodialysis - Insert.subclav.catheter
	G336	Dialysis - Revision of G324
	G325	Dialysis – Haemodialysis - Medical component (incl in unit fee)
	G326	Dialysis - Chronic, contin. haemodialysis or haemofiltration each
	G327	Insertion of femoral catheter for dialysis
	G860	Chronic hemodialysis hospital location
	G862	Hospital self care Chronic hemodialysis
	G863	Chronic hemodialysis IHF location
	G865	Chronic Home hemodialysis
	G866	Intermittent hemodialysis treatment centre
	G099	Haemodialysis - insertion of permanent jugular dialysis catheter
	R825	Veins - Resec AV aneurysm/Fistula w/out Graft – Major
	R826	Veins - Resec AV aneurysm/fistula w/out graft – minor
	R827	Dialysis/Veins - Anastomosis - Creation of A.V. fistula
	R833	Dialysis/Veins - Ligation/remov bypass graft
	R840	Dialysis - Same as R851 with - autogenous vein
	R841	Dialysis/Veins - Anastomosis - Obliteration of A.V. fistula

	R843	Dialysis - Removal of cannula or A.V. shunt
	R848	Dialysis Cannula insertion under vision into central line
	R851	Dialysis - By-pass graft haemodialysis – synthetic
	Z450	Dialysis - Revision Scribner Shunt – single
	Z451	Dialysis - Revision Scribner Shunt – both
	Z452	Dialysis - De-clotting Scribner Shunt
	G330	Peritoneal dialysis - Acute (up to 48 hrs)
	G331	Peritoneal dialysis - Repeat acute (up to 48 hrs) max. 3
	G332	Peritoneal dialysis - Chronic (up to 48 hrs)
	G861	Chronic peritoneal dialysis hospital location
	G864	Chronic Home peritoneal dialysis
	R852	Peritoneal dialysis - Insert peritoneal cannula by laparotomy
	R853	Peritoneal dialysis - Insert Tenchkov catheter chronic etc.
	R854	Peritoneal dialysis - Removal Tenchkov catheter
	R885	Removal of peritoneal cannula by laparotomy
	G082	Continuous venovenous haemodialfiltration
	G083	Continuous venovenous haemodialysis
	G085	Continuous venovenous haemofiltration
	G090	Veneovenous slow continuous ultrafiltration
	G091	Continuous arteriovenous haemodialysis
	G092	Continuous arteriovenous haemodiafiltration
	G093	Haemodiafiltration - Contin. Init & Acute (repeatx3)
	G094	Haemodiafiltration - Contin. Chronic
	G095	Slow Continuous Ultra Filtration - Initial & Acute (repeat)
	G096	Slow Continuous Ultra Filtration – Chronic
	G294	Arteriovenous slow continuous ultrafiltration init and acute

	G295	Continuous aterivenous haemofiltration initial and acute
	G333	Home/self-care dialysis
	H540	OOP Renal Dialysis Out-patient visit
	H740	PRE-APPROVED OOC Out-patient Renal dialysis

Appendix C. Codes used to identify maternal and fetal outcomes using diagnostic and procedural codes

Condition	Database	Codes	Validity
Maternal Outcomes			
Gestational Hypertension	CIHI (ICD-9)	6429	
	CIHI (ICD-10)	O1300	Sensitivity: 68.2%[10] Specificity: 99.6%[10] Positive predictive value: 94.4%[10]
Preeclampsia	CIHI (ICD-9)	6424, 6425	Sensitivity: 69.7-100%[11;12] Specificity: 98.9-100%[11;12] Positive predictive value: 77-100%[11;12]
	CIHI (ICD-10)	O1400	
Eclampsia	CIHI (ICD-9)	6426	Sensitivity: 50-100%[11;13] Specificity: 99.9-100%[11;13] Positive predictive value: 100%[11;13]
	CIHI (ICD-10)	O1500, O1510, O1520,	
	OHIP DxCode	642	
Fetal Outcomes			
Small for gestational age (SGA), intrauterine growth restriction (IUGR)	CIHI (ICD-9)	6565, 7640, 7641, 7649	
	CIHI (ICD-10)	P0590, P0591, P0599	

Appendix D. Codes used to identify exploratory and tracer outcomes

Condition	Database	Codes	Validity
Exploratory Outcomes			
Gestational Diabetes Mellitus	CIHI – diagnostic	**ICD-9** 6480	
		ICD 10 024	
Caesarean section	CIHI – diagnostic	**ICD-9** 6697	
		ICD-10P034	
	CIHI – procedural	**CCP**86, 86.0, 86.1, 86.2	
		CCI 5MD60	Sensitivity:99%[14] Specificity: 100%[14] Positive Predictive Value: 100%[14]
	OHIP – procedural	**OHIP fee code** P018, P041, P042	
Placental abruption	CIHI – diagnostic	**ICD-9** 6412	Sensitivity: 89%[12] Positive Predictive Value: 89%[12]
		ICD-10 O45	
Tracer Outcomes			
Placenta previa	CIHI – diagnostic	**ICD 9** 6410, 6411	
		ICD 10 O44	
Post partum hemorrhage	CIHI – diagnostic	**ICD 9** 6660	
		ICD 10 072	

Appendix E: Data dictionary for pregnancy outcomes
Walkerton Health Study data dictionary for pregnancy outcomes

Variable Name	Variable Description	Variable Details
studyid	Unique identifier for Walkerton Health Study	
IPDATE	Date of Year 1 interview for Walkerton Health Study (Index Date)	dd/mm/yyyy
ENTRY	Year entered Walkerton Health Study	1=Year 1 to 7=Year 7
diohip	OHIP number	
didob	Date of birth	dd/mm/yyyy
disex	Sex	1=Male, 2=Female
EXPCASE3	Exposure, by severity of gastroenteritis	Severe: Confirmation of symptoms of severe gastroenteritis by prior health records =2 Mild: Self reported symptoms, not verified =1 Asymptomatic: Not ill during outbreak =0
ageentry	Age at study entry	
CTFhus	Diagnosed with hemolytic uremic syndrome during outbreak	0=No, 1=Yes
PHNSMOKE	Smoking, at least 100 cigarettes in lifetime and smoked in the year before the outbreak	0=No, 1=Yes
PHNHTN	Self reported hypertension prior to outbreak	0=No, 1=Yes
PHNKIHD	Self reported kidney failure prior to outbreak	0=No, 1=Yes
PHNKI	Self reported poor kidney function prior to outbreak	0=No, 1=Yes
FHNHTN	Self reported family history of hypertension prior to outbreak	0=No, 1=Yes
HTN_CVDmeds	Self reported cardiovascular or hypertension medications prior to outbreak	0=No, 1=Yes
PHNhtmd	Self-reported hypertension medications prior to outbreak	0=No, 1=Yes
PHMDACE	Self-reported ACE inhibitor prior to outbreak	0=No, 1=Yes
PHMDALP	Self-reported Alpha-adrenergic blockers prior to outbreak	0=No, 1=Yes
PHMDARB	Self-reported Angiotension receptor blockers prior to outbreak	0=No, 1=Yes
PHMDBB	Self-reported Beta blockers prior to outbreak	0=No, 1=Yes
PHMDCCB	Self-reported Calcium channel blockers prior to outbreak	0=No, 1=Yes
PHMDDIU	Self-reported Diuretics prior to outbreak	0=No, 1=Yes
PHMDVAS	Self-reported Vasodilators prior to outbreak	0=No, 1=Yes
PHMDASA	Self reported Aspirin >1 time/week prior to outbreak	0=No, 1=Yes
PHNsalt	Self-reported low sodium diet one year prior to outbreak	0=No, 1=Yes
FIRSTBMI	BMI at index date (kg/m^2)	Obesity defined as BMI ≥ 30
PHNCHOL	Self-reported high blood cholesterol prior to outbreak	0=No, 1=Yes
CRS_1	Serum Creatinine, umol/L	studyid 343 is a valid outlier (not data entry error)
Y1age_CRS	Age at time of Y1 CRS test (to calc eGFR)	
PROTU_1	Urine protein, g/d	studyid's 318 and 412 are valid outliers (not data entry errors)
MICR_1	Urine albumin to creatinine ratio, mg/mmol	studyid's 4310 and 412 are valid outliers (not data entry errors)

Table 1. Flow diagram for inclusion in the study

A.Exposed group	Include	Exclude
All female participants enrolled in the Walkerton Health Study (disex, 2=female, entry =1, EXPCASE = 2 or 1)	Ab	
Female participants between 10 and 42 years of age at index date '1' (ageentry≥10 and ≤42)		b
Study eligible participants in the Walkerton Health Study	A	
Exclude from 'A' if B1:		
Invalid IKN		B11
Missing date of birth		B12
Missing gender		B13
Date of death in RPDB <index date '1'		B14
Date of last contact is less than Index date '1'		B15
If diagnosed with hemolytic uremic syndrome during outbreak (CFThus=1)		B16
No evidence of ≥ 1 childbirth between≥ 6 months AFTER index date '1' and Jan 6, 2011 using MOM BABY dataset		B17
Evidence of childbirth in MOM BABY dataset < 6 months after index date '1'.		B18
Evidence of a date of deathBEFORE last childbirth in MOM BABY dataset prior to Jan 6, 2011		B19
Evidence of hypertension from Jan 1, 1991 to May 17, 2000.		B20
Evidence of hypertension from May 18, 2000 to index date '1'.		B21
Evidenceof previous pregnancy complicated by a hypertensive disorderfrom Jan 1, 1991 to May 17, 2000.		B22
Evidence of previous pregnancy complicated by a hypertensive disorderfrom May 18, 2000 to index date '1'.		B23
Evidence of diabetes from Jan 1, 1991 to May 17, 2000.		B24
Evidence of diabetes from May 18, 2000 to index date '1'.		B25
Evidence of kidney disease or dialysis from Jan 1, 1991 to May 17, 2000.		B26
Evidence of kidney disease or dialysis from May 18, 2000 to index date '1'.		B27
Evidence of chronic gastrointestinal disease from Jan 1, 1991 to May 16, 2000		B28
Evidence of any multiple gestation(twins, triplets etc.) from index date '1' to Jan 6, 2011, using variables in MOM BABY dataset.		B29
Date of death in RPDB < 4 months AFTER index date '1'		B30
Date of last contact is < 4 months AFTER index date '1'		B31

Diabetes from index date '1' to last birth code in follow-up prior to Jan 6, 2011		B32
Hypertension fromindex date '1' to last birth code in follow-up prior to Jan 6, 2011		B32
Evidence of kidney disease or dialysis from index date '1' to last birth code in follow-up prior to Jan 6, 2011		B33
Included symptomatic women from Walkerton Health Study	**C=A- (B1+ B2+B3)**	

B. Unexposed group'1'	Include	Exclude
All female participants enrolled in the Walkerton Health Study (disex, 2=female entry =1, EXPCASE = 0)	Da	
Female participants between 10 and 42 years of age at index date '1' (ageentry≥10 and ≤42)		a
Included asymptomatic participants from Walkerton Health study	D	
Exclude from 'D' if E1:		
Invalid IKN		E11
Missing date of birth		E12
Missing gender		E13
Date of death in RPDB is less than index date '1'		E14
Date of last contact is less than Index date '1'		E15
No evidence of ≥ 1 childbirth between≥ 6 months AFTER index date '1' and Jan 6, 2011 using MOM BABY dataset		E16
Evidence of childbirth in MOM BABY dataset < 6 months after index date '1'.		E17
Evidence of a date of deathBEFORE last childbirth in MOM BABY dataset prior to Jan 6, 2011.		E18
Evidence of hypertension from Jan 1, 1991 to May 17, 2000.		E19
Evidence of hypertension from May 18, 2000 to index date '1'.		
Evidence of previous pregnancy complicated by a hypertensive disorderfrom Jan 1, 1991 to May 17, 2000.		E20
Evidence of previous pregnancy complicated by a hypertensive disorderfrom May 18, 2000 to index date '1'.		E21
Evidence of diabetes from Jan 1, 1991 to May 17, 2000.		E22
Evidence of diabetes from May 18, 2000 to index date '1'.		E23
Evidence of kidney disease or dialysis from Jan 1, 1991 to May 17, 2000.		E24
Evidence of kidney disease or dialysis from May 18, 2000 to index date '1'.		E25

Evidence of chronic gastrointestinal disease from Jan 1, 1991 to May 16, 2000		E26
Evidence of any multiple gestation(twins, triplets etc.) from index date '1' to Jan 6, 2011, using variables in MOM BABY dataset.		E27
Date of death in RPDB < 4 months AFTER index date '1'		E28
Date of last contact is < 4 months AFTER index date '1'		E29
Diabetes from index date '1' to last birth code in follow-up prior to Jan 6, 2011		E30
Hypertension from index date '1' to last birth code in follow-up prior to Jan 6, 2011		E31
Evidence of kidney disease or dialysis from index date '1' to last birth code in follow-up prior to Jan 6, 2011		E32
Included asymptomatic women from WHS	**F=D-(E1+E2+E3)**	

C. Unexposed group'2'	Include	Exclude
Women in RPDB from the postal codes as attached in Appendix A (valid gender field in RPDB)	Gc	
Did not have at least one encounter with health care professional Q2 1998 to Q2 2000		c
Exclude if 'H1':		
Participating in Walkerton health study (OHIP number also identified in Walkerton Health data set(diohip)		H1a
Invalid IKN		H1b
Missing date of birth		H1c
Missing gender		H1d
Study eligible participants from surrounding communities	I=G-(H1)	
Randomly assign index date '1', exclude if 'J1':		
Females<10 and >42 years of age at index date '1'		J11
Date of death in RPDB is less than index date '1'		J12
Date of last contact is less than Index date '1'		J13
No evidence of ≥ 1 childbirth between≥ 6 months AFTER index date '1' and Jan 6, 2011 using MOM BABY dataset		J14
Evidence of childbirth in MOM BABY dataset < 6 months after index date '1'		J15

Evidence of a date of deathBEFORE last childbirth in MOM BABY dataset prior to Jan 6, 2011.		J16
Evidence of hypertension from Jan 1, 1991 to May 17, 2000.		J17
Evidence of hypertension from May 18, 2000 to index date '1'.		J18
Evidence of previous pregnancy complicated by a hypertensive disorderfrom Jan 1, 1991 to May 17, 2000.		J19
Evidence of previous pregnancy complicated by a hypertensive disorderfrom May 18, 2000 to index date '1'.		J20
Evidence of diabetes from Jan 1, 1991 to May 17, 2000.		J21
Evidence of diabetes from May 18, 2000 to index date '1'.		J22
Evidence of kidney disease or dialysis code from Jan 1, 1991 to May 17, 2000		J23
Evidence of kidney disease or dialysis from May 18, 2000 to index date '1'.		J24
Evidence of chronic gastrointestinal disease from Jan 1, 1991 to May 16, 2000		J25
Evidence of any multiple gestation(twins, triplets etc.) from index date '1' to Jan 6, 2011,usingvariables in MOM BABY dataset.		J26
Date of death in RPDB < 4 months AFTER index date '1'		J27
Date of last contact is < 4 months AFTER index date '1'		J28
Date of last contact is less than index date '1'		J29
Diabetes from index date '1' to last birth code in follow-up prior to Jan 6, 2011		J30
Hypertension fromindex date '1' to last birth code in follow-up prior to Jan 6, 2011		J31
Evidence of kidney disease or dialysis from index date '1' to last birth code in follow-up prior to Jan 6, 2011		J32
Included asymptomatic women from surrounding communities	**K=1-(J1+J2+J3)**	

Table 2. Characteristics of exposed and unexposed

A. Baseline characteristics of exposed and unexposed at time of index date '1'				
Characteristic	**Exposed**	**Unexposed group '1'**	**Unexposed group '2'**	**P value**
Age				
Mean ± SD				
Median (IQR)				
20-29				
30-39				
< 20				
≥ 40				
Income quintile				
1				
2				
3				
4				
5				
Number of ADG groups				
Mean ± SD				
Median (IQR)				
0				
1-2				
3-5				
≥6				
Number of childbirth between Jan 1, 1991 and May 17, 2000				
0				
1				
≥2				
Number of childbirth between May 18, 2000 and index date '1'				
0				
1				
≥2				

Years since last childbirth to index date '1' †				
Mean ± SD				
Median (IQR)				
† if there was previous child birth				
B. Characteristics of pregnancies in the exposed and unexposed groups at time of index date '2'				
[The values with n (%) in table below refer to a denominator of the number of pregnancies (not number of women)]				
Characteristic	**Exposed**	**Unexposed** group '1'	**Unexposed** group '2'	**P value**
Number of unique pregnancies, n				
Number of unique women, N	76	37		
Maternal age				
Mean ± SD				
Median (IQR)				
20-29				
30-39				
< 20				
≥ 40				
Year of child birth				
2002, n (%)				
2003, n (%)				
2004, n (%)				
2005, n (%)				
2006, n (%)				
2007, n (%)				
2008, n (%)				
2009, n (%)				
2010, n (%)				
2011, n (%)				
Number of previous pregnancies prior to current				

pregnancy since index date '1', n (%)				
0				
1				
≥2				
Number of years (time) since last pregnancy, n (%)				
Mean ± SD				
Number of years (time) since index date '1', n (%)				
Mean ± SD				
Number of prenatal visits in 42 weeks prior to index date '2'				
Mean ± SD				
Number of abdomen/pelvic ultrasounds in 42 weeks prior to index date '2'				
Mean ± SD				
Birth weight				
Mean ± SD				
C. Characteristics of pregnant exposed and unexposed at time of index date '2'				
**Restrict to first pregnancy: Unit of analysis = woman = first pregnancy after index date '1'				
Characteristic	**Exposed, n=76**	**Unexposed** group '1', **n=37**	**Unexposed** group '2', **n=1,416**	**P value**
Maternal age				
Mean ± SD				
Median (IQR)				
20-29				
30-39				
< 20				
≥ 40				

Year of child birth				
2002, n (%)				
2003, n (%)				
2004, n (%)				
2005, n (%)				
2006, n (%)				
2007, n (%)				
2008, n (%)				
2009, n (%)				
2010, n (%)				
2011, n (%)				
Number of previous pregnancies prior to current pregnancy since index date '1',n (%)				
0				
1				
≥2				
Number of years (time) since last pregnancy, n (%)				
Mean ± SD				
Number of years (time) since index date '1', n (%)				
Mean ± SD				
Number of prenatal visits in 42 weeks prior to index date '2'				
Mean ± SD				
Number of abdomen/pelvic ultrasounds in 42 weeks prior to index date '2'				
Mean ± SD				

Birth weight				
Mean ± SD				

D. Additional Characteristics of Exposed and Unexposed group '1' from WHS from first year survey			
Characteristic	**Exposed, n=76**	**Unexposed group '1' N=37**	**P value**
***Smoking, n (%)**			
Missing, n (%)			
***Hypertension, n (%)**			
Missing, n (%)			
***Family history of hypertension, n (%)**			
Missing, n (%)			
***History of kidney failure or poor kidney function, n (%)**			
Missing, n (%)			
***Number on ≥1 cardiovascular/ hypertension medications, n (%)**			
Missing, n (%)			
***Taking aspirin>1time/week, n (%)**			
Missing, n (%)			
Obesity at index date'1', **n (%)**			
Missing, n (%)			
***Low sodium diet, n (%)**			
Missing, n (%)			
Serum creatinine at index date'1'(umol/L)			
Mean ± SD			
Median (IQR)			

Missing, n (%)			
Urine protein at index date'1' **(g/d)**			
Mean ± SD			
Median (IQR)			
Missing, n (%)			
Urine albumin creatinine ratio at index date'1' (mg/mmol)			
Mean ± SD			
Median (IQR)			
Missing, n (%)			

*self reported prior to outbreak

Table 3A. Primary and Secondary Outcomes

Outcomes	Number of events	Total pregnancies	Event rate per 100 pregnancies	Unadjusted Relative Risk (95% CI)	Adjusted Relative Risk (95% CI)
Maternal Outcomes					
Primary: Hypertensive disorder of pregnancy					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Secondary Outcomes					
Gestational hypertension					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Preeclampsia					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Eclampsia					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Maternal deaths					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Fetal Outcomes					
Single stillbirth					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Neonatal death					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Perinatal mortality					
Exposed					
Unexposed group '1'					

Unexposed group '2'				-	-
Premature births					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Low birth weight					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
SGA/IUGR					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-

Table 3B. Exploratory and Tracer Outcomes

Outcomes	Number of events	Total pregnancies	Event rate per 100 pregnancies	Relative Risk (Unadjusted)	95% Confidence intervals
Gestational Diabetes Mellitus					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Caesarean Section					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Placental abruption					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Post-partum Hemorrhage					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Placenta previa					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-

Table 3C. Outcomes Restricted to the First Pregnancy after Index Date '1'

Outcomes	Number of events	Total pregnancies	Event rate per 100 pregnancies	Relative Risk (Unadjusted)	95% Confidence intervals
Maternal Outcomes					
Primary: Hypertensive disorder of pregnancy					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Gestational hypertension					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Preeclampsia					

Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Eclampsia					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Maternal deaths					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Fetal Outcomes					
Single stillbirth					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Neonatal death					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Perinatal mortality					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Premature births					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Low birth weight					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
SGA/IUGR					
Exposed					
Unexposed group '1'					

Unexposed group '2'				-	-
Gestational Diabetes Mellitus					
Exposed					
Unexposed					
Unexposed group '2'				-	-
Caesarean Section					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Placental abruption					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Post-partum Hemorrhage					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-
Placenta previa					
Exposed					
Unexposed group '1'					
Unexposed group '2'				-	-

Table 4 A. Subgroup analysis for primary outcome

Variables	Cohort	# of pregnancies	# of pregnancies with event	Relative Risk (RR) *	(95% CI) *	(Test for interaction) p- value
Age ≥ median	exposed					
	Unexposed group '1'					
	Unexposed group '2'					
Age < median	exposed					
	Unexposed group '1'					
	Unexposed group '2'					
Primigravida	exposed					
	Unexposed group '1'					
	Unexposed group '2'					
Multigravida	exposed					
	Unexposed group '1'					
	Unexposed group '2'					
Time since "index 1" ≥ median	exposed					
	Unexposed group '1'					
	Unexposed group '2'					
Time since "index 1" < median	exposed					
	Unexposed group '1'					
	Unexposed group '2'					

B. Subgroup analysis for primary outcome restricting to first pregnancy after index date '1'‡

Variables	Cohort	# of women	Event	Relative Risk (RR) *	(95% CI) *	(Test for interaction) p- value
Age ≥ median	exposed					
	Unexposed group '1'					
	Unexposed group '2'					
Age < median	exposed					
	Unexposed group '1'					
	Unexposed group '2'					
Primigravida	exposed					
	Unexposed group '1'					
	Unexposed					

	group ‘2’					
Multigravida	exposed					
	Unexposed group ‘1’					
	Unexposed group ‘2’					
Time since "index 1" ≥ median	exposed					
	Unexposed group ‘1’					
	Unexposed group ‘2’					
Time since "index 1" < median	exposed					
	Unexposed group ‘1’					
	Unexposed group ‘2’					
	Unexposed group ‘2’					

* report point estimate and upper and lower confidence interval values to 3 decimal points.
‡ this will be done depending on adequacy of sample size (to be discussed later).

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Chapter 4

Manuscript acceptable for submission to a journal for peer review attached below.

Hypertension in Pregnancy Following Live Kidney Donation: A Prospective Cohort Study

ABSTRACT

Introduction: A common concern of young women wanting to become a kidney donor is whether removal of one kidney will affect future pregnancy outcomes. This is the protocol of a prospective cohort study to examine this issue.

Methods and analysis: We will conduct this study in two stages. In stage 1 of this study we will assess the feasibility of prospectively measuring blood pressure and urine protein bi-weekly beginning at 16-weeks gestation through to 8-weeks postpartum —in both kidney donors and a group of non-donating women who are in similar health to the donors (i.e. non-donors). We will also collect detailed information including medical history, maternal and fetal outcomes through medical-chart review. Outcomes will be adjudicated by a committee blinded to donation status. After successful completion of stage 1, in stage 2, we will extend recruitment to follow the pregnancies of an adequate number of donors and non-donors to reliably answer the question of whether donating a kidney increases a woman's risk of developing hypertension in pregnancy.

Ethics and dissemination: We have obtained ethics approval for this study at 12 transplant centres in Canada, and to date (January 12, 2013) we have recruited 59 women prior to donation: 45 donors and 14 non-donors. Seven have since become pregnant in follow up.

BACKGROUND

Living kidney donation: "A Gift of Life"

Patients with kidney failure either have the treatment option of transplantation or dialysis.[1] Many physicians prefer transplantation because it is known to reduce the risk of death in patients with kidney failure by 70%, improve their quality of life, and reduce health care costs compared with dialysis.[2-4] To gain these benefits, rates of living kidney donation have nearly doubled over the last 10 years and continue to rise.[5, 6] Approximately 27,000 living kidney donations take place around the world each year.[7] To optimize the practice, any adverse outcomes of living kidney donation need to be understood and minimized.

Living kidney donation is practiced with the understanding that the minimal risk of short- and long-term harm to the donor is outweighed by the clear advantage to the recipient. While the life expectancy of living donors is similar to matched non-donors, [8] outcomes such as hypertension and proteinuria also require consideration. These measures are strongly predictive of cardiovascular disease and kidney failure in the general population, and are monitored annually following donation.[9-13]

The complex, co-dependent relationships between kidney function and high blood pressure are well recognized. For this reason, the risk of hypertension after kidney

donation has frequently been investigated; some, but not all studies identify an increase in risk after donation.[9] In a meta-analysis of 5145 donors from 48 studies, post-donation blood pressure was 5 mm Hg higher in donors than in non-donors after an average follow-up of 5 years despite being similar at baseline.[9]

Could living kidney donation increase the risk of hypertension in pregnancy?

Hypertension in pregnancy includes gestational hypertension, preeclampsia, and eclampsia. Collectively, these disorders affect ~5-10% of pregnancies and remain a leading cause of maternal and perinatal morbidity and mortality worldwide.[14-16]

The importance of blood pressure control during pregnancy is undeniable. Clinical practice guidelines recommend that blood pressure be judiciously measured throughout pregnancy and be managed with anti-hypertensive medication when elevated.[17] Also, the observation of elevated blood pressure among non-pregnant living kidney donors is concerning.[9]

Pregnancy places additional demands on the kidney, including a 50-80% increase in kidney blood flow, and 50% increase in glomerular filtration rate (GFR).[18, 19] The kidneys are key regulators of salt and water, and pregnancy is a time of net salt and water retention.[20] After nephrectomy, the GFR of the remaining kidney increases by 40%, and its response to hormones such as angiotensin II is altered.[21] These alterations in vascular function along with the physiologic demands of pregnancy mean that living kidney donors could be at increased risk for hypertension in pregnancy.[22-26]

METHODS

Study overview

We are conducting this prospective cohort study in two stages with an ultimate goal of determining whether women have a higher risk of hypertension in the first pregnancy after kidney donation compared with similar healthy non-donors. Hypertension in pregnancy refers to a composite outcome of gestational hypertension, preeclampsia or eclampsia. In the first stage of the study we will assess protocol feasibility with respect to patient recruitment and data collection. This first stage of the study will be deemed successful if we have data collection completed for at least 20 pregnancies by 2014. If we are successful, we will continue into the second stage of the study, where we continue the recruitment to a total target of 286 donors and 143 non-donors to have adequate statistical power to examine hypertension in pregnancy in a reliable way.

To efficiently conduct this study we are partnering with the Living Kidney Donor (LKD) Study, a prospective cohort study designed to evaluate the long-term outcomes of living kidney donors (participant eligibility included in **Appendix A**). The LKD Study is actively recruiting live donors and healthy non-donors from 12 major transplant centres in Canada. Each year the LKD study enrolls 60 female donors and 30 female non-donors under the age of 45years. We are inviting eligible female participants of the LKD Study to participate in the LKD Pregnancy Study. Key aspects of the LKD Study that are relevant to the proposed LKD Pregnancy Study are described below briefly.

The LKD study: Long-term outcomes of living kidney donors

Design: Multi-centre prospective cohort study. Living kidney donors and healthy non-donors are followed annually.

Participants: Donors are those adults without risk factors such as hypertension who are approved by the nephrology team for kidney donation. Non-donor participants are healthy relatives, spouses or friends of donors or recipients, some of whom come forward for donation, but were ineligible due to blood group or cross match reasons. Non-donors must meet the same inclusion and exclusion criteria as the donors to help ensure they are of 'equivalent' health. Study eligibility is assessed through a 2-hour screening visit where participants complete a standardized questionnaire and undergo a physical exam, blood pressure assessment, and laboratory testing.

Data Collection: The same method of data collection is used for both donors and non-donors. In brief, participants complete an annual mailed survey and home blood pressure measurements in a standardized fashion. Missing or discrepant data are followed-up by telephone.

Pregnancy outcomes in living kidney donors

Participants: The eligibility criteria for the LKD pregnancy study are detailed in **Table 1**. Enrollment into this study occurs in two steps. Pre-pregnancy eligibility is first assessed during enrollment into the main LKD Study. A research coordinator describes the Pregnancy Study to women who meet pre-pregnancy eligibility criteria, and obtains written, informed consent for study participation. Permission to access the participant's and baby's medical records is obtained, and participants are asked to complete a medical-information release form.

The research coordinator asks the participants to contact the research team as soon as they know they are pregnant. Only the first pregnancy of the participant after study enrolment

is considered. As pregnancy outcomes among the same women can be correlated, we chose to examine only the first pregnancy in follow-up as this simplifies the analysis. Also, the risk of developing hypertension in pregnancy is higher in the first pregnancy and decreases with increasing parity. To facilitate re-contacting the participant at the time of early pregnancy, the coordinator also asks for permission to make additional contact, which includes mailing a reminder card every four months. A second set of eligibility criteria is assessed at the time of pregnancy (**Table 1)**. Eligibility criteria are the same for donors and non-donors.

Data collection

At study entry: At LKD study entry, all participants complete a baseline assessment that includes a physical exam and measurement of height, weight, and blood pressure. Participants also complete a survey with questions on previous obstetric and gynecological history and risk factors for hypertension in pregnancy (survey attached; **Appendix B**).[27, 28]

During pregnancy: Participants who become pregnant receive a kit in the mail (preferably before 14 weeks of gestation). The kit contains a congratulations card, a self-monitoring blood pressure machine, a bottle of urine dipsticks, 14 data-collection sheets (**Appendix C**) and a visit log chart (**Appendix D**). Each item in the kit is accompanied by detailed instructions on how and when to perform the tests, how to record the results, and how to interpret, confirm and notify health professionals about abnormal results. Participants are instructed to call their family doctor or obstetrician in cases of abnormal blood pressure or

urine protein readings. To facilitate the surveillance of abnormal results, and to improve the quality of data collection, the research coordinator telephones or emails the participants every two weeks to collect the readings recorded during the previous two-week period.

After childbirth: After delivery, participants receive a personalized note thanking them for participating in the study. Finally, participants are asked to complete a self-administered questionnaire 8 weeks after childbirth. This survey contains questions on prenatal, fetal, and maternal outcomes during pregnancy and after delivery (survey attached; **Appendix E**). We also provide a pre-addressed, postage-paid envelope to mail the questionnaire and completed data-collection sheets to the central coordinating centre. Once all data is completed, the coordinator reviews every participant's medical chart for accuracy of data collected (**Appendix F**). Charts are reviewed for the period of pregnancy and 8 weeks after childbirth.

Measurements

Blood pressure: Participants measure their blood pressure using the automated Omron self-monitoring device fitted with an appropriately sized cuff for each individual's arm circumference. This automated device has proved reliable and accurate compared to the standard mercury sphygmomanometer when recording blood pressure in women during pregnancy.[29] This has also been proved reliable in our main LKD study so far. During pregnancy, we ask participants to measure their blood pressure on two consecutive days

(twice per day: morning and evening) every two weeks. Similarly, after childbirth we ask participants to measure their blood pressure twice at 4-weeks and 8-weeks post-partum.

Urine protein: Participants test for urine protein using the Bayer Multistix 8 SG dipstick. The test for urine protein is conducted once every two weeks during pregnancy and twice at 4 weeks and 8 weeks post partum. For dipstick values ≥ 1+, we advise participants to repeat the test after waiting six hours.[30] A dipstick test result of ≥ 2+ implies significant proteinuria in antenatal care.[31] If abnormal results are obtained, the participant is referred to their primary care physician to rule out evidence of a urinary tract infection (symptoms of dysuria, positive urine culture). If confirmed to be a urinary tract infection the proteinuria will be disregarded and measured again when infection has been treated.

Additional laboratory measures: We also ask participants to provide blood and urine samples on one occasion during pregnancy (between 24-28 weeks) for storage in a long-term biorepository. When possible, we coordinate this visit with the participants' scheduled lab visit for routine prenatal care. Alternatively, we arrange a lab visit at a convenient time and location close to the participant. The lab then ships the samples to the central lab at the London Health Sciences Centre, London, Canada. These 4 ml samples are securely stored for future analysis of other measures of kidney function such as cystatin C, uric acid and novel biomarkers of pre-eclampsia (such as soluble FLT-1, endoglin, placental protein 13, thromboxane synthase, urinary podocytes; see **Table 2**).[32-35] We understand that there may be several years between the collection time and measurement of biomarkers. To reduce laboratory variability, we will test the biomarkers

at the time of study completion. Storage vial integrity and location will be checked annually. Even though there is a concern of biodegradation of the stored samples, we expect it to be less than 1%.[36-38]

Retention strategies

Participant retention is vital. A variety of retention strategies are employed to all participants in the LKD study. Additional strategies to improve data collection and retention for the pregnancy study are described in **Appendix G**. In the few situations where data collection may not be complete, we will collect available data from the medical charts of participants after childbirth.

Primary outcome: Hypertension in pregnancy

The ideal primary outcome for the second stage definitive study is a composite of gestational hypertension, preeclampsia and eclampsia (**Table 3**). Gestational hypertension is the most common of these disorders, affecting about 6-8% of pregnancies, and is usually the earliest to develop.[39] Gestational hypertension is defined as new-onset hypertension (SBP/DBP ≥ 140/90 mm Hg), which develops for the first time after 20 weeks of gestation.[40] Preeclampsia affects 2-3% of pregnancies, and is diagnosed when gestational hypertension is accompanied by proteinuria (dipstick protein ≥ 1+ or 24-hour urine protein ≥ 300 mg in the absence of urinary infection).[40] Eclampsia is the least common, but most serious of all hypertension complication during pregnancy. Eclampsia affects fewer than 0.1% of pregnancies and is diagnosed when gestational hypertension or preeclampsia is accompanied by new-onset tonic-clonic seizures.[40]

Secondary outcomes

Pregnancy outcomes such as placental abruption, preterm birth, small for gestational age, intrauterine growth restriction, stillbirth, and caesarean section are assessed for donors and non-donors.[41, 42] The number of antenatal visits for the two groups is also collected to evaluate the potential for any information bias (surveillance bias) given that donors may be more carefully monitored than non-donors.

Ascertainment and adjudication of outcomes

The research coordinator records details and date of onset for each diagnosis. Names and dosages of all prescription medications are also recorded. Primary and secondary outcomes will be centrally adjudicated by a committee blinded to donor status. The committee will review data from participant interviews, blood pressure readings, urine protein tests, medical records (in-patient, out-patient), and birth records. All records are first reviewed by a research assistant who blacks-out any information related to donor status.

Data analysis

We will use SAS version 9.2 (SAS Institute Inc., Cary, NC) for all statistical analysis. We will summarize normally distributed data by the mean and standard deviation (SD), and skewed distributions by the median and interquartile range (IQR). At the time of final analysis (completion of first and second stages of this study) we will use a log binomial regression model to estimate the relative risk of the composite primary outcome (gestational hypertension, preeclampsia or eclampsia).[43, 44] The following confounders

will be considered for statistical adjustment: race, age at the time of current pregnancy, multiple gestation in current pregnancy. Other confounders measured prior to donation date will also be considered for adjustment: smoking status, parity, socioeconomic status, body mass index, mean arterial blood pressure [defined as twice the diastolic pressure, added to the systolic pressure, and divided by 3 (2*DBP+SBP)/3], eGFR, and hypertension in previous pregnancies.[45-57] However, mean arterial blood pressure, eGFR and proteinuria after donation will not be adjusted in the model as they may be on the causal pathway. We will reduce models using backward elimination at alpha=0.15 unless elimination changes the effect measure by >10%.[58-62] This method achieves a conservative balance between the negative consequences of decreased model efficiency due to over-fitting and the positive consequences of minimizing bias by including all possible confounders. The individual components of the primary outcome will be examined separately in supplementary analyses, as well each of the secondary outcomes using similar statistical methods.

Statistical power: In the general population, the incidence of hypertension in pregnancy worldwide is approximately 10%. This estimate was derived from nine prospective studies, which reported on the incidence of gestational hypertension, preeclampsia and eclampsia using a similar definition as described in the current proposal.[47, 49, 52, 63-70] Using a weighted average of the incidence rates of hypertension in pregnancy from the general population, a sample size of 286 donors and 143 non-donors in the ratio of 2:1 will provide 80% power to establish whether living kidney donors, compared to non-donors, have a twofold or higher risk in their development of hypertension in pregnancy

(**Appendix H**).[71] Stage 1 will determine study feasibility, following which the participants from this stage will be carried over to stage 2 and recruitment continues to meet the total required number of participants for an adequately powered study. In the unlikely event of inadequate number of participants becoming pregnant during follow-up, we will 1) consider a one-sided sample size calculation and 2) we will calculate the average mean arterial pressure [defined as twice the diastolic pressure, added to the systolic pressure and divided by 3, MAP=(2*DBP+SBP)/3] for each woman at four intervals during pregnancy: 14-19 weeks gestation, 20-29 weeks gestation, 30-35 weeks gestation, and >36 weeks gestation.[72-75] These intervals correspond to known patterns of blood pressure change that are documented in studies of the general population. In univariate analysis, we will compare the mean arterial pressure between donors and non-donors at each time interval using 95% confidence intervals. Assuming a sample-size of 40 pregnant donors and 20 pregnant non-donors, we are powered to detect a minimum difference of 5 to 7 mm Hg in mean arterial pressure (two-sided α=0.05; 1-β=0.8), which is similar to reported differences between women who develop gestational hypertension or preeclampsia compared with those who do not (between-group difference in mean arterial pressure ranges between 5 and 11 mm Hg over the course of pregnancy).[40, 76]

Recognized limitations

1) Composite outcomes: Composite outcomes are useful because they increase the statistical precision for assessment of several important outcomes, and can provide insight

into an overall disease process. However, the level of inference for an increased risk can only be applied to the cluster of events within the composite, and not to its individual components.[77, 78] Although we plan to examine each component of the composite separately in supplementary analyses; these analyses will have low statistical power.

2) Potential for loss to follow-up/missing data: Loss to follow-up is a major limitation in most prospective studies. We will use a number of proven retention strategies to maximize follow-up. In the rare event that participants cannot complete the study requirements independently, we will schedule home visits. We will follow up missing survey responses with telephone interviews, and missing blood pressure/urine measurements with medical charts and therefore anticipate minimal missing data.

3) Accurate assessment of urine protein: Identifying urine protein is a key component of screening for and diagnosing preeclampsia. Although some consider the 24-hour urine collection to be the gold standard for measuring urine protein, the 24-hour collection is a cumbersome and time-consuming method, which can negatively impact recruitment and retention, and is not practical for multi-centre clinical studies. Although dipstick values ≥ 1+ have a positive predictive value of 92% for predicting ≥300 mg protein in a 24-hours urine collection, negative or trace values do not rule out significant proteinuria (the negative predictive value is only 34% in hypertensive patients).[30] Thus, for participants who have a high blood pressure recording and a negative urine dipstick, the research coordinator will contact the participant's family doctor or obstetrician for additional urine protein testing. These medical notes will then be acquired for central adjudication.

Ethical considerations, feasibility and study significance

All data will be collected by accredited research staff in accordance with the highest ethical standards. An independent Data Safety Monitoring Board (DSMB) supervises the safety of the study participants in the LKD study and oversees the data integrity and analysis. The DSMB will review reports on the ethical considerations, and will be notified immediately about any serious unanticipated adverse events or major protocol violations in the LKD pregnancy sub study.

Study progress

As of January 12, 2013 we have enrolled 59 women (45 donors and 14 non-donors) into the LKD Pregnancy Study, seven of these women have since become pregnant in follow-up, (data collection in progress for all seven women has been presented in Table 4 below) and we are collecting detailed measures on maternal and fetal outcomes.

Conclusion

By accepting healthy persons into the role of a donor, our health care system takes on additional responsibility beyond our 'normal' tasks of curing, or at least helping patients with a disease.[79] Living kidney donation is a unique model to help clarify the role of reduced nephron mass in the development of hypertension in pregnancy. From a clinical perspective, our study will provide new information that will improve donor selection, informed consent, and best practices for caring for donors who become pregnant.

Table 1. Eligibility criteria for the LKD pregnancy study (donors and non-donors)

1. Pre-pregnancy	2. During Pregnancy
• Participant of the LKD Study	• First pregnancy after enrollment into the LKD Study
• Non-pregnant female 18 to 50 years	• Gestational age less than 28 weeks
• No known reason not to conceive*	• Intent to carry pregnancy to term

*Does not include women on temporary methods of contraception; however, women who are unable to become pregnant because of tubal ligation, hysterectomy or for other reasons are ineligible to participate.

Table 2. Biomarkers

Biomarkers	**Blood**	**Urine**
Soluble FLT-1[80]	√	
s-Endoglin[81]	√	
Placental protein 13[81]	√	
Thromboxane synthase[81]	√	
Podocytes[33]		√

Table 3. Composite outcome: Hypertension in pregnancy[a]

Clinical Findings (>20 weeks pregnant)	Gestational Hypertension	Preeclampsia	Eclampsia
Hypertension[b]	+	+	+
Proteinuria[c]	–	+	+
Seizures[d]	–	–	+

[a]Hypertension in pregnancy: gestational hypertension, preeclampsia, or eclampsia.
[b]Hypertension: two measurements of systolic or diastolic blood pressure ≥140/90 mm Hg, separated by 6-hours.
[c]Proteinuria: two dipstick values ≥1+, separated by 6-hours, or ≥ 300 mg of protein in a 24-hour urine collection, in the absence of urinary infection.
[d]Seizures: new-onset of tonic-clonic seizures in a woman with preeclampsia.

Table 4. Data collected so far

Patient No	Baseline survey	Pre-delivery biweekly measurement	Lab measurement (24-28 weeks)	Time of delivery (year, quarter)	Post-delivery measurement	Post-delivery survey	Chart review
1	Completed	13 sets	Collected	2011, fourth quarter	Completed	Completed	In progress
2	Completed	11 sets	Collected	2011, fourth quarter	Completed	Completed	In progress
3	Completed	11 sets	Collected	2011, first quarter	Completed	Completed	Completed
4	Completed	10 sets	Not Collected	2011, first quarter	Completed	Completed	In progress
5	Completed	2 sets	Not Collected	2012, first quarter	Not Completed	Mailed	In progress
6	Completed	In progress	Not collected	-	Not completed	-	In progress
7	Completed	In progress	Not collected	-	Not completed	-	In progress

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Appendix A: Inclusion/Exclusion Criteria of the LKD study

Inclusion Criteria

To be eligible to participate subjects must:

- Be able to speak and read English and/or French, and
- Be able to provide informed consent, and
- Be between the ages of 18 – 70

AND

Subjects must either:
A: Be approved as a standard-criteria living donor
OR
B: Meet study eligibility for controls as follows:

- Meet blood pressure criteria as follows;

(Blood pressure < 140 mmHg systolic and < 90 mmHg diastolic based on an average of last 3 blood pressure measurements taken during the interview) or (Average blood pressure < 140 mmHg systolic and < 90 mmHg diastolic based on a minimum of 12 readings taken at home.)

- Meet local lab criteria as follows;

(Documented pre-donation serum creatinine < 115µmol/L (1.3 mg/dL) in men or < 90µmol/L (1.0mg/dL) in women, or Cockroft-Gault estimated glomerular filtration rate > 80 mL/min) or (Urine dipstick test for protein is negative or a random urine albumin to creatinine ratio < 8 mg/mmol (70 mg/g) or (Urine dipstick test for hematuria is negative. Test should not occur during menses, and test should be repeated if there is evidence of urinary tract infection.)

- Have a Body Mass Index of < 35 kg/m^2

Exclusion Criteria

To be eligible to participate, subjects must not:

- Be involved in another clinical study that would affect the outcome of this study

AND

Participants must not:

- Ever have received dialysis, even for a short period of time, or
- Ever have had a kidney transplant, or
- Be taking any hypertension class medication for any reason, or have any history of hypertension, currently or in the past, or
- Have plasma glucose of >7 mmol/L after a 6 hr fast, or a two hour oral glucose test of 11.1 mmol/L, or
- Have a history of diabetes during pregnancy, or
- Have been symptomatic for kidney stones any time in the past 3 years, or
- Have a known contraindication to anesthesia or surgery, or
- Be currently pregnant or have been pregnant in the past month, or
- Have a medical condition that would prevent him or her from becoming a kidney donor, eg:
  - History of renal disease
  - Permanent protein in urine
  - Cancer other than cured non-melanoma skin cancer
  - Cardiovascular disease
  - Pulmonary disease
- Principal Investigator or a member of the transplant team or study research team does not think that the potential participant would be a good candidate for this long term follow-up study

Appendix B

LKD 3 - ☐☐☐ - ☐☐☐ - 1 Participant Initials: ☐☐☐ Assessment #: 0 1 Form #: 0

STUDY Centre ID Participant ID F M L

PREGNANCY SUBSTUDY: (Baseline) PREGNANCY FORM

(Form to be completed over the phone)

1) Are you currently pregnant?

☐ No ☐ Yes ☐ Unknown

→ For how many weeks? ☐☐ WEEKS ☐ Unknown

→ Are you pregnant with multiples (i.e. twins, triplets, etc)?

☐ No ☐ Yes ☐ Unknown

→ ☐☐ NUMBER OF CHILDREN

2) Date of first day of your last menstrual cycle: ☐☐ / ☐☐☐ / ☐☐☐☐
DAY (e.g. 01) MONTH (e.g. OCT) YEAR

3) Current weight: ☐☐☐.☐ ☐ kilograms ☐ pounds

4) Did you receive any medications/treatment for fertility reasons (example: medications, artificial insemination, IVF etc)?

☐ No ☐ Yes

5) Previous to this pregnancy, but after donation (for non-donors the date of enrollment into the study), have you been diagnosed with hypertension (high blood pressure)?

☐ No ☐ Yes

6) Previous to this pregnancy, but after donation (for non-donors the date of enrollment into the study), have you been diagnosed with diabetes (high blood sugar)?

☐ No ☐ Yes

7) During this pregnancy were you ever told by a doctor that you had the following?

a) **Hypertension, pre-eclampsia or toxemia** (high blood pressure with or without protein in the urine)**?**

☐ No ☐ Yes

→ Date first told: ☐☐☐ / ☐☐☐☐
MONTH (e.g. OCT) YEAR

→ Started on any medications during the pregnancy:

☐ No ☐ Yes

→ Name of medication(s) you have ever taken during the pregnancy for this condition:

_____

→ Were you admitted to the hospital for this?

☐ No ☐ Yes

→ Date of first admission: ☐☐☐ / ☐☐☐☐
MONTH (e.g. OCT) YEAR

→ Name of main physician who provided care (**if known**):_____

→ Hospital name and location:_____

→ For how many days were you in hospital: ☐☐
DAYS

b) **Gestational diabetes** (high blood sugar only during the pregnancy)**?**

☐ No ☐ Yes

→ Date first told: ☐☐☐ / ☐☐☐☐
MONTH (e.g. OCT) **YEAR**

→ Started on any medications during the pregnancy (pills or insulin):

☐ No ☐ Yes

→ Name of medication(s) (pills or insulin) you have ever taken during the pregnancy for this condition:

_____

→ Were you admitted to the hospital for this condition?

☐ No ☐ Yes

→ Date of first admission: ☐☐☐ / ☐☐☐☐
MONTH (e.g. OCT) **YEAR**

→ Name of main physician who provided care (**if known**):_____

→ Hospital name and location:_____

→ For how many days were you in hospital: ☐☐
DAYS

Person completing form (please print): _____ _____
last name **first initial**

Appendix C

LKD STUDY 3 - ☐☐☐ - ☐☐☐ - 1 Participant Initials: ☐☐☐ Assessment #: 0 1 Form #: 0

Centre ID | Participant ID | F M L

PREGNANCY SUBSTUDY: BIWEEKLY HOME COLLECTION CHART

1) Number of weeks pregnant OR number of weeks after delivery: ☐☐

2) Blood pressure recording:

	Morning	**Evening**
2a. Day #1 ☐☐/☐☐☐/☐☐☐☐ (DAY (e.g. 01) / MONTH (e.g. OCT) / YEAR)		
(Systolic/Diastolic mmHg)	☐☐☐/☐☐☐	☐☐☐/☐☐☐
2b. Day #2 ☐☐/☐☐☐/☐☐☐☐ (DAY (e.g. 01) / MONTH (e.g. OCT) / YEAR)		
(Systolic/Diastolic mmHg)	☐☐☐/☐☐☐	☐☐☐/☐☐☐

3) Urine protein by dipstick

3a. Reading #1: ☐ negative ☐ trace ☐ (1+) ☐ (2+) ☐ (3+) ☐ >3+

Date: ☐☐/☐☐☐/☐☐☐☐ (DAY (e.g. 01) / MONTH (e.g. OCT) / YEAR)

3b. Reading #2: ☐ negative ☐ trace ☐ (1+) ☐ (2+) ☐ (3+) ☐ >3+

Date: ☐☐/☐☐☐/☐☐☐☐ (DAY (e.g. 01) / MONTH (e.g. OCT) / YEAR)

Appendix D
PREGNANCY STUDY: CAREGIVER(S) VISIT LOG

Please, record all visits to your caregiver(s) that occur during and after your pregnancy (8 weeks) until the end of the study period.

Pregnancy Caregiver(s) Visit Date Log			
Visits	Date of visit to the family physician (day/month/year)	Date of visit to the obstetrician (day/month/year)	Date of visit to other pregnancy caregiver (midwife/nurse) (day/month/year)
1	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
2	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
3	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
4	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
5	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
6	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
7	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
8	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
9	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR
10	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	☐☐/☐☐☐/☐☐☐☐ DAY (e.g. 01) MONTH (e.g. OCT) YEAR

Please, record all admissions to the hospital(s) that occur during and 8 weeks after your pregnancy until the end of the study period.

Admissions	Name of hospital	Date of admission to the hospital (day/month/year)	Date of discharge from the hospital (day/month/year)	Reason for admission (if known)
1		□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	
2		□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	
3		□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	
4		□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	
5		□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	□□/□□□/□□□□ DAY (e.g. 01) MONTH (e.g. OCT) YEAR	

Appendix E
PREGNANCY SUBSTUDY: AFTER PREGNANCY FORM

1) During your pregnancy, were you ever told by a doctor you had/have gestational hypertension (high blood pressure developing after 20 weeks of pregnancy)?

☐ No ☐ Yes

→ Date first told: ☐☐ / ☐☐☐ / ☐☐☐☐
DAY (e.g. 01) MONTH (e.g. OCT) YEAR

→ Started on any medications:

☐ No ☐ Yes

→ Name of medication(s) you have ever taken for this condition:

_____

2) During your pregnancy, were you ever told by a doctor you have pre-eclampsia or toxemia (high blood pressure developing after 20 weeks of pregnancy with protein in your urine)?

☐ No ☐ Yes

→ Date first told: ☐☐ / ☐☐☐ / ☐☐☐☐
DAY (e.g. 01) MONTH (e.g. OCT) YEAR

→ Started on any medications:

☐ No ☐ Yes

→ Name of medication(s) you have ever taken for this condition:

_____

3) During your pregnancy, did you ever have convulsions or a seizure with high blood pressure (eclampsia)?

☐ No ☐ Yes

→ Date first told: ☐☐ / ☐☐☐ / ☐☐☐☐
DAY (e.g. 01) MONTH (e.g. OCT) YEAR

→ Started on any medications:

☐ No ☐ Yes

→ Name of medication(s) you have ever taken for this condition:

_____

4) During your pregnancy, were you ever told by a doctor you have gestational diabetes (high blood sugar during pregnancy)?

☐ No ☐ Yes

→ Date first told: ☐☐ / ☐☐☐ / ☐☐☐☐
DAY (e.g. 01) MONTH (e.g. OCT) YEAR

→ Started on any medications (pills or insulin):

☐ No ☐ Yes

→ Name of medication(s) you have ever taken for this condition:

_____

5) Were you ever admitted to a hospital during your pregnancy for any reason related to the pregnancy?

☐ No ☐ Yes (If yes, please ensure that your log form is up-to-date / complete)

Pregnancy Outcomes:

6) Were you pregnant with multiples? (i.e. twins, triplets, etc.) ☐ Yes ☐ No

→ ☐
NUMBER OF CHILDREN

7) How many times did you have any of the following events occur (**if never write 0**)?

Event	Number
a) Full-term live birth (pregnancy longer than 37 weeks and baby lived more than 28 days after birth)?	☐
b) Pre-mature live birth (pregnancy shorter than 37 weeks and baby lived more than 28 days after birth)?	☐
c) Stillbirth (pregnancy lasting longer than 20 weeks but fetus was not alive)?	☐
d) Neonatal death (child died within 28 days after live birth)	☐

8) Date of delivery: ☐☐ / ☐☐☐ / ☐☐☐☐

DAY (e.g. 01) **MONTH (e.g. OCT)** **YEAR**

9) Weeks of gestation at time of delivery: ☐☐ ☐ unknown

WEEKS

10) Method of delivery:

☐ vaginal (includes forceps, episiotomy, vacuum extraction)

☐ C-Section

☐ vaginal / C-Section (sometimes both can happen during multiple gestation delivery)

11) Birth weight of Child 1: ☐☐.☐☐ kg (or) ☐☐ lbs ☐☐ ounces

Child 2: ☐☐.☐☐ kg (or) ☐☐ lbs ☐☐ ounces

Child 3: ☐☐.☐☐ kg (or) ☐☐ lbs ☐☐ ounces

12) Where did you give birth?

☐ Home ☐ Birthing centre

☐ Hospital ☐ other (specify)

Person completing form (please print):_____ _____

Last name **first initial**

Appendix F
PREGNANCY SUBSTUDY: CENTRAL ADJUDICATION FORM

(This form is to be completed after receiving all the materials from the participants 8 weeks after delivery)

DATA COLLECTED: THE FIRST PREGNANCY POST DONATION GREATER THAN 20 WEEKS INTO PREGNANCY.

1) Had hypertension prior to pregnancy?

☐ No ☐ Yes ☐ Not Available

→ Was on blood pressure medication prior to pregnancy?

☐ No ☐ Yes ☐ Not Available

→ Name of medication(s):

_____

2) Had gestational hypertension?

☐ No ☐ Yes ☐ Not Available

→ Observed:

☐ antenatally ☐ during labour

☐ post-partum ☐ Not Available

→ Received medication?

☐ No ☐ Yes ☐ Not Available

→ Name of medication(s):

_____

3) Had pre-eclampsia?

☐ No ☐ Yes ☐ Not Available

→ Observed:

☐ antenatally ☐ during labour

☐ post-partum ☐ Not Available

→ Received medication(s)?

☐ No ☐ Yes ☐ Not Available

→ ☐ Outpatient ☐ Inpatient

→ Name of medication(s):

_____

4) Had eclampsia?

☐ No ☐ Yes ☐ Not Available

→ Observed:

☐ antenatally ☐ during labour

☐ post-partum ☐ Not Available

→ Was on medication(s)?

☐ No ☐ Yes ☐ Not Available

→ Name of medication(s):

_____

5) a) Was there evidence of multiple birth? (i.e. twins, triplets, etc)

☐ Yes ☐ No

→ ☐

NUMBER OF CHILDREN

b) Please indicate the outcomes for the pregnancy (**if never**, write 0):

Event	Number
a) Full-term live birth (pregnancy longer than 37 weeks and baby lived more than 28 days after birth)	☐
b) Pre-mature live birth (pregnancy shorter than 37 weeks and baby lived more than 28 days after birth)	☐
d) Stillbirth (pregnancy lasting longer than 20 weeks but fetus was not alive)	☐
e) Neonatal death (child died within 28 days after live birth)	☐

c) Date of delivery: ☐☐ / ☐☐☐ / ☐☐☐☐

DAY (e.g. 01) MONTH (e.g. OCT) YEAR

d) Weeks of gestation at delivery: ☐☐

WEEKS

e) Method of delivery (check one of the boxes below):

☐ vaginal (includes vacuum extraction, assisted forceps, delivery with episiotomy)

☐ C-Section

☐ both vaginal and C-Section (in some cases of multiple gestation)

f) If there was a C-section, indication(s) for it available:

☐ No ☐ Yes ☐ Not Available

(check all boxes that applies)

☐ Breech presentation ☐ Fetal distress

☐ Placenta praevia ☐ CPD cephalopelvic disproportion

☐ Previous caesarean ☐ preeclampsia, toxemia, eclampsia

☐ Not Available ☐ Other (specify _____)

g) Birth weight of : Child 1: ☐☐.☐☐kg (or) ☐☐ lbs ☐☐ ounces ☐ N/A

Child 2: ☐☐.☐☐kg (or) ☐☐ lbs ☐☐ ounces ☐ N/A

Child 3: ☐☐.☐☐kg (or) ☐☐ lbs ☐☐ ounces ☐ N/A

7) Was there any evidence of placental abruption?

☐ No ☐ Yes ☐ Not Available

8) Was there any evidence of placenta previa?

☐ No ☐ Yes ☐ Not Available

Person completing form (please print): _____ _____

last name **first initial**

Appendix G: Follow-up and Retention strategies for the LKD Pregnancy Study

- In follow-up, we will send a post card reminder every 4 months asking the participant to contact us if pregnant.
- When the participant informs us that she is pregnant, we will mail a congratulation card along with the kit.
- A coordinator personally will call or email (if available) each participant every 2 weeks until 8 weeks after child birth to follow up on blood pressure and urine protein measurements.
- We will mail a congratulation card soon after child birth.
- We will send a letter of sympathy with some flowers to participants who have a still birth or miscarriage or pregnancy loss.
- The post pregnancy survey is usually mailed to participants 8 weeks after child birth. Depending on participant's preference we also have the option of completing it over the phone with an interviewer.
- In an unlikely situation where a participant is unable to independently complete the study requirements we will schedule a home visit to help the participant.
- An extended search will be initiated for those who cannot be reached. This includes speaking to all the contacts the participant provided at the time of study entry.

Appendix H: Sample size calculations

Assuming, Proportion of hypertension in pregnancy among non-donors = 0.10
Two tailed Alpha=0.05
Power=80%; Relative risk=2

Ratio of donors to non-donors	**Donors**	**Non-donors**
1:1	199	199
2:1	286	143
3:1	372	124
4:1	460	115
5:1	545	109

Relative risk=2.5

Ratio of donors to non-donors	**Donors**	**Non-donors**
1:1	100	100
2:1	142	71
3:1	183	61
4:1	224	56

Chapter 5

Conclusions and Future Directions

This doctoral thesis explored the association between different kidney conditions and hypertension in pregnancy. A systematic review of literature, a retrospective cohort study using linked health care databases and a prospective multicentre study (protocol phase) were used to study this association. In this concluding section I summarize the three chapters of my thesis, discuss methodological limitations, and conclude with future directions.

Hypertension in pregnancy involves a cascade of physiological response (including the kidneys) and remains complex.[1] Future studies should relate to kidney playing a role in this process. Early intervention could reduce health care costs by reducing the number of hospital admissions for perinatal complications.[2]

5.1 SUMMARY OF CONCLUSIONS

5.1.1 **Chapter 2: Pregnancy Outcomes in Women with Chronic Kidney Disease**

This systematic review synthesized thirteen studies that described the association between CKD and adverse maternal and fetal outcomes with the use of an internal comparator group. Women with CKD appear to have at least a two-fold higher risk of developing

adverse maternal outcomes compared with women without CKD. Given the potential for risk, women with CKD who wish to become pregnant should have preconception counselling and antenatal care with a multidisciplinary "high-risk pregnancy" team.[1] This review is an efficient way for clinicians to become aware of the current published literature and to understand the limitations of available literature. They can integrate the information with their clinical expertise when counselling women with CKD about pregnancy.[3]

5.1.2 **Chapter 3: Walkerton Health Study: Pregnancy Outcomes**

This unique retrospective cohort study studied the association between pre-pregnancy E.*coli* exposure and the long term adverse pregnancy outcomes. For the purpose of this study, we linked data collected from Walkerton residents to provincial healthcare databases (2002-2011). This allowed us for a follow-up of nine years. We studied the pregnancies of three groups of women: two groups from Walkerton (those with and without acute gastroenteritis during outbreak) and a third group from neighbouring rural communities unaffected by the outbreak (referent group).

No significant association between *E. coli* O157:H7 exposure and the composite risk of gestational hypertension and preeclampsia in pregnancies were observed. However, the risk of preeclampsia was lower than expected in the referent group and overall there were small number of events in all the groups. Although outbreaks of toxigenic *E. coli* continue to occur worldwide, to our knowledge the Walkerton Health Study is the first study to examine the risk for hypertension in pregnancy after *E. coli* O157:H7 gastroenteritis.

Pregnant women with a history of toxigenic *E. coli* infection may benefit from additional prenatal surveillance.

5.1.3 **Chapter 4: Hypertension in Pregnancy after Kidney Donation**

I designed this prospective cohort study protocol as a sub-study to the already recruiting Living Kidney Donor study. The living kidney donor (LKD) Pregnancy Study is recruiting women at 12 major transplant centres throughout Canada. To date (January 12, 2013), 59 women (45 donors and 14 non-donors) have been enrolled into the LKD Pregnancy Study. We are in the process of collecting data for seven pregnancies so far which has proved feasible. This first stage of the study will be deemed successful if we have data collection for at least 20 pregnancies by the end of 2014.

5.2 METHODOLOGICAL LIMITATIONS

5.2.1 **Chapter 2: Pregnancy Outcomes in Women with Chronic Kidney Disease**

As with all systematic reviews, the quality of the primary studies inherently limits the results of the review.[4] All the included studies were observational studies as it is not possible to conduct randomized trials to study the research question posed. Many of the included studies did not adequately address for confounders. Statistical techniques used to adjust for correlation of pregnancies within the same women were not explained in many of the studies that included all pregnancies in a woman.[5, 6] Also, we were not able to study

the dose-response relationship between pregnancy outcomes and the degree of kidney function before pregnancy.

5.2.2 **Chapter 3: Walkerton Health Study: Pregnancy Outcomes**

One major issue that I came across while conducting this study included the limited number of events in the study population. There was not adequate statistical power to rule out clinically important differences on the outcomes between groups. I designed very stringent inclusion exclusion criteria to minimize confounding where ever possible. Adjustment for potential confounders such as age, parity, comorbidities and socio-economic status did not substantively affect the measures of association. However, we were not able to adjust for factors such as family history of preeclampsia, pre-pregnancy body mass index, and pre-pregnancy renal function, which were unavailable in our data sources.

5.2.3 **Chapter 4: Hypertension in Pregnancy after Kidney Donation**

In this prospective cohort study, where blood pressure and urine protein measurements are taken during pregnancy, loss to follow-up can be a major limitation.[7] So far there has been no loss to follow-up in the pregnancy study. In order to prevent loss to follow-up, I have discussed a number of proven retention strategies in detail in Chapter 4 of my thesis. Also, another challenge in prospective data collection can be missing data. To maximize complete data collection certain strategies such as home visits, following up missing survey responses with telephone interviews, and following up missing blood

pressure/urine measurements with medical charts have also been discussed in detail in the proposal.

5.3 FUTURE DIRECTIONS

5.3.1 **Pregnancy Outcomes in Women with Chronic Kidney Disease**

Pregnancy in women with CKD is uncommon and pregnancy outcomes in such women may not be desirable. Given existing data, it is quite evident that the current literature is of low quality, indicating the need for future research.[3] The adverse maternal and fetal outcomes in such women are certainly high. This review summarizes current evidence to guide physicians in their care of women with CKD who are pregnant or wish to become pregnant. Rigorously conducted multicenter prospective cohort studies should be done in the future recognizing the time, effort and funding involved in conducting such studies. Nonetheless, perinatal outcomes depend largely on the degree of renal dysfunction at the time of pregnancy rather than the specific disease itself. Future studies should measure baseline renal function at the time of pregnancy to determine if there is a dose response relationship between renal function and adverse maternal outcomes. If a dose response relationship is found, it should be quantified.

5.3.2 **Walkerton Health Study: Pregnancy Outcomes**

The unique circumstances of the Walkerton outbreak provided a rare opportunity to study the natural history following exposure to *E. coli* O157:H7 within a single cohort. The risk

for preeclampsia when examined separately was significantly elevated. The results from this study deserve confirmation from future studies. Outbreaks of toxigenic *E. coli* continue to occur world wide and the harmful long term effects of this pathogen on pregnancy needs further research.

5.3.3 **Hypertension in Pregnancy after Kidney Donation**

By accepting healthy persons into the role of a donor, our health care system takes on additional responsibility beyond our 'normal' tasks of curing, or at least helping patients with a disease. Living kidney donation is a unique model to help clarify the role of reduced nephron mass in the development of hypertension in pregnancy. From a clinical perspective, our study will provide new information that will improve donor selection, informed consent, and best practices for caring for donors who become pregnant. Once risk estimates are obtained from this study we will be able to have some perspective towards future research directions.

Reference List

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2. Backe B, Buhaug H. Societal costs of antenatal and obstetrical care. Acta Obstet Gynecol Scand 1994;73(9):688-694.

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4. Egger M DSGADe. Systematic Reviews in Health Care. BMJ 2001. 2001. Ref Type: Serial (Book,Monograph)

5. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. BMJ 2005;330(7491):565.

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7. Kristman V, Manno M, Cote P. Loss to follow-up in cohort studies: how much is too much? Eur J Epidemiol 2004;19(8):751-760.