THE ASSOCIATION BETWEEN VARYING DEGREES OF HYPERGLYCEMIA DURING PREGNANCY AND ADVERSE SHORT- AND LONG-TERM OUTCOMES

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. I was responsible for the conceptualization of the research presented in this thesis, including the development of study objectives and design. I conducted all data analyses and played the lead role in interpreting the results. I also drafted the manuscripts that form the core chapters of this thesis. My supervisor and TAC members provided guidance both in both epidemiological and statistical methodologies. While co-authors offered valuable feedback and suggestions, the intellectual direction, analytical decisions, and written content are primarily my own contributions.

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Since high school, I've been drawn to the health field—a decision deeply shaped by my experience as a caregiver for my younger brother. That role instilled in me a desire to better understand illness and be more helpful to those in need. This early experience laid the foundation for my journey, which began as a registered nurse and evolved into a passion for public health research. I realized that through research, I could impact not just individual lives, but broader health policies and systems. That realization led me to pursue a master's degree in Epidemiology at the School of Epidemiology and Public Health in 2019 and then transit to be a PhD student in 2020.

I am profoundly grateful to my supervisors, Dr. Shi Wu Wen, and Dr. Daniel J. Corsi. Their mentorship and support have had a lasting influence on me and will continue to shape my path as a researcher. They have guided me through the complexities of epidemiological and statistical methods, and provided invaluable expertise on my thesis research, which focuses on evaluating gestational diabetes mellitus criteria—a topic I care deeply about, given its implications for both maternal and fetal health. Beyond academic guidance, they've also supported my professional development with generosity, passion, and a commitment to excellence that have inspired my own aspirations in academia. Words truly cannot capture the depth of my appreciation and respect for them.

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Abstract

Background

Women with gestational diabetes mellitus (GDM) are at increased risk of adverse perinatal outcomes and long-term outcomes, including type 2 diabetes mellitus (T2DM) and cardiovascular diseases (CVD). However, evidence on the association between GDM and stillbirth is inconclusive, and recent studies suggest that women with mild hyperglycemia not meeting a clinical diagnosis of GDM may also confer increased risks of short- and long-term outcomes. Given the lack of universal screening and diagnostic criteria for GDM, relying solely on a GDM diagnosis may underestimate true risks. This thesis aimed to: (1) assess the association of varying degrees of hyperglycemia and risk of stillbirth through a systematic review and meta-analysis; (2) explore the impact of early- and late-onset GDM on late stillbirth using a retrospective cohort study; (3) evaluate the association of varying degrees of hyperglycemia and risk of diabetes via a systematic review and meta-analysis; and (4) examine the association of hyperglycemia during pregnancy and risk of T2DM in a nested case-control study.

Methods

Objectives 1 and 3 involved comprehensive searches in MEDLINE, EMBASE, and CINAHL. Meta-analyses were conducted to generate pooled odds ratios (ORs) and 95% confidence intervals (CIs) if the included studies were homogeneous. For objectives 2 and 4, the Cerner Real-World Dataset (CRWD), which comprises the United States electronic health record (EHR) data from 2000 to 2016, was used as the data source. Objective 2 included a cohort of women with a liveborn or stillborn surviving ≥ 28 weeks. The study outcome of late stillbirth was defined as death of a fetus occurred 28 weeks or more completed weeks of gestation. Maternal

and clinical characteristics among women with early GDM (0-23 weeks), late GDM (24-28 weeks) and non-GDM were described. Logistic regression was performed to determine the association of early GDM and late GDM with the risk of late stillbirth. Objective 4, a nested case-control study was conducted among women with glucose challenge test (GCT), or oral glucose tolerance test (OGTT) measured during pregnancy. A case was defined as having T2DM after index delivery until the end of follow-up time, while a control was defined as not being identified as having T2DM during the same follow-up period. Optimal variable matching was used for matching controls to cases (5:1) without replacement based on maternal age at delivery (±1 year), birth year (±1 year), and gestational age at delivery (±1 week) of the index pregnancy. Patients were divided into four groups: normal GCT, normal OGTT but abnormal GCT, one abnormal value on OGTT, and two or more abnormal values on OGTT. For these groups with one abnormal value and these with two or more abnormal values on OGTT, further analyses were conducted after stratifying by fasting glucose status. Maternal and clinical characteristics were compared between cases and controls. Conditional logistic regression was used to derive the best estimate of the association between glucose level and risk of T2DM.

Results

For the study on the association between maternal hyperglycemia and risk of stillbirth, the systematic review found that pooled ORs for risk of stillbirth among women with at least two abnormal values on OGTT and women with just one abnormal value or normal OGTT but abnormal GCT under the Carpenter & Coustan (C&C) criteria were 3.65 (95% CI, 1.74, 7.64), and 1.29 (95% CI, 0.56, 2.98), respectively, as compared to women with normal GCT. For women with at least one abnormal value diagnosed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, the pooled OR for the risk of stillbirth was 1.19

(95% CI, 0.94, 1.49), as compared to women with normal glucose level. Our cohort study found that the adjusted ORs (aORs) for the risk of late stillbirth among women with early GDM and late GDM were 3.50 (95% CI, 2.85, 4.29) and 3.20 (95% CI, 2.71, 3.76), respectively, as compared to women without GDM.

For the study on the association between maternal hyperglycemia and future risk of diabetes, the systematic review found that the pooled ORs for the risk of diabetes among women with at least two abnormal values and one abnormal value on 100-g OGTT were 12.29 (95% CI, 8.36, 18.07), and 4.44 (95% CI, 2.71, 7.28), respectively, as compared to women with normal GCT or OGTT. The nested case-control study found that aORs for the risk of T2DM among women with normal OGTT but abnormal GCT, one abnormal value on OGTT, and those with at least two abnormal values on OGTT diagnosed by the National Diabetes Data Group (NDDG) criteria were 1.66 (95% CI,1.35, 2.04), 5.69 (95% CI, 4.34, 7.47), and 13.20 (95% CI, 10.52, 16.57), respectively. Whereas the aORs for the risk of T2DM among women with normal OGTT but abnormal GCT, one abnormal value, and these with at least two abnormal values diagnosed by the Carpenter & Coustan (C&C) criteria were 1.21 (95% CI, 0.94, 1.55), 2.62 (95% CI, 1.95, 3.51), and 11.69 (95% CI, 9.61, 14.23), respectively. For women with at least two abnormal values on OGTT by either C&C or NDDG criteria, abnormal fasting glucose imposed additional risk of T2DM.

Conclusion and Implications

Women with borderline hyperglycemia during pregnancy that did not meet current diagnostic criteria for GDM (i.e., one abnormal value on OGTT) in some jurisdictions may be associated with increased risks of stillbirth and (especially) diabetes, although the associations were not as strong and as consistently observed in women with universally accepted diagnosis (i.e., two or

more abnormal values on OGTT) of GDM. Although no immediate medical intervention is needed, attention should be paid to women with borderline hyperglycemia during pregnancy for recommendations to intensify lifestyle changes to reduce their risk of adverse short- and long-term outcomes. Large scale studies assessing the association of fasting glucose status, along with the number of abnormal values, with adverse short- and long-term outcomes, should be conducted to confirm the preliminary findings from this thesis. Since universal screening policies are now in place in most countries/jurisdictions, prenatal care offers many young adult women opportunities of first contact with health care providers for screening of glucose levels. Pregnancy is a good time to detect women at increased risk of adverse short- and long-term outcomes, with targeted interventions to reduce the burden of the diseases for the affected women and their offspring.

This thesis work focused on stillbirth and T2DM, two of the most prominent adverse outcomes associated with hyperglycemia during pregnancy. As the next step, I plan to conduct a retrospective cohort study using large population health databases in Ontario, Canada to assess the association between maternal hyperglycemia and CVDs.

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LIST OF ABBREVIATIONS

ACOG: American College of Obstetricians and Gynecologists

ADA: American Diabetes Association

BMI: Body mass index

C&C: Carpenter & Coustan

CI: Confidence Interval

CRWD: Cerner Real-World Data™

CVD: Cardiovascular disease

EHR: electronic health records

GDM: Gestational diabetes mellitus

GCT: Glucose challenge test

G: Gram

GRADE: Grading of Recommendations Assessment, Development and Evaluation

HAPO: Hyperglycemia and Adverse Pregnancy Outcomes

HIPAA: Health Insurance Portability and Accountability Act

H: Hour

IADPSG: International Association of Diabetes and Pregnancy Study Groups

IFGO: International Federation of Gynecology and Obstetrics

NIH: National Institute of Health

NDDG: National Diabetes Data Group

NOS: Newcastle-Ottawa Scale

OGTT: Oral glucose tolerance test

OR: Odds ratio

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RevMan: Review Manager 5

T2DM: Type 2 Diabetes Mellitus

USPSTF: United States Preventive Services Task Force

WHO: World Health Organization

Chapter 1

INTRODUCTION

1.1 Background

Women with elevated glucose levels indicating glucose intolerance with onset or first recognition during pregnancy are typically diagnosed as having gestational diabetes mellitus (GDM), although diagnostic criteria for GDM remain somewhat variable.[1-2] GDM affects 4.4% pregnancies overall, varying from 2.9% to 10.6%.[3] Maternal glucose levels increase with the onset of insulin resistance in the second trimester and peak in the third trimester due to a progressive reduction in insulin sensitivity, particularly evident in women with impaired endocrine pancreatic function.[4-5] In the absence of uniform international standards for GDM screening and diagnosis, different jurisdictions have adopted varying criteria and approaches. The diagnostic criterion for GDM was initially established by O'Sullivan and Mahan over 60 years ago to identify women with a higher risk of type 2 diabetes (T2DM). This procedure involved a 50-gram (g) 1-hour (h) glucose challenge test (GCT) as a screening tool, followed by a 100-g 3-h oral glucose tolerance test (OGTT) for diagnosis in women with positive GCT results.[6] For the GCT, glucose level was measured at 1 hour after ingestion of the 50-g glucose load. For the OGTT, glucose levels were measured at baseline (fasting), and at 1-, 2-, and 3-hours following ingestion of the 100-g glucose load. This practice was based on the relationship between maternal glucose level in 100-g 3-h OGTT and the subsequent occurrence of diabetes. Modified versions of which were in use in the United States and some other areas after adjustment for the measurement of glucose in plasma instead of whole blood (National Diabetes Data Group (NDDG) criteria)[7], or the use of an enzymatic method (Carpenter & Coustan (C&C)

criteria)[8]. The International Association of Diabetic Pregnancy Study Groups (IADPSG) developed GDM criteria in 2010 based on a large, multi-center Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study, in which the odds of pre-defined adverse outcomes occurring reached 1.75 (an increase of 75%) when glucose was above the mean glucose level in the study population.[9] The IADPSG criteria was endorsed by the World Health Organization (WHO) and the International Federation of Gynecology and Obstetrics (IFGO), and have since been incorporated into clinical practice in many parts of the world.[10-11] This shift has led to a substantial increase in GDM rates, as only one abnormal value on a 75-g 2-h OGTT is sufficient to meet the diagnosis of GDM, rather than at least two abnormal values on a 100-g 3-h OGTT required by previous criteria.[10] The American Diabetes Association (ADA) recommended implementing the IADPSG criteria as a one-step strategy to screen and diagnose GDM as an alternative to existing criteria in 2015.[12] As interventional studies have not demonstrated the superiority of such a one-step approach, the two-step approach remains the preferred strategy and is recommended in some national guidelines.[13-14]

Before 2013, the ADA recommended screening for GDM in pregnant women with risk factors, while allowing for the omission of screening in those considered low-risk, in whom all the following factors were present: age younger than 25 years; body mass index (BMI) less than 25 before pregnancy; not of Hispanic, African American, American Indian, South or East Asian, or Pacific Islander descent; no first-degree relative with diabetes; no history of abnormal glucose tolerance; and no history of poor obstetric outcomes.[15-17] Universal screening for GDM after 24 weeks of gestation has been recommended since 2013 in the United States.[14,18] Meanwhile, the National Institutes of Health (NIH) and the American College of Obstetricians and Gynecologists (ACOG) advocated that women with significant risk factors of diabetes, such as

BMI >35 kg/m^2 or maternal age ≥ 40 years, and previous macrosomia must be screened for unrecognized T2DM before 24 weeks of gestation.[14,19] This practice has led to the identification of some cases of GDM before 24 weeks of gestation, referred to as early-onset GDM. The diagnostic criteria for early-onset GDM are similar to those used for GDM diagnosed between 24 and 28 weeks, although early-onset GDM cases tend to be more common among women with increased risk factors due to the nature of early screening.[20]

Screening and diagnosis for GDM are designed to identify women and their offspring who are at increased risk of short- and long-term adverse outcomes in the context of maternal hyperglycemia, coupled with treatment to reduce these complications and improve health-related quality of life. The two-step criterion for screening and diagnosing GDM in the United States were created based on a relatively small sample size over 60 years ago.[6] With increasing rates of obesity and lifestyle changes in modern society, it is essential to evaluate the effects of maternal elevated glucose levels on pregnancy outcomes and long-term health risks, such as stillbirth, T2DM, and cardiovascular diseases (CVDs). Consequently, the United Status Preventive Services Task Force (USPSTF) called for additional research on how screening for GDM directly influence maternal and infant health outcomes.[21] Although IADPSG was developed based on maternal glucose levels and perinatal outcomes, stillbirth was not assessed given the limited sample size in the prospective cohort study.[9] A recent systematic review showed that GDM was associated with slight higher risk of stillbirth, but with high probability due to chance, and the result of which was controversial given the high degree of heterogeneity and the possibility of important biases, especially immortal time bias in some of the included studies.[22]

Elevated maternal glucose levels during pregnancy, whether reaching a diagnosis of GDM, is believed to increase the risk of a variety of adverse health outcomes in pregnant women and their

offspring.[23-24] It has been reported that different degrees of gestational glucose intolerance may correspond to differential risks of short- and long-term outcomes.[23,25-26] However, it remains unclear at what level of maternal hyperglycemia may increase the risk of adverse perinatal outcomes and long-term maternal outcomes. Several recent studies have shown that the risk of diabetes differed based on type of abnormal glucose values on OGTT, including post-load (1-h, 2-h, 3-h) and fasting glucose.[24,27] However, the available evidence on these associations has not been appropriately synthesized through a comprehensive systematic review/meta-analyses. A simple diagnosis of GDM may provide limited information on prediction of risks of adverse short- and long-term adverse outcomes. This highlights the importance of quantifying the risk of adverse outcomes based on the degree of hyperglycemia during pregnancy.

1.2 Hypothesis and significance

Based on thorough literature review, we hypothesized that 1) GDM is associated with risk of stillbirth if appropriate methodology is used in the analysis; 2) borderline maternal hyperglycemia that did not meet current diagnostic criteria for GDM (i.e., one abnormal value on OGTT) in some jurisdictions is associated with increased risks of stillbirth and (especially) diabetes, although the associations are not as strong and as consistent for universally accepted diagnosis (i.e., two or more abnormal values on OGTT) of GDM. The findings of this thesis would bear significant public health implications. Pregnancy affords an excellent opportunity to detect women at increased risks of developing adverse pregnancy outcomes, T2DM, and CVDs, with targeted interventions to reduce the burden of these diseases in affected women and their offspring.[28] Since universal screening policies are now in place in most countries, prenatal care provides an opportunity for glucose screening and diagnosis of GDM in pregnant women at the point of first contact with their health care providers. Since maternal hyperglycemia is a

modifiable risk, an in-depth study of the association between varying degrees of hyperglycemia during pregnancy and adverse short- and long-term outcomes could yield useful information in policy development to guild optimal maternity care in the future.

1.3 Objectives

The objectives of my thesis research are as follows:

Objective 1: To provide an overview of variation in hyperglycaemia during pregnancy and risk of stillbirth through a systematic review and meta-analysis of existing evidence.

Objective 2: To determine whether pregnant women diagnosed with GDM have an increased risk of late stillbirth, and to examine whether this risk differs between early- and late-onset GDM.

Objective 3: To characterize the occurrence of varying degrees of hyperglycaemia during pregnancy and associated risk of diabetes through a systematic review and meta-analysis.

Objective 4: To quantify the risk of T2DM based on both the number of abnormal values on OGTT and fasting glucose status during pregnancy.

1.4 Structure of dissertation

This dissertation is manuscript based, with findings for objectives 1, 2, 3, and 4 presented in chapters 2-5, respectively. Specifically, chapter 2 presents findings of my systematic review of the association of varying degrees of hyperglycemia and risk of stillbirth. Chapter 3 presents findings for women with early- and late-onset of the GDM and risk of stillbirth based on a large retrospective cohort analysis. Chapter 4 presents a systematic review on the association of various degree of hyperglycemia during pregnancy and risk of diabetes. The findings of a nested case-control study which quantify the risk of T2DM based on the number and type of abnormal values on 100-g OGTT during pregnancy is then described in chapter 5. Chapter 6 presented an overarching discussion of the implications of these findings for public health policy and clinical

practice. The next steps following this thesis, including a systematic review protocol on the association between varying degrees of hyperglycemia and the risk of CVDs, as well as a grant application proposal to investigate this association, are presented in Chapter 7.

Chapter 2

Varying degrees of hyperglycaemia during pregnancy and risk of stillbirth: a systematic review and meta-analysis

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Abstract

Background Recent studies have found that women with hyperglycemia, not reaching a diagnosis of gestational diabetes mellitus (GDM), are still at increased risk for adverse perinatal outcomes. Therefore, relying on a binary GDM diagnosis to predict risk of adverse outcomes provides limited insight, particularly given that growing emphasis on personalized care. A recent systematic review did not find that women with GDM had higher risk of stillbirth as compared to women without GDM. However, several studies included in that systematic review were subject to immortal time bias, which could have led to biased effect estimates. In light of these limitations, this study aims to appropriately assess the association of varying degrees of hyperglycemia, based on the number and type of abnormal values on oral glucose tolerance test (OGTT), and risk of stillbirth through a systematic review and meta-analysis, with improved methodology.

Methods Comprehensive searches were conducted in MEDLINE, EMBASE, and CINAHL to retrieve relevant papers. The study population of the included study should be women who have been screened for GDM using a glucose challenge test (GCT) or OGTT during pregnancy. Statistical heterogeneities were assessed by using I^2 (I^2>50% indicate substantial heterogeneity) and Cochrane's Q test (P<0.2). Meta-analyses were conducted to generate pooled odds ratios (ORs) and 95% CIs if the included studies were homogeneous.

Results Eighteen studies were included in this review, whereas 17 studies were included in the meta-analyses**.** A total of 243,033 pregnant women were included in meta-analyses. The pooled ORs for risk of stillbirth among women with at least two abnormal values, and one abnormal value or normal OGTT but abnormal GCT under the Carpenter &Coustan (C&C) criteria were 3.65 (95% CI, 1.74, 7.64) and 1.29 (95% CI, 0.56, 2.98), retrospectively, as compared to women

with normal GCT. For the comparison of women with at least one abnormal value diagnosed by International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria and women with normal OGTT, the pooled OR for the risk of stillbirth was 1.19 (95%CI, 0.94, 1.49).

Conclusion Women with at least two abnormal values on OGTT by the C&C criteria had about four times higher risk of stillbirth as compared to women with normal glucose level, whereas women with one abnormal value or normal OGTT but abnormal GCT by the C&C criteria, and women with one abnormal value by the IADPSG criteria had slightly higher risk of stillbirth, very likely due to chance. It is worth to mention that inadequate power hindered the ability to draw conclusion on the association of varying degrees of hyperglycemia with stillbirth. Further research is needed to quantify the risk of stillbirth based on number and type of abnormal values on OGTT by different criteria vias large scale studies.

2.1 Background

Stillbirth has been reported to occur in approximately 14 in 1000 births globally in 2021.[1] In 2014, the World Health Assembly endorsed Every Newborn Action Plan, which includes a global target of 12 or fewer third trimester (late) stillbirths per 1,000 births in every country by 2030.[2] Fifty-six countries–the majority of which are low-income–had not yet met the target by 2021 and might not meet the target if further efforts are not made.[3] Stillbirth causes psychological costs, especially to women and their families, such as maternal depression, financial consequences, as well as stigma and taboo.[3] The effect of maternal hyperglycemia begins in utero, where it can lead to a relative fetal hypoxia, increasing the risk of stillbirth.[4] The risk of stillbirth among pregnant women with pre-existing diabetes is about four times higher compared to the general obstetric population.[5-6] However, whether women with gestational diabetes mellitus (GDM) are at increased risk of stillbirth remains inconclusive.

There are currently not universally accepted diagnostic criteria for GDM. Women with glucose intolerance with onset or first recognition during pregnancy are often diagnosed as GDM.[7] GDM affects around 3.6% to 11.4% of pregnant women worldwide, and differences in diagnostic criteria result in variable estimates.[8] There is an increasing trend of GDM, a result of changes in the criteria for diagnosing GDM[8], and the obesity epidemic[9]. A recent systematic review and meta-analysis reported that there was no association of GDM and risk of stillbirth, but considerable heterogeneity has been found across studies.[10] However, some large cohort studies included in the systematic review were subject to immortal time bias, which may have led to the misleading conclusion that GDM has a protective effect against the risk of stillbirth.[11-13] Specifically, in these studies, a clinical diagnosis of GDM was used without examining the actual glucose test data. Under this scenario, pregnancies in the exposed group must have "survived"

until 24–28 weeks of gestation for the women to be screened for and diagnosed with GDM. In contrast, women who were not diagnosed with GDM, including those whose fetuses did not survive to 24-28 weeks of gestation and therefore were never screened, were classified as the non-exposed group. According to the United States Centers for Disease Control and Prevention (CDC) National Vital Statistics Reports, stillbirths occurring between 20 weeks and 27 weeks of gestation accounted for about half of all stillbirths from 20 weeks to delivery between 2014 and 2022.[14] As a result, the overall stillbirth rate may ironically appear higher in the non-GDM group, which introduced immortal time bias and resulted in biased outcome estimates.[15] Moreover, lumping studies with different diagnostic criteria for GDM into one meta-analysis might further contribute to the heterogeneity and make the study results hard to interpret. Our systematic review will address these issues by including only studies in which the study population consists of pregnant women who were screened for GDM, thereby minimizing immortal time bias. Additionally, we will conduct separate meta-analyses based on the different diagnostic criteria used to further reduce heterogeneity.

It has been reported that women with hyperglycemia, not reaching a diagnosis of GDM, are at increased risk of short- and long-term adverse outcomes. A recent review demonstrated that maternal fasting glucose concentrations are more strongly associated with adverse perinatal outcomes than post-load glucose levels.[16] Therefore, relying on a binary GDM diagnosis to predict stillbirth risk provides limited insight, particularly in light of the growing emphasis on personalized care. We aim to assess the impact of varying degrees of hyperglycemia during pregnancy, based on the number and type of abnormal glucose values on oral glucose tolerance test (OGTT), on the risk of stillbirth through a systematic review and meta-analyses.

2.2 Methods

Eligibility criteria The study population of the included studies should consist of women who were screened for GDM during pregnancy using a glucose challenge test (GCT) and/or a 75-gram(g) or 100-g OGTT. Studies that used a diagnosis of GDM without specifying the diagnostic criteria or in which hyperglycaemia was not determined based on glucose measurements, were excluded. Studies in which glucose was not measured in plasma were excluded. Additionally, studies focusing on high-risk population with specific pre-existing conditions, and those in which GDM screening occurred before 24 weeks of gestation, were also excluded from this review. The outcome of this systematic review was stillbirth, defined as any fetal death occurring after 20 weeks of gestation. Studies were excluded if stillbirth was only reported as part of a composite adverse outcome. No restrictions were placed on study setting, publication date or language. All observational studies, including cohort studies, case-control studies, and cross-sectional studies were eligible for inclusion. In contrast, case reports, case series, commentaries, editor letters, reviews without original data, and animal studies were excluded.

Information sources and search strategy A comprehensive literature search was conducted across EMBASE, MEDLINE, and CINAHL databases. The date of publication was set from inception to June 29, 2024. Reference lists of included studies and relevant systematic reviews were also scanned to identify additional eligible studies. The search strategy was developed with the aid of a medical librarian experienced in systematic review research. Medical Subject Headings [MeSH] and keywords related to study population, exposure, outcome, and study type were combined to create search strategies. The complete search strategies for each database are provided in Supplement 1. Abstract screening and full-text review were conducted independently by two reviewers (NZ, JY) using Covidence, based on the pre-defined eligibility criteria.

Duplicates were removed automatically, with additional manual checks to eliminate remaining duplicates. Discrepancies regarding the screening processes were resolved with the involvement of a third reviewer (SWW). Reasons for exclusion were documented at the full-text screening stage.

Data extraction Two reviewers (NZ, JY) independently extracted data from the included studies. A data extraction form was created as a data collection tool and was modified based on group feedback. A calibration exercise was implemented by reviewers to ensure consistent methods of assessment between reviewers. The principal investigator (SWW) was involved wherever any discrepancies arose between reviewers. The following details were extracted from each study: (1) Studies characteristics: first author's last name, year of publication, study design, country of study, study period; (2) Study population: sample size; high-risk population or universal; (3) Outcome measures: definition of stillbirth (time period), including antepartum stillbirth (refers to the death of a fetus in the womb before labor begins); (4) Exposure measures: type of glucose tests (e.g., GCT, 75-g OGTT or 100-g OGTT), measurement time, diagnostic criteria for GDM (details in Table 2.1); (6) Comparison groups: available comparison group of interest were listed.

Table 2.1 GDM diagnostic criteria published in the literature*

	No. of abnormal values to qualify as a diagnosis of GDM	Fasting glucose (mmol/l)	1-h post-load (mmol/l)	2-h post-load (mmol/l)	3-h post-load (mmol/l)
100-g OGTT					
C&C	≥2	5.3	10.0	8.6	7.8
NDDG	≥2	5.8	10.6	9.2	8.0
75-g OGTT					
IADPSG	≥1	5.1	10.0	8.5	N/A
WHO 2013	≥1	5.1	N/A	8.5	N/A

WHO 1999	≥1	7.0	N/A	7.8	N/A
CDA	≥1	5.3	10.6	9.0	N/A
ADIPS (1998)	≥ 1	5.5	N/A	8.0	N/A

*All criteria were recommended screening at 24-28 weeks of gestation, with some starting with 50-gramGCT while others directly undergoing OGTT; For OGTT: any of these test (fasting, 1-h, 2-h, and 3-h post-load) occurred at one visit to the clinical site; C&C, Carpenter & Coustan; NDDG, National Diabetes Data Group; IADPSG, International Association of Diabetes and Pregnancy Study Groups; WHO, World Health Organization; CDA, Canadian Diabetes Association; ADIPS, The Australasian Diabetes in Pregnancy Society; OGTT, oral glucose tolerance test. N/A: Not Available.

Quality assessment The Newcastle-Ottawa Scale (NOS) was used to assess the methodological quality of the included studies. Three versions of NOS tailored for cohort studies, case-control studies and cross-sectional studies were applied for each type of study. The NOS evaluates the risk of bias across three domains: selection, comparability, and ascertainment of outcome or exposure, which consisted of eight items. In the selection and ascertainment of outcome or exposure domain, a maximum of one star could be awarded per item, while the comparability domain allowed for up to two stars. Based on the total score, studies were categorized as follows: high quality (7–9 stars), moderate risk of bias (4–6 stars), and high risk of bias (0–3 stars). Two reviewers (NZ, JY) independently conducted the quality assessment, with any disagreements resolved through consultation with a third reviewer (SWW).

Data synthesis and Analysis Patient and study characteristics were managed and analyzed by Review Manager 5 (RevMan). Statistical heterogeneity was assessed by using I^2 statistic, with values greater than 50% indicating substantial heterogeneity, and Cochrane's Q test, with a significance threshold of $P < 0.2$.[17] Pooled crude Odds Ratios (ORs) and 95% confidence interval (CI) were calculated. Random effects models were used to determine weights for meta-analysis. The Mantel-Haenszel method was used for the random effects model. The pooled effect measures were calculated separately for each GDM diagnostic criteria based on the number of abnormal values on OGTT and fating glucose status. For studies using 100-g OGTT, participants

were categorized into: (1) two or more abnormal values on OGTT; (2) one abnormal value on OGTT or normal OGTT but abnormal GCT; and (3) normal GCT (reference). For the 75-g OGTT, women were categorized as having (1) one or more values on OGTT, and (2) normal OGTT or normal GCT (reference). Exposure groups were further categorized based on the fasting glucose status. A qualitative (narrative) synthesis was performed when heterogeneity was substantial. Subgroup analyses were conducted based on geographic region of the included study (Asian, North American, and Europe) to explore potential sources of heterogeneity. Reporting bias was assessed by comparing reported outcomes in published protocols with those in final manuscript. If protocols were unavailable, outcomes described in the methods sections were compared with those reported in the results. Publication bias was evaluated using funnel plots when ten or more studies were included; otherwise, it was not assessed due to limited statistical power. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was used to evaluate the overall strength of the evidence. This systematic adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A PRISMA flow diagram was used to present the study selection process. The protocol for this systematic review was registered in an international systematic review registry—PROSPERO (registration number: CRD42024565162).

2.3 Result

Figure 2.1 Study selection

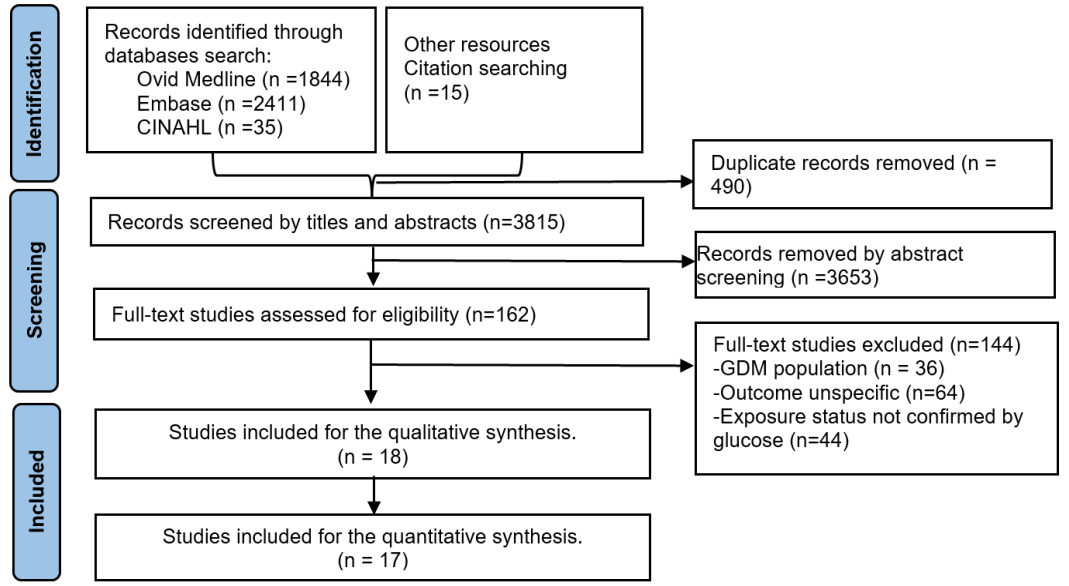


Table 2.2 Characteristics of included studies

First Author & Year	Country	Study design	Hospital/ Registry-based	Study period	Sample size	GDM criteria	Universal /High-risk	Stillbirth Definition	Timing of GDM assessment	Glucose type	Comparison groups of interest
100-g OGTT											
Yesildager (2014)[18]	Turkey	Prospective cohort	Hospital	2010-2011	192	C&C	Universal	Antepartum stillbirth	24-28w	100-g OGTT, GCT	Normal OGTT abnormal GCT vs. Normal GCT
Simsek (2021)[19]	Turkey	Retrospective cohort	Hospital	2016-2020	1,638	C&C	Universal	Stillbirth (>22w)	24-28w	100-g OGTT	1.One abnormal value vs. Normal GCT 2.Two abnormal values & above vs. Normal GCT
Hossein-Nezhad (2007)[20]	Iran	Prospective cohort	Hospital	N/A	2,416	C&C	Universal	Stillbirth	24-28w	100-g OGTT	Two abnormal values & above vs. Normal GCT
Grotegut (2008)[21]	USA	Retrospective cohort	Hospital	2003-2005	330	C&C	Universal	Antepartum stillbirth	24-28w	100-g OGTT, GCT	[One abnormal value + Normal OGTT] vs. Normal GCT
Magee (1993)[22]	USA	Prospective cohort	Registry	1985-1986	881	C&C	Universal	Stillbirth	24-32w	100-g OGTT	1.Two abnormal values & above vs. Normal GCT 2.[One abnormal value + Normal OGTT] vs. Normal GCT
Stamilio (2004)[23]	Germany	Retrospective cohort	Hospital	1995-1997	1,825	NDDG	Universal	Antepartum stillbirth	24-28w	100-g OGTT	[One abnormal value + Normal OGTT] vs. Normal GCT
Ethridge Jr (2014)[24]	USA	Retrospective cohort	Hospital	2007-2012	2,459	C&C	Universal	Stillbirth	> 24w	100-g OGTT	1.Two abnormal values & above vs. Normal GCT 2.[One abnormal value + Normal OGTT anormal GCT] vs. Normal GCT
75-g OGTT											
Alfadhhi (2015)[25]	Saudi Arabia	Prospective cohort	Hospital	2011-2014	573	IADPSG	Universal	Stillbirth (>22w)	24-28w	75-g OGTT	One abnormal value & above vs. normal OGTT
Koning (2018)[26]	Netherlands	Retrospective cohort	Hospital	2011-2016	4,431	WHO 1999/WHO 2013	High-risk	Stillbirth	24-28w	75-g OGTT	One abnormal value & above vs. normal OGTT
Donovan (2017)[27]	Canada	Retrospective cohort	Registry	2008-2012	181,616	IADPSG/CDA	Universal	Stillbirth	24-28w	75-g OGTT, GCT	1.Normal OGTT abnormal GCT vs. Normal GCT 2.One abnormal value & above vs. Normal GCT
Li (2020)[28]	China	Retrospective cohort	Hospital	2016	3,154	IADPSG	Universal	Stillbirth	24-28w	75-g OGTT	One abnormal value & above vs. Normal OGTT
Nayak (2013)[29]	India	Prospective cohort	Hospital	2011-2012	304	IADPSG	Universal	Stillbirth	24-32w	75-g OGTT	One abnormal value & above vs. Normal OGTT
Jiang (2017)[30]	Australia	Retrospective cohort	Hospital	2011-2015	4,081	ADIPS	Universal	Stillbirth	> 20w	75-g OGTT, GCT	One abnormal value & above vs. Normal OGTT anormal GCT

Shen (2020)[31]	China	Retrospective cohort	Hospital	2014-2015	15,097	IADPSG	Universal	Stillbirth (>20w)	26-28w	75-g OGTT	One abnormal value & above vs. Normal OGTT
Wahabi (2017)[32]	Saudi Arabia	Prospective cohort	Hospital	2013-2015	9,305	IADPSG	Universal	Stillbirth	24-34w	75-g OGTT	One abnormal value & above vs. Normal OGTT
Young (2020)[33]	China	Retrospective cohort	Hospital	2016	733	IADPSG/ WHO 2013	High-risk	Stillbirth	24-28w	75-g OGTT	One abnormal value & above vs. Normal OGTT
Prakash (2017)[34]	India	Prospective cohort	Hospital	2012-2014	271	IADPSG	Universal	Stillbirth	N/A	75-g OGTT	One abnormal value & above vs. Normal OGTT
Pan (2015)[35]	China	Prospective cohort	Registry	2010-2012	17,808	IADPSG	Universal	Stillbirth	24-28w	75-g OGTT, GCT	One abnormal value & above vs. (Normal OGTT anormal GCT +normal GCT)

C&C, Carpenter &Coustan; NDDG, National Diabetes Data Group; IADPSG, International Association of Diabetes and Pregnancy Study Groups; WHO, World Health Organization; CDA, Canadian

Diabetes Association; ADIPS, The Australasian Diabetes in Pregnancy Society; OGTT, oral glucose tolerance test; GCT, glucose challenge test; N/A: Not Available

The study selection process is illustrated in the flow chart (Figure 2.1). Following abstract screening, 162 full-text articles were reviewed for eligibility. Of these, 18 studies were included in the qualitative synthesis, and 17 were included in the quantitative meta-analysis. The characteristics of the included studies are summarized in Table 2.2. Geographically, 11 studies were conducted in Asia, two in Europe, four in North America, and one in Australia. Regarding glucose measurements, seven studies used 100-g OGTT, while 11 studies used 75-g OGTT. Among studies using 100-g OGTT, six applied the Carpenter and Coustan (C&C) criteria, and one used the National Diabetes Data Group (NDDG) criteria. Regarding studies using 75-g OGTT, nine studies employed the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, while two studies used the World Health Organization (WHO) 2013 criteria, one study used the WHO 1998 criteria, and one study assessed both the Canadian Diabetes Association (CDA) and the Australasian Diabetes in Pregnancy Society (ADIPS) criteria. In total, 247,114 pregnant women who underwent glucose testing were included in the review, with 243,033 of them included in the meta-analyses. All included studies were rated as high quality, with 11 studies scoring nine points, two studies scoring eight and five studies scoring seven (Table 2.3).

Table 2.3 Quality assessment

Author& Year	Study design	Selection	Comparability	Outcome	Total score
Yesildager (2014)	Prospective cohort	★★★★	★★	★★★	9
Simsek (2021)	Retrospective cohort	★★★★	★★	★★★	9
Hossein-Nezhad (2007)	Prospective cohort	★★★★	★★	★★★	9
Grotegut (2008)	Retrospective cohort	★★★	★	★★★	7
Magee (1993)	Prospective cohort	★★★★	★★	★★★	9
Stamilio (2004)	Retrospective cohort	★★★★	★★	★★★	9
Ethridge Jr (2014)	Retrospective cohort	★★★★	★★	★★	8
Alfadhhi (2015)	Prospective cohort	★★★★	★	★★	7
Koning (2018)	Cross-sectional study	★★★	★	★★★	7
Donovan (2017)	Retrospective cohort	★★★★	★	★★★	8
Li (2020)	Retrospective cohort	★★★★	★★	★★★	9
Nayak (2013)	Retrospective cohort	★★★	★★	★★★	8
Jiang (2017)	Retrospective cohort	★★★★	★★	★★★	9

Shen (2020)	**Prospective cohort**	★★★★	★★	★★★	**9**
Wahabi (2017)	**Retrospective cohort**	★★★★	★★	★★★	**9**
Young (2020)	**Prospective cohort**	★★★★	★★	★★★	**9**
Prakash (2017)	**Prospective cohort**	★★★	★	★★★	**7**
Pan (2015)	**Prospective cohort**	★★★★	★★	★★★	**9**

100-g OGTT

Compared to women with normal GCT, those with at least two abnormal values on 100-g OGTT based on the C&C criteria had a significantly increased risk of stillbirth, with a pooled OR of 3.65 (95% CI, 1.74, 7.64) (Figure 2.2). For women with one abnormal value or normal OGTT but abnormal GCT under the C&C criteria, the pooled OR for stillbirth was 1.29 (95% CI, 0.56, 2.98) (Figure 2.2). Only one study conducted in Germany assessed the risk of stillbirth based on the number of abnormal values under the NDDG criteria.[23] This study reported that women with one abnormal value on the 100-g OGTT had a statistically non-significant increased risk of stillbirth, with an adjusted OR of 4.61 (95%CI, 0.77, 27.48), compared to women with normal GCT. None of the included studies evaluated the risk of stillbirth based on both the number and type of abnormal values on 100-g OGTT.

When stratified by region, the pooled ORs for stillbirth among women with at least two abnormal values by the C&C criteria was 4.26 (95% CI, 1.80, 10.11) in Asian countries and 2.21 (95% CI, 0.47, 10.33) in North American countries (Supplement 2, 3). In the comparison between women with one abnormal value and those with normal GCT under the C&C criteria, the pooled ORs was 3.36 (95% CI, 0.71, 15.85) in Asia and 0.81 (95% CI, 0.27, 2.41) in North America (Supplement 2, 3). No reporting bias was identified. Publication bias was not assessed due to the small number of included studies ($n < 10$).

75-g OGTT

Compared to women with normal OGTT or GCT, women with at least one abnormal value on 75-g OGTT by the IADPSG criteria had a pooled OR of 1.19 (95%CI, 0.94, 1.49) for risk of

stillbirth (Figure 2.3). For women diagnosed using the WHO 2013 criteria, the pooled OR for stillbirth was 1.87 (95% CI, 0.79, 4.42) compared to those with normal OGTT (Figure 2.4). One study applying the WHO 1999 criteria reported women with at least one abnormal value had statistically non-significant lower risk of stillbirth as compared to women with normal OGTT (OR: 0.55 (95%CI, 0.12, 2.42).[26] The study using the ADIPS criteria reported an OR of 1.02 (95% CI, 0.22, 4.82) for stillbirth among women with at least one abnormal value as compared to women with normal OGTT.[30] Similarly, a Canadian study applying the CDA criteria found an OR of 1.24 (95% CI, 0.84, 1.83) for the same comparison.[27] None of the included studies evaluated the risk of stillbirth based on both the number and type of abnormal values on 75-g OGTT.

For studies applying the IADPSG criteria in Asia countries, the pooled OR for stillbirth among women with at least one abnormal value compared to those with normal OGTT or GCT was 1.17 (95% CI, 0.84, 1.62) (Supplement 4). In North America, one study[27] reported an OR of 1.21 (95% CI, 0.87, 1.67) for the same comparison (Supplement 5). Under the WHO 2013 criteria, the ORs for stillbirth among women with at least one abnormal glucose value were 1.21 (95% CI, 0.87, 1.67) in a study conducted in Europe and 5.27 (95% CI, 0.87, 31.82) in a study from Asia (Supplement 6, 7). No reporting bias was identified. Publication bias was not assessed due to the small number of included studies (n < 10).

Figure 2.2 Pooled odds ratio of stillbirth for the comparison of at least one abnormal value on 100-g OGTT vs normal GCT by C&C criteria

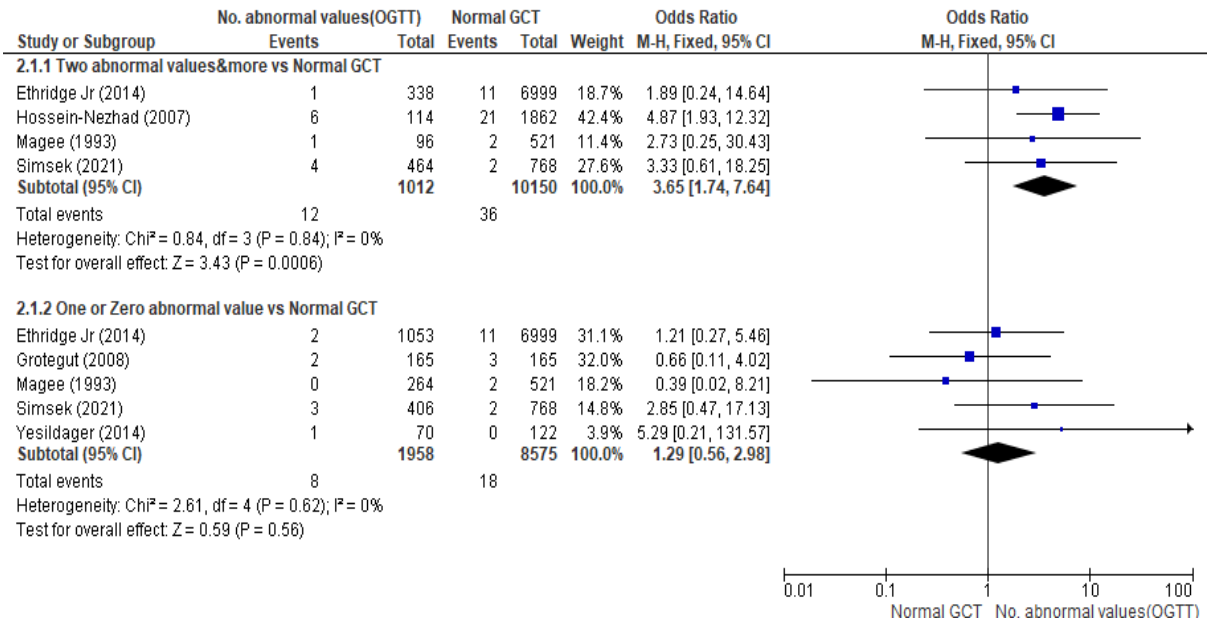


Figure 2.3 Pooled odds ratio of risk of stillbirth for the comparison of at least on abnormal value on 75-g OGTT vs. normal OGTT/GCT under IADPSG criteria

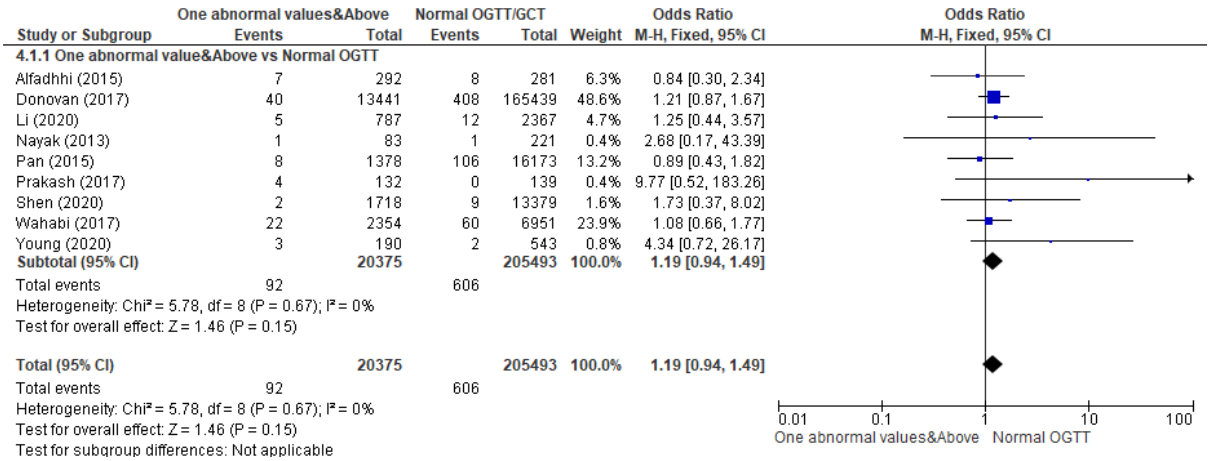
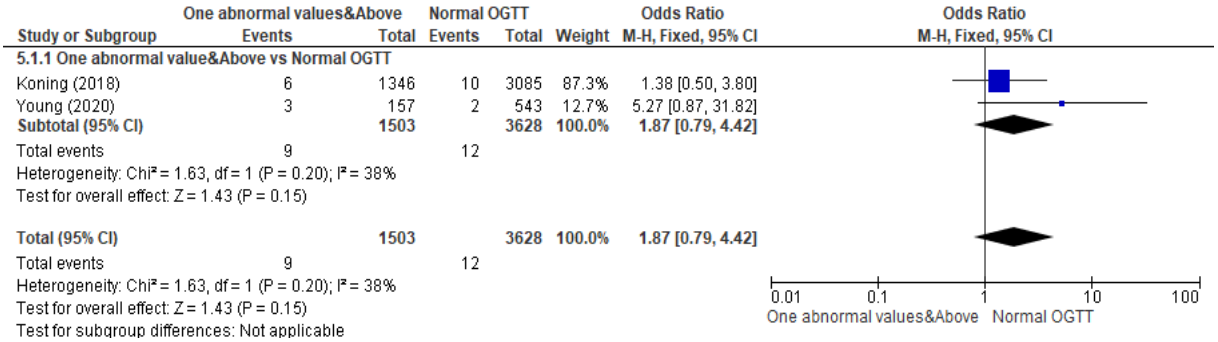


Figure 2.4 Pooled odds ratio of risk of stillbirth the comparison of at least on abnormal value on 75-g OGTT vs. normal OGTT under WHO 2013



2.4 Discussion

Women with at least two abnormal values on 100-g OGTT based on the C&C criteria had about four times higher risk of stillbirth compared to women with normal GCT. However, the risk of stillbirth among women with one abnormal value on OGTT value or normal OGTT but abnormal GCT was slightly higher as compared to women with normal GCT, but with high probability due to chance. Furthermore, women with at least one abnormal value on 75-g OGTT diagnosed by the IADPSG criteria was also at slightly higher risk of having stillbirth compared to women with normal OGTT/GCT, with high probability due to chance.

Our systematic review addresses key limitations in previous research. The previous systematic review did not find an association between GDM and stillbirth.[10] However, the findings from the previous meta-analysis were inconclusive due to substantial heterogeneity across studies (I^2 > 85% for both case-control and cohort studies) and the presence of immortal time bias in several included studies. The immortal time bias may be the main reason for the failure to accurately assess the relationship between GDM and stillbirth. Specifically, several retrospective studies included in the previous review reported a counterintuitive protective effect of GDM on the risk of stillbirth; due to their large sample sizes, these studies disproportionately influenced the pooled estimates and skewed the results in the opposite direction of what would be clinically expected.[11-13] Our systematic review addressed these issues in two keyways. First, we limited inclusion to studies where GDM status was determined by GCT or OGTT measurements, ensuring that both exposed and unexposed groups survived until the testing period (24–28 weeks), thereby minimizing the risk of immortal time bias. Second, we conducted separate meta-analyses for each diagnostic criteria, which reduced heterogeneity and improved interpretability. As a result, included studies within each meta-analysis were more methodologically consistent,

with no observed heterogeneity ($I^2 = 0$).

In our systematic review, we found that the severity of hyperglycemia was positively associated with the risk of stillbirth. GDM diagnosed by the C&C criteria (at least two abnormal values on 100-g OGTT) represented women with severe hyperglycemia as it has higher thresholds as compared to women with GDM diagnosed by the IADPSG criteria (at least one abnormal value). Although there was difference in the glucose load for the OGTT test (75-g vs 100-g), there were only slight difference in mean glucose level between pregnant women tested by the 100-g OGTT (1-, 2-, 3-h post-load, 8.4 mmol/dl, 7.5 mmol/dl, 6.6 mmol/dl) and 75-g OGTT (1-, 2-, 3-h post-load, 8.3mmol/dl, 7.0mmol/dl and 5.6 mmol/dl).[36] Our review did not find women with one abnormal value on 100-g OGTT by C&C criteria, representing a less severe form of hyperglycemia, had higher risk of stillbirth as compared to women with normal GCT, which might be due to smaller effect size, or small sample size for the meta-analysis.

Strengths and weaknesses This is the first systematic review and meta-analyses assessed the association between hyperglycaemia during pregnancy and stillbirth based on the number of abnormal values under different diagnostic criteria, of which the findings were relevant to stakeholders and jurisdictions adopting different criteria. Since we excluded studies relied solely on clinical diagnosis of GDM without actual glucose measurement data, the pooled estimate may be accurate as immortal time bias was minimized among the included studies. However, our review had much smaller sample size (only 48 events in all included studies) compared with previous review, which limited the confidence of the evidence regarding those women with at least two abnormal values by the C&C criteria had about four times higher risk of stillbirth compared to women with normal GCT. The sample size was also very limited for the meta-analysis on examining the association of borderline hyperglycemia, represented by those with

one abnormal value or normal OGTT but abnormal GCT under the C&C criteria, and the risk of stillbirth. Given the population we interested in this review are pregnant women screened with GCT and/or OGTT, which would lead to that the study population in this review need to survive until the glucose measurement time, therefore most of the stillbirth cases in this review occurred at 24-28 weeks and after. Although all included studies were observational ones, overall rating of the evidence should still be considered medium given the magnitude of the effect and the existence of dose response relationship, and low risk of bias. However, inadequate power hindered the ability to draw conclusion on the association of GDM defined by different criteria with stillbirth.

2.5 Conclusion and Implications

Our systematic review indicated that women with at least two abnormal values on 100-g OGTT diagnosed by the C&C criteria have about four times higher risk of stillbirth compared to women with normal GCT. Therefore, surveillance and intervention should be targeted on these high-risk women. However, risk of stillbirth for women with one abnormal value and normal OGTT but abnormal GCT diagnosed by the C&C criteria or women with at least one abnormal value by the IADPSG criteria had slightly higher risk of stillbirth as compared with women of normal glucose levels, but with high probability due to chance. Large scale studies assessing the association of number and type of abnormal values on OGTT under different criteria with stillbirth should be conducted to confirm some of the preliminary findings from this systematic review. Once we quantify the risk based on both the number and type of abnormal values under each diagnosis criteria, then further clinical practice suggestions could be provided for patients and care providers.

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2003 May;81(2):169-73. doi: 10.1016/s0020-7292(03)00031-6. PMID: 12706274.

2.7 Supplements

Supplement 1

1.1 Search Strategy - Ovid MEDLINE(R) <1946 to June 28, 2024>

#	Searches	
1	Blood glucose/	187340
2	glucose intolerance/	10095
3	glucose tolerance test/	37111
4	(Glucose adj3 (tolera* or intolera* or fast* or value* or level* or	174649
5	(blood adj3 (sugar or glucose)).ti,ab,kf.	118251
6	OGTT.ti,ab,kf.	10782
7	(dysglyc?emi* or hyperglyc?emi*).ti,ab,kf.	81367
8	1 or 2 or 3 or 4 or 5 or 6 or 7	372607
9	pregnancy/	1016407
10	(pregnan* or gestation* or trimester or antepartum or gravidity).ti,ab,kf.	769580
11	9 or 10	1233561
12	8 and 11	25781
13	Case-Control Studies/ or Control Groups/ or Matched-Pair Analysis/ or	996055
14	cohort studies/ or longitudinal studies/ or follow-up studies/ or prospective studies/ or retrospective studies/ or cohort.ti,ab. or longitudinal.ti,ab. or prospective.ti,ab. or retrospective.ti,ab.	3528780

15	**Cross-Sectional Studies/ or Prevalence/ or (cross-sectional or prevalence or transversal).ti,ab,kf.**	**1488221**
16	**13 or 14 or 15**	**5340866**
17	**12 and 16**	**9938**
18	**exp animals/ not humans.sh.**	**5235946**
19	**17 not 18**	**9433**
20	**Pregnancy Outcome/**	**59637**
21	**Stillbirth/**	**6782**
22	**Perinatal Death/**	**2288**
23	**Fetal Death/**	**24914**
24	**(pregnancy adj1 outcome*).ab,ti,kf.**	**35850**
25	**(stillbirth* or stillborn*).mp.**	**2114**
26	**(((late or adult* or matur* or slow or stabl*) adj3 onset) and diabet*).tw.**	**5687**
27	**((f?etal or f?etus or intra?uterine) adj1 death*).ab,ti,kf.**	**12492**
28	**fetal demise.ab,ti,kf.**	**2237**
29	**peri?natal death*.ab,ti,kf.**	**5283**
30	**20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29**	**126824**
31	**30 and 19**	**1844**

1.2 Search Strategy - Embase < 1974 to 2024 June 28 >

#	Searches	
1	Blood glucose/	271721
2	glucose intolerance/	22421
3	glucose tolerance test/	27909
4	(Glucose adj3 (tolera* or intolera* or fast* or value* or level* or test)).ti,ab,kw.	260837
5	(blood adj3 (sugar or glucose)).ti,ab,kw.	174552
6	OGTT.ti,ab,kw.	20467
7	(dysglyc?emi* or hyperglyc?emi*).ti,ab,kw.	121310
8	1 or 2 or 3 or 4 or 5 or 6 or 7	537962
9	pregnancy/	711911
10	(pregnan* or gestation* or trimester or antepartum or gravidity).ti,ab,kw.	983846
11	9 or 10	1214272
12	8 and 11	35133
13	exp cohort analysis/	1183706
14	exp longitudinal study/	216263
15	exp prospective study/	925037
16	exp follow up/	2210295
17	cohort$.tw.	1596352
18	exp case control study/	237872

19	**(case$ and control$).tw.**	**934749**
20	**(cross sectional or questionnaire or questionnaires or survey or epidemiological**	**2234839**
21	**13 or 14 or 15 or 16 or 17 or 18 or 19 or 20**	**6754079**
22	**12 and 21**	**12493**
23	**(exp animal/ or animal experiment/ or nonhuman/) not (exp human/ or human**	**7463075**
24	**22 not 23**	**12270**
25	**Pregnancy Outcome/**	**88230**
26	**Stillbirth/**	**24744**
27	**Perinatal Death/**	**5341**
28	**Fetal Death/**	**25370**
29	**(pregnancy adj1 outcome*).ab,ti,kw.**	**52290**
30	**(stillbirth* or stillborn*).mp.**	**34139**
31	**((f?etal or f?etus or intra?uterine) adj1 death*).ab,ti,kw.**	**15103**
32	**fetal demise.ab,ti,kw.**	**4077**
33	**peri?natal death*.ab,ti,kw.**	**7039**
34	**25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33**	**156511**
35	**24 and 34**	**2411**

1.3 Search Strategy – CINAHL

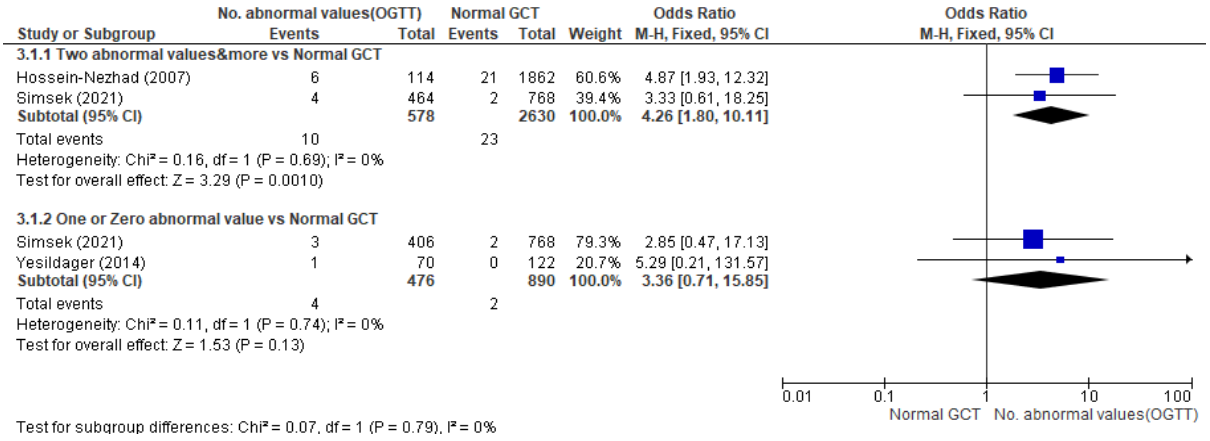
#	Searches

S1	**MH "Blood glucose"**	**42633**
S2	**MH "glucose intolerance"**	**3,529**
S3	**MH "glucose tolerance test"**	**8,031**
S4	**TI (Glucose N3 (tolera* or intolera* or fast* or value* or level* or test)) OR AB**	**37,646**
S5	**TI blood N3 (sugar or glucose)) OR AB blood N3 (sugar or glucose))**	**27,311**
S6	**TI OGTT OR AB OGTT**	**2,514**
S7	**TI (dysglyc?emi* or hyperglyc?emi*) OR AB (dysglyc?emi* or hyperglyc?emi*)**	**2,934**
S8	**S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7**	**76,582**
S9	**MH "pregnancy"**	**228,002**
S10	**TI (pregnan* or gestation* or trimester or antepartum or gravidity) or AB**	**206,476**
S11	**S9 OR S10**	**306,890**
S12	**S8 AND S11**	**6,386**
S13	**TI ((animal or animals or canine* or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep*)**	**129,552**
S14	**S12 NOT S13**	**6,070**
S15	**(MH "Case Control Studies+") or (MH "Control Group") or (MH "Matched-Pair Analysis") or (TI (case or cases) n5 TI (control or controls)) OR (AB (case or cases) n5 AB (control or controls)) OR (TI (case or cases) n3 TI (matched)) OR (AB (case or cases) n3 AB (matched)) OR TI (control group*)**	**110,713**
S16	**cohort[TIAB] OR Cohort Studies[Mesh] OR longitudinal[TIAB] OR**	**45**
S17	**Cross-Sectional Studies[Mesh] OR "cross-sectional"[TIAB] OR "prevalence**	**2**

S18	**S15 OR S16 OR S17**	**110,758**
S19	**S14 AND S18**	**406**
S20	**MH "Pregnancy Outcome"**	**0**
S21	**MH "Stillbirth"**	**0**
S22	**MH "Perinatal Death"**	**9,316**
S23	**MH "Fetal Death"**	**91**
S24	**TI (pregnancy N1 (outcome*)) OR AB (pregnancy N1 (outcome*))**	**14,955**
S25	**TX (stillbirth* or stillborn*)**	**6,322**
S26	**TI ((f?etal or f?etus or intra?uterine) N1 (death*)) OR AB ((f?etal or f?etus or intra?uterine) N1 (death*))**	**85**
S27	**TI (fetal demise) OR AB (fetal demise)**	**818**
S28	**TI (peri?natal death*) OR AB (peri?natal death*)**	**312**
S29	**S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28**	**26,295**
S30	**S19 AND S29**	**35**

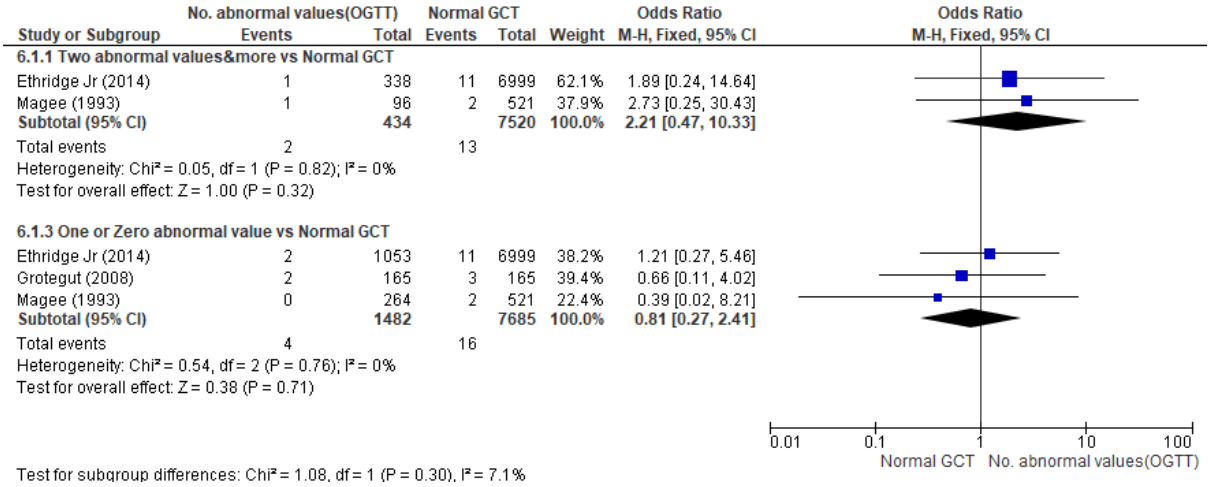
Supplement 2

Pooled odds ratio of stillbirth based on the number of abnormal values on 100-g OGTT to normal GCT in C&C criteria by Asia countries



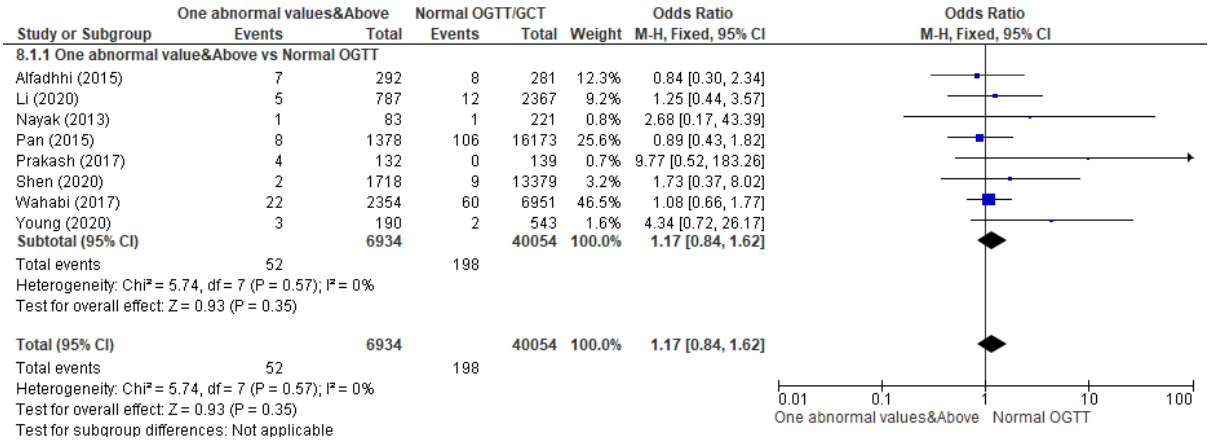
Supplement 3

Pooled odds ratio of stillbirth based on the number of abnormal values on 100-g OGTT to normal GCT in C&C criteria by North America countries



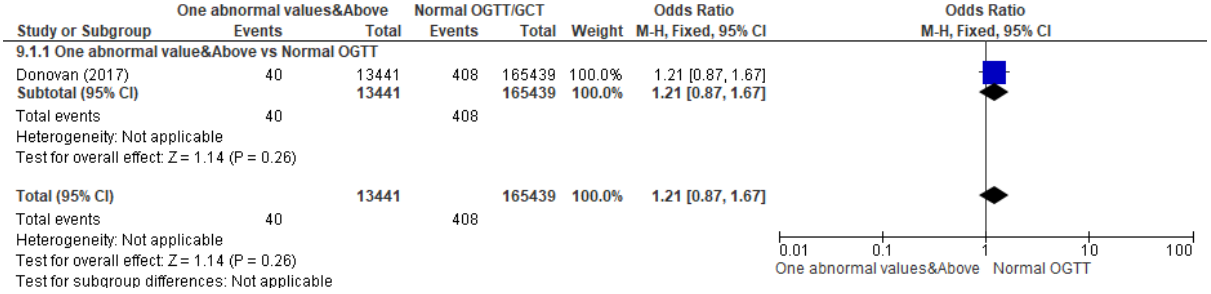
Supplement 4

Pooled odds ratio of risk of stillbirth of number of abnormal values on 75-g OGTT vs. normal OGTT under IADPSG criteria by Asia Countries



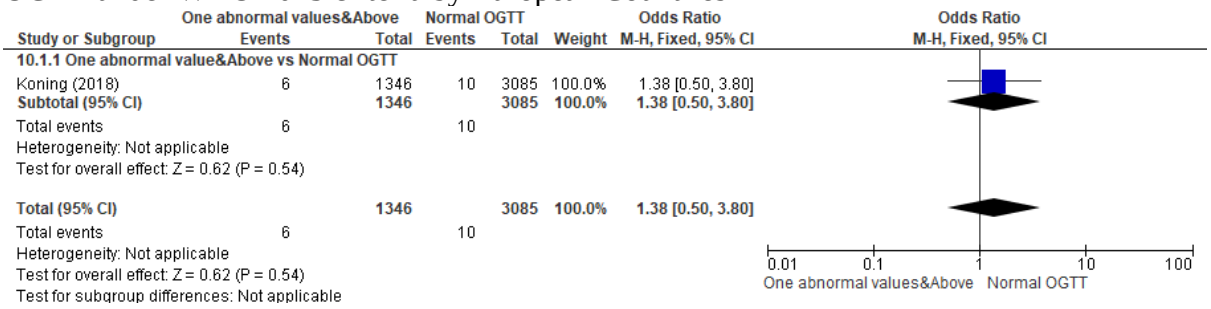
Supplement 5

Pooled odds ratio of risk of stillbirth of number of abnormal values on 75-g OGTT vs. normal OGTT under IADPSG criteria by North America Countries



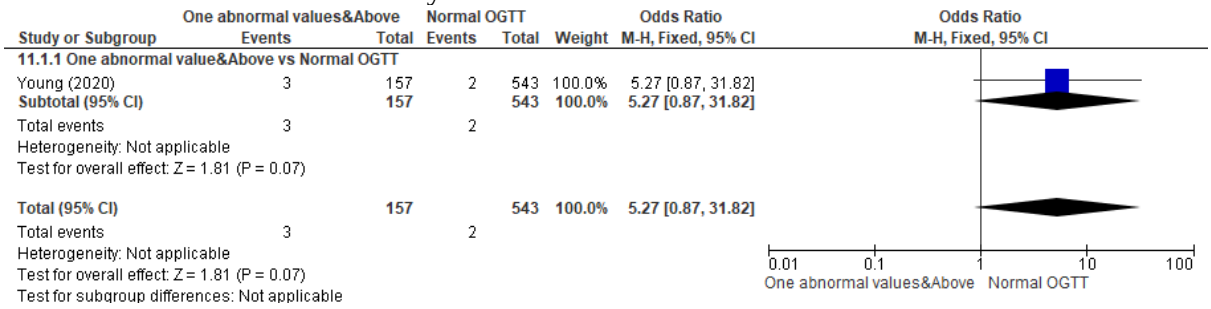
Supplement 6

Pooled odds ratio of risk of stillbirth of number of abnormal values on 75-g OGTT vs. normal OGTT under WHO 2013 criteria by European Countries



Supplement 7

Pooled odds ratio of risk of stillbirth of number of abnormal values on 75-g OGTT vs. normal OGTT under WHO 2013 criteria by Asia Countries



Chapter 3

Association between early- and late-onset gestational diabetes mellitus and risk of stillbirth: a retrospective cohort study using a large administrative database from the United States

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Abstract

Background Our recent systematic review found that women with gestational diabetes mellitus (GDM) diagnosed by the Carpenter & Coustan (C&C) criteria had higher risk of stillbirth as compared to women with normal glucose level. However, the confidence of this evidence was limited by the small sample size – only 11,162 pregnant women and 48 events in all included studies in the meta-analysis. This study aims to investigate the association of GDM with risk of late stillbirth by using a large health administrative database in the United States, and to compare the association between early- and late-onset GDM.

Methods We conducted a retrospective cohort study of women with at least one liveborn or stillborn who survived until 28 weeks of gestational age captured by Cerner Real-World Data™ (CRWD) from the United States. Maternal and clinical characteristics among women with early GDM (0-23 weeks), late GDM (24-28 weeks) and non-GDM were described. The study outcome of late stillbirth was defined as death of a fetus occurred 28 weeks or more completed weeks of gestation. Logistic regression was performed to determine the association between early GDM, late GDM and the risk of late stillbirth.

Results A total of 866,510 pregnant women who registered with the CRWD were included in this study. The adjusted odds ratio (aOR) for the risk of late stillbirth among women with early GDM and late GDM were 3.50 (95% confidence interval (CI), 2.85, 4.29) and 3.20 (95% CI, 2.71, 3.76), respectively, as compared to women without GDM.

Conclusion Women with early- and late-onset GDM had more than three times higher risk of late stillbirth as compared to women without GDM. Intensified effort in maternity care should target on these women to reduce the risk of stillbirth.

3.1 Background

The risk of stillbirth among pregnant women with pre-existing diabetes was about four times higher compared to general obstetric population.[1] However, it remains controversial whether gestational diabetes mellitus (GDM) is associated with an increased risk of stillbirth. A cohort study found that the incidence of stillbirth between 36 and 42 weeks of gestation was 17.4 per 10,000 deliveries among women with GDM, compared to 12.7 per 10,000 deliveries among women without GDM.[2] A recent systematic review and meta-analysis did not find an increased risk of stillbirth among women with GDM. However, considerable heterogeneity has been found across included studies as some large studies included in the meta-analysis were subject to immortal time bias and studies had different diagnostic criteria for GDM.[3-4]

Currently, there is no universally accepted standard for GDM diagnosis. About 4% to 8% of the obstetric population in the United States were affected by GDM under the Carpenter & Coustan (C&C) criteria.[5] Our systematic review found that women diagnosed with GDM using the C&C criteria had a significantly higher risk of stillbirth compared to women with a normal glucose challenge test (GCT), with a pooled odds ratio (OR) of 3.65 (95% CI, 1.74, 7.64). However, the confidence of this evidence was limited due to small sample size of the meta-analysis: only 48 events in 11,162 pregnant women in all included studies. Therefore, further research with well-designed studies using large data is needed to confirm the association between GDM and the risk of stillbirth.

Although the screening for GDM is generally recommended between 24 and 28 weeks of gestation, women with high-risk profiles for diabetes, such as a history of GDM, known impaired glucose metabolism or obesity (body mass index (BMI) of 30 or more) are recommended to undergo earlier screening before 24 weeks to identify overt diabetes.[6] Early-

onset GDM can be identified during the process of early screening conducted before 24 weeks of gestation. The diagnostic criteria for early-onset GDM, which occurred before 24 weeks of gestation, are like those used for GDM diagnosed between 24 and 28 weeks.[7] However, few studies have assessed the risk of stillbirth among women with early-onset GDM. For women with early GDM, fetus has longer exposure to hyperglycemia environment, which may affect the form of the heart and therefore lead to stillbirth.[8] On the other hand, the development of GDM after 24 weeks is primarily attributed to hormonal changes associated with pregnancy,[9] whereas early-onset GDM may be more closely linked to pre-existing high-risk factors, such as obesity or impaired glucose metabolism. We hypothesize that women with early-onset GDM may have a different risk of stillbirth as compared to those diagnosed with GDM between 24 and 28 weeks of gestation. Therefore, this study aims to: (1) assess whether late-onset GDM (24-28 weeks) is associated with an increased risk of late stillbirth, and (2) determine whether the risk of late stillbirth differs between women with early- and late-onset GDM.

3.2 Methods

Study Population & Database This retrospective cohort study was conducted by using the Cerner Real-World Data™ (CRWD), a large health administrative database representing approximately 20% of the United States population.[10] It comprises longitudinal electronic health records (EHR) from over 480 institutions covering most geographical regions in the United States between 2000 and 2016. This health administrative database includes information on maternal demographics, health behaviours, pre-existing health conditions, obstetric complications, intrapartum events, and birth outcomes. This study received ethical approval from the Ottawa Health Science Network Research Ethics Board (20220430-01H).

Women with at least one delivery (live birth or stillbirth) in which the pregnancy period starting at January 1st, 2001, and ending before December 31st, 2016 were included in the study. To ensure inclusion of women registered in hospitals within the CRWD during the study period, the study population was restricted to deliveries with at least one encounter during pregnancy and at least one encounter during delivery. Multifetal pregnancies were excluded from the study population. Since the exposure window for late GDM is between 24 and 28 weeks of gestation, including early stillbirth (fetal deaths occurring between 24 and 28 weeks) could introduce immortal time bias. We therefore included only pregnant women with deliveries at a gestational age of at least 28 weeks to avoid immortal time bias (Hutcheon JA et al., 2013).[4] Deliveries involving pre-existing diabetes (including type 1 and type 2), diagnosed from 180 days before conception up to 90 days after, were excluded. The algorithm by Andrade SE et al. was modified by excluding the use of anti-diabetic medications to identify pre-existing diabetes, in order to avoid misclassification, as women with GDM may also be prescribed with these medications.[11] Multifetal pregnancies and pre-existing diabetes were identified using International Classification of Diseases(ICD)-9 and ICD-10 codes (Supplement 2). To further reduce immortal time bias, women diagnosed with GDM beyond 28 weeks of gestation were excluded from the study population.[4] Specifically, pregnant women with late-onset GDM must survive until the time of glucose screening, which makes early stillbirths (occurring before this point) extremely rare in the late GDM group compared to the non-GDM group, particularly among those who were not screened for GDM. According to the United States Centers for Disease Control and Prevention (CDC) National Vital Statistics Reports, stillbirths occurring between 20 weeks and 27 weeks of gestation accounted for about half of all stillbirths from 20 weeks to delivery between 2014 and 2022.[12] This indicates that including stillbirths prior to 28 weeks in the study

could introduce significant bias into the results. If a woman had multiple eligible pregnancies, the first one was included.

Exposure GDM was identified using a combination of ICD-9 and ICD-10 diagnosis codes. The algorithm used for GDM diagnosis has been validated in previous studies, demonstrating a specificity of > 90% and a positive predictive value of > 80%.[13-14] Early GDM was defined as GDM diagnosed before 24 weeks of gestation, whereas late GDM was defined as GDM diagnosed between 24 and 28 weeks. Gestational age at the time of GDM diagnosis was determined based on the first recorded occurrence of a GDM diagnosis code during the pregnancy period.

Outcome The outcome of this study was late stillbirth, defined as fetal death occurring at 28 or more completed weeks of gestation. Validated algorithms were used to identify live births and stillbirths and to estimate the corresponding pregnancy periods, as gestational age is not routinely captured in the CRWD.[15-16]

Covariates Covariates in this study included maternal characteristics such as maternal age at delivery (<20, 20-24, 25-29, 30-34, ≥35 years), race (Caucasian, Black, Hispanic, Asian/Pacific Islander, Native American), marital status (single, widowed/divorced, married/life partner), birth year (2000-2004, 2005-2008, 2009-2012, 2013-2016), type of health insurance (private insured, self-insured, Medicaid, government/other)), hospital bed size at delivery (<100, 100-199, 200-299, 300-499, ≥ 500), census region of residence (Midwest, Northeast, South, West), and urbanization of delivery hospital (rural/urban). Maternal health behaviours included self-reported substance use during pregnancy (alcohol use, tobacco smoking, and drug use). Physical health conditions, identified through validated algorithms, included pre-pregnancy obesity[17], pre-existing hypertension[18], polycystic ovary syndrome[19]. Mental health conditions included

anxiety[20], depression[20], bipolar disorder[21], schizophrenia[22].

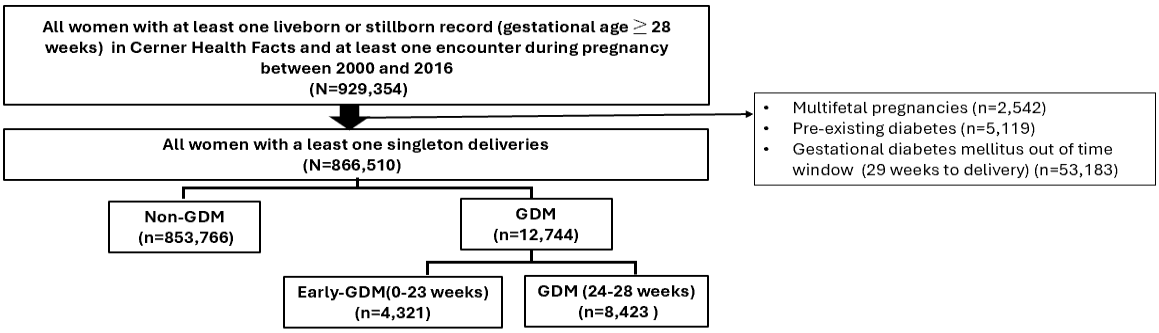
Statistical Analysis Categorical variables were reported as frequencies and percentages, and continuous variables as means with standard deviations. Covariates were compared among women with early GDM, late GDM, and non-GDM using chi-square test or ANOVA. Covariates were also compared between women with live births and stillbirths. The effects of early- and late-onset GDM (with non-GDM as the reference) on stillbirth were estimated by logistic regression models, with both crude and adjusted ORs and their 95% CI reported. Confounders were carefully selected to avoid the occurrence of overadjustment.[23-24] The multivariable logistic regression models adjusted for a range of covariates potentially associated with GDM and stillbirth, with covariate selection based on statistical significance ($p < 0.05$).[25] Women younger than 12 or older than 54 years at the time of pregnancy were recoded as missing, as these records were likely erroneous. Before conducting multivariable regression analyses, multiple imputation using a fully conditional specification method was applied to create five complete datasets.[26-27] Specifically, both categorical and continuous variables were imputed using discriminant methods. The imputation process ran for 100 iterations to ensure convergence. All statistical analyses were performed using Statistical Analysis System (SAS) for Windows, version 9.4 (SAS Institute, Cary, NC).

3.3 Results

We initially identified a cohort of 929,354 pregnant women who registered in the CRWD and had at least one delivery between 2000 and 2016. The detailed process of study population selection is shown in Figure 3.1. After excluding women with multifetal pregnancies, pre-existing diabetes, or GDM diagnosed after 28 weeks, the final cohort included 866,510 women. Among them, 4,321 were identified with early GDM and 8,423 with late GDM. The number of

late stillbirths observed among women with early GDM, late GDM, and non-GDM were 99, 156, and 5,162, respectively.

Figure 3.1 Study Population Selection



Women with early GDM or late GDM were more likely to be older at delivery, identify Asian/Pacific Islande or Native American, reside in the western of the United States, and delivery at hospitals with fewer than 100 beds, compared to women without GDM. They were also more likely to report tobacco and drug use, and to have pre-existing physical health and mental health conditions. Compared to women with late-onset GDM, women with early-onset GDM had higher rates of pre-pregnancy obesity (31.4% vs. 17.0%) and were frequently covered by Medicaid (35.7% vs. 28.5%). Full distributions of maternal and clinical characteristics by GDM status are provided in Table 3.1.

Table 3.1 Maternal characteristics by exposure status

Maternal characteristics (n,%)	Early GDM n= 4,321	Late GDM n= 8,424	Non-GDM n=853,766	P value
Age*, Y (M±SD)	30.32 ± 5.68	29.43 ± 5.37	27.91 ± 9.08	<.0001
Age*, Y n (%)				<.0001

	<20	113 (2.62)	257 (3.05)	80,423 (9.42)
	20-24	547 (12.66)	1,305 (15.49)	199,565 (23.37)
	25-29	1,229 (28.44)	2,573 (30.55)	228,912 (26.81)
	30-34	1,532 (35.45)	2,995 (35.56)	193,061 (22.61)
	35-39	694 (16.06)	1,014 (12.04)	92,059 (10.78)
	≥40	194 (4.49)	270 (3.21)	29,612 (3.47)
	Unknown	12 (0.28)	9 (0.11)	30,134 (3.53)
Race				<.0001
	Caucasian	2,647 (61.26)	5,640 (66.96)	535,488 (62.72)
	Black	572 (13.24)	709 (8.42)	133,548 (15.64)
	Hispanic	157 (3.63)	266 (3.16)	36,996 (4.33)
	Asian/Pacific Islander	229 (5.30)	731 (8.68)	31,152 (3.65)
	Native American	80 (1.85)	245 (2.91)	9,766 (1.14)
	Unknown	636 (14.72)	832 (9.88)	106,816 (12.51)
Marital status				<.0001
	Single	1,559 (36.08)	2,569 (30.50)	357,778 (41.91)

Widowed/Divorced	144 (3.33)	209 (2.48)	26,746 (3.13)	
Married/Life Partner	2,326 (53.83)	4,833 (57.38)	391,620 (45.87)	
Unknown	292 (6.76)	812 (9.64)	77,622 (9.09)	
Health insurance				<.0001
Commercial	1,660 (38.42)	4,025 (39.19)	340,238 (39.85)	
Self-insured	78 (1.81)	183 (2.17)	21,945 (2.57)	
Medicaid	1541 (35.66)	2,396 (28.45)	254,222 (29.78)	
Government (Others)	109 (2.52)	147 (1.75)	34,164 (4.00)	
Unknown	933 (21.59)	1672 (19.85)	203,197(23.80)	
Physical health conditions				
Obesity	1,355 (31.36)	1,430 (16.98)	46,994 (5.50)	<.0001
Hypertension	314 (7.27)	293 (3.48)	24,160 (2.83)	<.0001
Polycystic Ovary Syndrome	117 (2.71)	149 (1.77)	3,754 (0.44)	<.0001
Mental health conditions				
Anxiety	200 (4.63)	337 (4.00)	23,472 (2.75)	<.0001
Bipolar disorder	54 (1.25)	73 (0.87)	7,401 (0.87)	0.0258
Depression	272 (6.29)	389 (4.62)	27,235 (3.19)	<.0001
Schizophrenia	8 (0.09)	8 (0.19)	1,240 (0.15)	0.3790
Substance use				

Tobacco smoking	445 (10.30)	750 (8.90)	69,197 (8.10)	<.0001
Alcohol use	17 (0.39)	39 (0.46)	4,080 (0.48)	0.7110
Drug use	140 (3.24)	183 (2.17)	23,732 (2.78)	0.0006
Birth year				<.0001
2000-2004	133 (3.08)	366 (4.35)	46,417 (5.44)	
2005-2008	372 (8.61)	884 (10.50)	97,953 (11.47)	
2009-2012	1,186 (27.45)	2,273 (26.99)	302,361 (35.41)	
2013-2016	2,630 (60.87)	4,900 (58.17)	407,035 (47.68)	
Census region				<.0001
Midwest	1,299 (30.16)	2,588 (30.79)	189,272 (22.18)	
Northeast	1,171 (27.19)	2,320 (27.61)	263,833 (30.92)	
South	697 (16.18)	1,255 (14.93)	241,072 (28.25)	
West	1,140 (26.47)	2,241 (26.67)	159,134 (18.65)	
Hospital bed-size				
<100	1,028 (23.79)	2,487 (29.53)	163,336 (19.13)	<.0001
100-199	618 (14.30)	1,236 (14.67)	145,344 (17.02)	
200-299	791 (18.31)	1,578 (18.73)	178,613 (20.92)	
300-499	1,022 (23.65)	1,734(20.59)	199,660 (23.39)	
500+	814(18.84)	1,189 (14.12)	162,734 (19.06)	

Unknown	48 (1.11)	199 (2.36)	4,079 (0.48)	
Hospital's urbanization				<.0001
Urban	3,485 (80.91)	6,479 (77.09)	698,116 (81.81)	
Rural	822 (19.09)	1,925 (22.91)	155,195 (18.19)	

Compared to women with live births, those with stillbirths were more likely to be older, identify as Black, delivery in urban hospitals, and give birth in hospitals with 500 or more beds. They were less likely to be married or have a life partner and less likely to have commercial insurance. Additionally, women with stillbirths were more likely to report tobacco and drug use, be obese, have pre-existing hypertension, and experience depression during pregnancy. Detailed distributions of maternal and clinical characteristics by outcome status are presented in Table 3.2.

Table 3.2 Maternal and clinical characteristics by outcome status

Maternal characteristics (n,%)	**Stillbirth n=5,411**	**Live Birth n= 860,391**	**P value**
Age*, Y (M, SD)	28.70 ± 7.668	27.94 ± 59.05	<.0001
Age*, Y (n, %)			<.0001
<20	498 (9.19)	80,295 (9.32)	
20-24	1,208 (22.30)	200,209 (23.25)	
25-29	1,347 (24.87)	231,367 (26.87)	
30-34	1,263 (23.32)	196,325 (22.80)	

35-39	755 (13.94)	93,012 (10.80)	
≥40	295 (5.45)	29,781 (3.46)	
Unknown	51 (0.94)	30,104 (3.50)	
Race			<.0001
Caucasian	2,902 (53.57)	540,873 (62.81)	
Black	1,402 (25.88)	133,427 (15.50)	
Hispanic	214 (3.95)	37,205 (4.32)	
Asian/Pacific Islander	165 (3.05)	31,947 (3.71)	
Native American	88,(1.62)	10,003 (1.16)	
Unknown	646 (11.93)	107,638 (12.50)	
Marital status			<.0001
Single	2,557 (47.20)	359,349 (41.73)	
Widowed/divorced	171 (3.16)	26,928 (3.13)	
Married/life partner	2,176 (40.17)	396,603 (46.06)	
Unknown	513 (9.47)	78,213 (9.08)	
Health insurance			<.0001
Commercial	1,963 (36.24)	343,960 (39.94)	
Self-insured	204 (3.77)	22,002 (2.56)	

Medicaid	1,774 (32.75)	256,385 (29.77)	
Government (Others)	203 (3.75)	34,217 (3.97)	
Unknown	1,273 (23.50)	204,529 (23.75)	
Physical health conditions			
Obesity	419 (7.73)	49,360 (5.73)	<.0001
Hypertension	241 (4.45)	24,526 (2.85)	<.0001
Polycystic Ovary Syndrome	27 (0.50)	3,993 (0.46)	0.7078
Mental health conditions			
Anxiety	149 (2.75)	23,860 (2.77)	0.9277
Bipolar disorder	65 (1.20)	7,463 (0.87)	0.0084
Depression	210 (3.88)	27,686 (3.22)	0.0060
Schizophrenia	11 (0.20)	1,245 (0.14)	0.2594
Substance use			
Tobacco smoking	591 (10.91)	69,801 (8.11)	<.0001
Alcohol use	35 (0.65)	4,101 (0.48)	0.0706
Drug use	250 (4.62)	23,805 (2.76)	<.0001
Birth year			0.0278
2000-2004	293,(5.41)	46,623 (5.41)	
2005-2008	663 (12.24)	98,546 (11.44)	
2009-2012	1,973 (36.42)	303,847 (35.29)	

2013-2016	2,488 (45.93)	412,077 (47.86)	
Census region			<.0001
Midwest	1,301 (24.02)	191,858 (22.29)	
Northeast	1,686 (31.13)	265,638 (30.87)	
South	1,598 (29.51)	241,426 (28.05)	
West	831(15.34)	161,684 (18.79)	
Hospital bed-size			
<100	815 (15.05)	166,036 (19.28)	<.0001
100-199	769 (14.20)	146,429 (17.01)	
200-299	1,099 (20.29)	179,883 (20.89)	
300-499	1,398 (25.81)	201,018 (23.34)	
500+	1,302 (24.04)	163,435 (18.98)	
Unknown	34 (0.63)	4,292 (0.50)	
Hospital's urbanization			<.0001
Urban	4,625 (85.40)	703,455 (81.74)	
Rural	791 (14.60)	157,151 (18.26)	

Women with early GDM had a significantly increased risk of late stillbirth, with an adjusted OR of 3.50 (95% CI, 2.85, 4.29), as compared to women without GDM. Similarly, women with late

GDM had approximately three times the odds of late stillbirth (adjusted OR:3.20; 95% CI, 2.71, 3.76).

Table 3.3. Odds ratios for risk of stillbirth among women with early GDM and late GDM to non-GDM

Exposure	Cases/N	Incidence	Crude OR (95% CI)	Adjusted OR* (95% CI)
Non-GDM	5,162/853,766	0.60	REF	REF
Early GDM	99/4,321	2.29	3.86 (3.15, 4.71)	3.50 (2.85, 4.29)
Late GDM	156/8,423	1.85	3.10 (2.64, 3.64)	3.20 (2.71, 3.76)

***Note:** adjusting for maternal age, maternal race, marital status, insurance, pre-existing obesity, pre-existing hypertension, census region, hospital's urbanization, and hospital bed size

3.4 Discussion

Our retrospective cohort study using a large United States population health database found that both early- and late-onset GDM were associated with a more than three times increased risk of late stillbirth as compared to women without GDM. These findings were consistent with some previous studies. For example, our meta-analysis covering four studies reported a pooled OR of 3.65 (95%CI, 1.64, 7.74) for the risk of stillbirth among women with GDM diagnosed by the C&C criteria, compared to women normal glucose. Although both the C&C criteria and the more stringent (National Diabetes Data Group) NDDG criteria are recommended in United States guidelines, our study findings provide partial confirmation of the results from the systematic review. A previous study found that women with early GDM had a higher risk of stillbirth as compared to women with late GDM, but the results lacked statistical power due to small sample size.[28] Our study found that the risk of late stillbirth was slightly higher among women with

early-onset GDM compared to those with late-onset GDM. This is biologically plausible, as prolonged fetal exposure to elevated glucose levels earlier in gestation can adversely affect placental function, fetal development, and oxygen transport, potentially leading to earlier fetal demise. Since our study focused exclusively on late stillbirth (≥28 weeks of gestation) to minimize immortal time bias, this focus may also partially explain the limited difference in risk between early and late GDM. It could also be explained by the possibility that women with severe undiagnosed late-onset GDM were excluded from our study population due to their higher likelihood of experiencing early stillbirth, which was not captured in our analysis.

There are several strengths to this study. First, the samples size was exceptionally large, approximately 900,000 pregnancies, making it the largest study in the field to date. To our knowledge, this is the first study with sufficient statistical power to compare the risk of late stillbirth associated with early versus late GDM. We carefully defined the eligible study population by excluding pregnancies that ended before 28 weeks of gestation and by including only GDM cases diagnosed between conception and 28 weeks of gestation.[4] This approach effectively eliminated the possibility of immortal time bias in our analysis. Immortal time bias occurred because individuals in the exposed group had to survive until the time of glucose screening, whereas individuals in the non-exposed group—particularly those who were not screened—did not. This led to a higher occurrence of early stillbirths in the non-exposed group. Notably, some previous studies reported a paradoxical protective effect of GDM on stillbirth, caused most likely by immortal time bias, as those studies did not restrict their study population to pregnancies that survived beyond 28 weeks of gestation.[29-31]

Despite these strengths, several limitations should be acknowledged. Currently, GDM screening before 24 weeks is typically performed in high-risk women to detect pre-existing diabetes.[32]

Some early GDM cases may have been misclassified as late due to delayed screening in high-risk women who should have been tested in the first trimester. Although we employed validated algorithms to differentiate pregestational diabetes from GDM, misclassification may still have occurred due to incomplete recording in administrative health datasets. Specifically, some early GDM cases may have been undiagnosed pre-existing diabetes. To mitigate this, we used all available clinical data from 180 days before conception to 90 days after conception during the index pregnancy to identify pre-existing diabetes, which likely minimized such misclassification. To avoid immortal time bias that may occur in late GDM and to facilitate comparison between early GDM and late GDM, in this study, we focused on GDM diagnosed before 29 weeks of gestation and late stillbirths occurring after 28 weeks. As a result, we were unable to assess early stillbirths or GDM cases diagnosed after 28 weeks. However, this approach was intentionally chosen to eliminate immortal time bias by prioritizing internal validity. In this study, we used clinical diagnosis of GDM rather than glucose measurements to ascertain exposure status. As a result, we were unable to compare the associations between stillbirth and GDM defined by different diagnostic criteria (C&C vs. NDDG). Further research is needed to explore the relationship between stillbirth and GDM under each diagnostic criterion.

3.5 Conclusion and Implication

Our study revealed a higher risk of late stillbirth among women with both early- and late-onset GDM. Therefore, intensified maternity care should be directed towards these women to reduce the risk of stillbirth. Further research is needed to assess the overall risk of stillbirth, including early stillbirths, among women with early-onset GDM.

3.6 References

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Chapter 4

The association of varying degrees of hyperglycemia during pregnancy and risk of diabetes among women: a systematic review and meta-analysis

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Abstract

Background Although the association between firmly diagnosed gestational diabetes mellitus (GDM) (i.e., those with at least two abnormal values on 100-gram (g) oral glucose tolerance test (OGTT) during pregnancy) and diabetes is well established, the association of women affected by "mild" hyperglycemia (e.g., a single abnormal value on 100-g OGTT during pregnancy) with diabetes remains unclear. The main objective of this systematic review and meta-analysis is to summarize evidence on the association of varying degrees of hyperglycemia, based on the number and type of abnormal values on OGTT during pregnancy, and the subsequent development of diabetes.

Methods Comprehensive searches were conducted in MEDLINE, EMBASE, and CINAHL to retrieve relevant papers from inception to December 31, 2023. We included all observational studies that included pregnant women who had undergone glucose challenge test (GCT) and/or OGTT (including 100-g and 75-g OGTT) during pregnancy. We extracted effect measures from eligible studies on OGTT results during pregnancy and diabetes during follow-up period.

Results Twenty studies were identified with a total of 620,633 women. Pooled odds ratios (ORs) among women with one abnormal value and at least two abnormal values on 100-g OGTT for risk of diabetes as compared to those with normal 100-g OGTT or GCT were 4.44 [95% confidence interval (CI) 2.71, 7.28] and 12.29 [95% CI, 8.36, 18.07], respectively. Similar results were found among women with glucose measured by 75-g OGTT during pregnancy.

Conclusions The risk of diabetes in women with only one abnormal value on OGTT during pregnancy is increased substantially. Attention should be paid to these women for intensified lifestyle changes to reduce their risk of developing diabetes.

4.1 Introduction

Diabetes is one of the most common chronic diseases globally. Diabetes affected an estimated 108 million people in 1980, which rose 422 million people in 2014, and is projected to reach 552 million by 2030.[1-2] Among adults aged 18 years and older, the prevalence of diabetes has increased dramatically—from 6.0% in 2010 to 14.7% in 2021.[3-4] This sharp rise is largely attributed to changes in lifestyle, including reduced physical activity, unhealthy diets, and rising rates of obesity.[5] According to the Unites States Centers for Disease Control and Prevention (CDC), approximately 1.85 million women of reproductive aged (18-44 years) were living with diabetes in 2002, with nearly 500,000 unaware of their disease.[6] Women with hyperglycemia during pregnancy are at particularly high risk for developing diabetes later in life, making it one of the strongest predictors of subsequent diabetes.

Women with elevated glycemia levels with onset or first recognized during pregnancy are often diagnosed as gestational diabetes mellitus (GDM).[7] GDM affects 4.4% pregnancies overall, varying from 2.9% to 10.6%.[8] A systematic review covering 675,000 pregnancies from 1960 to 2009 found that women with GDM had more than a sevenfold increased risk of developing type 2 diabetes mellitus (T2DM) compared women without GDM.[9] With the universal screening of GDM in place, pregnancy offers a critical opportunity to identify women at elevated risk for diabetes.

It has been under debate regarding the GDM diagnostic criteria over the past decade. The traditional two-step approach was developed approximately 60 years ago to identify women at increased risk for diabetes.[10] This method involves an initial glucose challenge test (GCT), followed by a 100-g oral glucose tolerance test (OGTT), with a diagnosis of GDM requiring at least two abnormal values (10). More recently, a one-step approach has been introduced, using a

75-g OGTT in which only one abnormal is sufficient to diagnose GDM. This method aims to identify women at risk of adverse pregnancy outcomes rather than long-term diabetes.[11] The two-step has been criticized for being overly conservative, as studies have shown that women with only one abnormal value on the 100-g OGTT may still face an increased risk of adverse outcomes.[12] Conversely, the one-step approach has raised concerns about potential overdiagnosis and increased healthcare burden due to its lower diagnostic thresholds with substantially increased GDM rate by this criterion.[13]

Although the association between GDM and future diabetes is well established, the predictive value on future risk of diabetes by a single abnormal value on OGTT during pregnancy remains uncertain. Recent studies have also shown that fasting glucose levels are a stronger predictor of future diabetes risk compared to post-load glucose values. Relying solely on a binary diagnosis of GDM provides limited insight—particularly in the context of evolving diagnostic criteria and the increasing emphasis on personalized care. Therefore, we conducted a systematic review and meta-analysis to quantify the risk of developing diabetes based on the number of and the type of abnormal values on OGTT. The protocol for this systematic review was registered on the PROSPERO International Prospective Register of Systematic Reviews (PROSPERO registration number: CRD42023420411).

4.2 Methods

Eligibility Criteria *S*tudies were eligible if they included pregnant women who underwent GCT and/or OGTT (including both 100-g and 75-g OGTT) during pregnancy. Studies lacking actual glucose measurements or whose reference group did not include women with normal GCT or OGTT were excluded. The primary outcomes of interest were diabetes and/or pre-diabetes (impaired glucose tolerance and/or impaired fasting glucose). There was no restriction on setting,

publication date or language. All observational studies, including cohort, case-control, and cross-sectional studies, were included, while case reports, case series, commentaries, editorials, and reviews without original data were excluded.

Information Sources and Search Strategy Comprehensive literature searches were conducted in EMBASE, MEDLINE, and CINAHL. The date of publication was set from inception to December 31, 2023. Reference lists of selected studies and systematic reviews of similar scope were also screened for additional eligible studies. The search strategy was developed with assistance from a medical librarian experienced in systematic review research. Medical Subject Headings [MeSH] and keywords related to the study population, exposure, outcome, and study type were combined to form the search strategy (detailed in Supplement 1). Abstract selection and full-text screening were performed independently by two reviewers (NZ, WW) using Covidence, based on pre-defined eligibility criteria. Duplicates were automatically removed, with additional manual checks for any remaining duplicates. Discrepancies during screening were resolved through discussion with a third reviewer (SWW). During full-text screening, a separate a separate Excel log was generated to track studies identified through reference scanning. Meanwhile, the reasons for exclusion were documented during the full-text screening. Study selection results are presented using a PRISMA flow diagram, in accordance with the PRISMA checklist.

Data extraction Two reviewers (NZ, WW) independently extracted data from the included studies. A data extraction form was created as a data collection tool and was modified based on group feedback. A calibration exercise was conducted to ensure consistency and reliability between reviewers. The principal investigator (SWW) was involved wherever any discrepancies arose between reviewers. The following details were extracted from each study: (1) Study

characteristics: first author, year of publication, study design, country of study, and study period; (2) Study population; (3) Diagnostic criteria for GDM; (4) Outcome measures (diabetes (specifying T2DM when reported), and/ or pre-diabetes), and length of follow-up; (5) Exposure measures: glucose tests (100-g OGTT, 75-g OGTT or GCT), and comparison groups; (6) Effect measures: adjusted risk ratios (RR), odds ratios (OR), or hazard ratios (HR) when available, otherwise, crude OR or HR were extracted; (7) Covariates: confounders were listed when adjusted effect estimates were reported.

Quality assessment The Newcastle-Ottawa Scale (NOS) was used to assess the methodological quality of included studies. Three versions of NOS tailored for cohort studies, case-control studies and cross-sectional studies were used for each type of study. The quality assessment covered three domains: selection, comparability, and ascertainment of outcome or exposure, comprising a total of eight items. In the selection and ascertainment of outcome or exposure domain, a maximum of one star could be assigned for each item, while in the comparability domain, up to two stars could be assigned. Studies were rated as high quality (7–9 stars), moderate risk of bias (4–6 stars), or high risk of bias (0–3 stars). Two reviewers (NZ, WW) independently assessed the quality of each study, and disagreements were resolved by consultation with a third reviewer (SWW).

Data synthesis and Analysis Patient and study characteristics were stored and analyzed by Review Manager 5 (RevMan). Statistical heterogeneities were assessed using I^2 (I^2> 50% indicate substantial heterogeneity) and Cochrane's Q test (P<0.2).[14] Pooled crude ORs along with the 95% confidence interval (CI) were calculated. Random effects models were used to determine weights for meta-analysis. The Mantel-Haenszel method was used for the random effects model. The pooled effect measures were made for each type of glucose tests (100-g

OGTT, 75-g OGTT, and GCT). As primary comparisons of interest, women with 100-g OGTT were categorized into following groups: normal GCT (if available), normal OGTT, one abnormal value on OGTT, and two or more abnormal values on OGTT (Supplement 2). If data for the normal GCT group were unavailable, the normal OGTT group served as the reference. Similar categorizations were used for studies employing the 75-g OGTT. Additionally, as a secondary comparison, we examined the risk of diabetes in women with abnormal GCT but normal OGTT versus those with normal GCT. When multiple studies utilized the same cohort, data from the most recent publication were included in the meta-analysis. Narrative synthesis was used in instances of substantial heterogeneity. Subgroup analyses were performed based on the number and type of abnormal value on OGTT: one abnormal value (isolated fasting, 1-hour (h), 2-h, or 3-h post-load), two abnormal values (overall, with abnormal fasting, without abnormal fasting); three abnormal values (overall, with abnormal fasting, without abnormal fasting), and four abnormal values on OGTT. Further subgroup analyses were conducted by outcome (diabetes vs. T2DM), GDM diagnostic criteria (National Diabetes Data Group (NDDG) vs. Carpenter-Coustan (C&C)), reference group (normal GCT vs. normal OGTT), follow-up period (<5 years 5–10 years, >10 years), study design (prospective cohort, retrospective cohort, cross-sectional) and region of study location (Asia, North American, Europe) to fully explore the source of heterogeneity. To assess the robustness of study results, sensitivity analyses were performed by implementing sequential and combination algorithms in case when I^2 exceeded 50% (Supplement 3).[15-16] Reporting bias was evaluated by comparing outcomes listed in protocols with those reported in publications. If protocols were unavailable, outcomes in the methods and results section were compared. Publication bias was assessed using funnel plots if the number of included studies is at least ten; otherwise, it was not evaluated given the limited power. The

Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was used to evaluate the overall strength of the body of evidence. This systematic review was conducted based on Cochrane Systematic Reviews. Our findings were reported following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines.

Patient and public involvement None

4.3 Results

Details of included and excluded studies

The study selection process is presented in the flow chart (Supplement 4). We identified 84 studies after abstract screening for full text review. Twenty studies were included in the systematic review and 9 were included for the meta-analysis. The characteristics of the included studies are summarized in Table 4.1. Ten studies were conducted in North America, and five were from Israel. Sixteen studies had a median follow-up time of at least three years. Given that five studies were based on the same cohort[20-21,23,32,35], but only the most recent study with the longest follow-up[23] was included in the meta-analysis to avoid duplication. In total, the review included 620,633 women with glucose measured by GCT and/or OGTT during pregnancy. All included studies were rated as high quality: five studies received a score of 9, twelve scored 8, and three scored 7 (Table 4.2)

Table 4.1 Characteristics of included studies

Author& Year	Country	Study design	Study period	Sample size*	GDM criteria	Outcome	Follow-up	Glucose test	Comparison & effect measures	Confounder
Hiersch et all (2022)[17]	Canada	Retrospective cohort	2007-2017	41,507*	Canada	Type 2 diabetes	4.4 y	75-g OGTT	Fasting glucose (I mmol/L increase): aHR 2.77 [2.14-3.58]; 1-hour post-load (I mmol/L increase): aHR 1.26 [1.15-1.37]; 2-hour post-load (I mmol/L increase): aHR 1.14 [1.04-1.25].	Maternal pre-existing hypertension, ethnicity, income, fetal sex.
Berezowsky et all (2022)[12]	Israel	Retrospective cohort	2007-2014	5,295	C&C‡	Type 2 diabetes	64 m	100-g OGTT	One abnormal value vs. normal OGTT: aOR 3.59 [1.96–6.58]; Two abnormal values & above vs. normal OGTT: aOR 11.38 [6.89–18.97].	Age, parity, ART, pre-pregnancy obesity, chronic hypertension, and hyperlipidemia.
Retnakaran et all (2009)[18]	Canada	Retrospective cohort	1999-2007	76,618*	Canada /C&C	Diabetes	6.4 y	100-g OGTT, GCT	One abnormal value & normal OGTT vs. normal GCT aHR 2.56 [2.28-2.87].	Matched for age at delivery, region of residence, social economic status, year of delivery.
Dubietyte et all (2022)[19]	Denmark	Prospective cohort	1991-2021	352*	Denmark¶	Diabetes	28 y	75-g OGTT¶	One abnormal value vs. normal OGTT: OR 2.61[1.44, 4.74].	NA
Retnakaran et all (2008)[20]	Canada	Prospective cohort	2004-2007	487	NDDG§	Diabetes/ Pre-diabetes	3.2 m	100-g OGTT, GCT	One abnormal value vs. normal GCT: OR 5.7[1.6, 21.1]; Two abnormal values & above vs. normal GCT: OR 14.3 [4.2, 49.1]; normal OGTT vs. normal GCT: OR 3.6[1.01-12.9].	Months postpartum, age, ethnicity, family history of DM, pre-pregnancy BMI, weight gain in pregnancy preceding OGTT.
Retnakaran et all (2009(1))[21]	Canada	Prospective cohort	2004-2007	259*	NDDG	Pre-diabetes	3.2 m	100-g OGTT, GCT	Normal OGTT vs. normal GCT: OR 3.42 [0.91,12.01].	NA
Carr et all (2008)[22]	US	Retrospective cohort	1985-2002	24,780*	C&C	Type 2 diabetes	8.8 y	100-g OGTT GCT	One abnormal value vs. normal OGTT: aHR 2.07[1.34-3.18]; GCT (5.4-6.2 mmol/l) vs. GCT (<5.4 mmol/l): aHR 1.72[1.09-2.70]; GCT (6.3-7.3 mmol/l) vs. GCT (<5.4 mmol/l): aHR 2.13[1.38-3.29]; GCT (>7.3 mmol/l) vs. GCT (<5.4 mmol/l): aHR 3.65 [2.42-5.52].	Age, primigravity, preterm delivery
Kramer et all (2014)[23]	Canada	Prospective cohort	2004-2010	337	NDDG	Diabetes/ Prediabetes	3 y	100-g OGTT, GCT	One abnormal value vs. normal GCT: OR 3.23[1.15, 9.09]; Two abnormal values & above vs. Normal GCT: OR 6.89 [2.74,17.35]; normal OGTT vs. normal GCT: OR 1.99 [0.73, 5.46].	NA
Vambergue et all (2007)[24]	France	Prospective cohort	1992-1999	1,009	C&C	Diabetes/ Prediabetes	6 y	100-g OGTT, GCT	One abnormal value vs. normal GCT: aOR 4.57 [1.47, 14.22]; Two abnormal values & above vs. normal GCT: aOR 6.62 [2.22,19.77].	Origin of parents, previous GDM, previous BMI>27kg/m^2, socioeconomic level
Corrado et all	Italy	Cross-sectional	1990-1999	122	C&C	Diabetes/ pre-	6.9 y	100-g OGTT	One abnormal value vs. normal OGTT: OR 4.12 [1.43, 11.92]; Two abnormal values & above vs. normal OGTT:	Matched with year of delivery and pre-pregnancy BMI.

(2007)[25]		study				diabetes			OR 5.37 [1.85, 15.59].	
Bardugo et all (2023)[26]	Israel	Retrospective cohort	2001-2019	177,241	C&C	Type 2 diabetes	10·8 y	100-g OGTT, GCT	One abnormal value vs. normal GCT: aHR 9.11[7.64–10.86]; Two abnormal values & above vs. normal GCT aHR 24.84[21.78–28.34].	Sociodemographic characteristics (residential socioeconomic position, years of education), adolescent BMI, and age at gestational screening.
Yefet et all (2021)[27]	Israel	Retrospective cohort	1991-2011	1,077	NDDG	Type 2 diabetes	12.4 y	100-g OGTT	One abnormal value vs. normal OGTT: aHR 5.5[2.6–11.6]; Two abnormal values & above vs. normal OGTT: aHR 10.5[5.2–21.4]	GDM diagnosis criteria, parity, birth number, body mass index (BMI) before Pregnancy, weight before pregnancy, OGTT values, macrosomia, type of birth, maternal age, maternal weight before pregnancy, delivery week, and insulin use.
Hakkarainen et all (2015)[28]	Finland	Retrospective cohort	1989-2009	874	Finland‖	Type 2 diabetes	7.3 y	75-g OGTT	One abnormal value vs. normal OGTT: aHR 17.6[1.9–162.3]; Two abnormal values & above vs. normal OGTT: aHR 72.9[9.6–553.7]	BMI, age, and follow-up time.
Vinograd et all (2022)[29]	Israel	Retrospective cohort	2005-2020	77,568	NDDG /C&C	Type 2 diabetes	6.07 y	GCT	GCT (6.7-7.1 mmol/l) vs. GCT (<6.7 mmol/l): aHR 1.46[1.20-1.78]; GCT (7.2-7.7 mmol/l) vs. GCT (<6.7 mmol/l): aHR 2.43[2.04-2.89]; GCT (7.8-8.3 mmol/l) vs. GCT (<6.7 mmol/l): aHR 3.92[3.35-4.59]; GCT (>8.3 mmol/l) vs. GCT (<6.7 mmol/l): aHR 12.05[10.73-13.54].	Maternal age, body mass index, socioeconomic status, and low-income status;
Hiersch et all (2021)[30]	Canada	Retrospective cohort	2007-2017	55,361	Canada† /IADPSG**	Type 2 diabetes	4.4 y	75-g OGTT	One abnormal value vs. normal OGTT; aHR 4.92 [4.31–5.62]; Two abnormal values vs. normal OGTT: aHR 10.05[8.79–11.48]; Three abnormal values vs. normal OGTT: aHR 24.57[21.26–28.39].	Maternal age at the time of index birth, ethnicity, income quintile, and fetal sex.
Retnakaran et all (2023)[31]	Canada	Retrospective cohort	2007-2017	141,858	Canada	Diabetes	3.5 y	GCT	GCT (1 mmol/L increment): aHR 1.38[1.37-1.39];	Age, income, rurality, and hypertension.
Retnakaran et all (2009(2))[32]	Canada	Prospective cohort	2004-2007	487	C&C /NDDG	Diabetes/ pre-diabetes	3 m	100-g OGTT	One abnormal value vs. normal OGTT: OR 2.27[0.98, 5.27]; Two abnormal values & above vs. normal OGTT: OR 6.75[3.48, 13.09].	NA
Yoles et all (2017)[33]	Israel	Retrospective cohort	2005-2015	6,929	NA	Diabetes	9 y	GCT	GCT (5.6-6.9 mmol/L) vs. GCT (≤5.5 mmol/L) OR 1.54[1.25, 1.90]; GCT (7.0-7.8 mmol/L) vs. GCT (≤5.5 mmol/L) OR 2.33[1.80, 3.04]; GCT (>7.9 mmol/L) vs. GCT (≤5.5 mmol/L) OR 7.36 [6.01, 9.02].	NA
Selen et all (2023)[34]	US	Retrospective cohort	1998-2018	16,836	C&C	Diabetes	8.4 y	100-g OGTT, GCT	One abnormal value vs. normal GCT: aHR 2.97 [2.07–4.27]; Two abnormal values & above vs. normal GCT: aHR 8.26 [6.49–10.51].	Age at first delivery, marital status, insurance status, race and ethnicity, parity, prenatal body mass index (BMI), and prenatal diastolic blood pressure (BP).

Retnakaran et all (2009(3))[35]	Canada	Prospective cohort	2004-2007	412	NDDG	Diabetes/ pre-diabetes	3 m	100-g OGTT, GCT	Fasting glucose (I mmol/L increase): aOR 1.17 [0.68-2.00]; 1-hour post-load (I mmol/L increase): aOR 1.31 [1.09-1.57]; 2-hour post-load (I mmol/L increase): aOR 1.54 [1.28-1.84]; 3-hour post-load (I mmol/L increase): aOR 1.30 [1.09-1.55];	Age, gestational weeks at OGTT, pre-pregnancy BMI, weight gain in pregnancy preceding OGTT, parity, and previous GDM/macrosomia.

*: Only non-GDM population were included in this study

†: During 1999-2002, the diagnosis test was a mix of 100-gr OGTT and 75-g OGTT with two abnormal values as the cutoff. Between 2007-2017, the 75-gram OGTT has been recommended, the normal cut-off values were fasting blood, 1-h and 2-h post glucose loading of < 5.3 mmol/L (95 mg/dL), < 10.6 mmol/L (190 mg/dL), and < 9.0mmol/L (162 mg/dL) respectively.[38]

‡: According to the Carpenter -Coustan criteria, the normal OGTT cut-off values were fasting blood glucose, 1-h, 2-h, and 3-h post glucose loading of < 5.3 mmol/L (95 mg/dL), < 10.0 mmol/L (180 mg/dL), < 8.6 mmol/L (155 mg/dL) and < 7.8 mmol/L (140 mg/dL), respectively.

§: According to National Diabetes Data Group criteria, the normal OGTT cut-off values were fasting blood glucose, 1-h, 2-h, and 3-h post glucose loading of < 5.8 mmol/L (105 mg/dL), < 10.6 mmol/L (190 mg/dL), < 9.2 mmol/L (165 mg/dL) and < 8.0 mmol/L (145 mg/dL), respectively.

¶: The OGTT was performed with seven-point measurements with the diagnosis threshold values of 6.4mmol/L(fasting), 13.6mmol/L(30min.), 13.7mmol/L(60min.), 11mmol/L(90min.), 10.2mmol/L(120min.), 9.7mmol/L(150min.), 8.5mmol/L(180min.). GDM was diagnosed if two capillary plasma glucose values exceeded the thresholds.[39]

||: The diagnostic criteria of GDM were as follows: until September 2001 lower limits of abnormal fasting, 1-h and 2-h capillary whole-blood glucose were 4.8, 10.0 and 8.7 mmol/L and since September 2001 the lower limits of fasting, 1-h and 2-h capillary plasma glucose were 4.8, 11.2 and 9.9 mmol/L.

**: The International Association of the Diabetes and Pregnancy Study Groups criteria, the normal OGTT cut-off values were fasting blood glucose, 1-h and 2-h post glucose loading of < 5.1 mmol/L (92 mg/dL), < 10.0 mmol/L (180 mg/dL) and < 8.6mmol/L (153 mg/dL) respectively.

Table 4.2 Quality assessment of the included studies by the Newcastle–Ottawa Scale

Author& Year	Study design	Selection	Comparability	Outcome	Total score
Hiersch 2022	Retrospective cohort	★★★★	★	★★★	8
Berezowsky 2022	Retrospective cohort	★★★★	★	★★★	8
Retnakaran 2009	Retrospective cohort	★★★★	★	★★★	8
Dubietyte 2022	Prospective cohort	★★★	★★	★★★	8
Selen 2021	Retrospective cohort	★★★	★★	★★★	8
Retnakaran 2008	Prospective cohort	★★★★	★★	★★	7
Retnakaran 2009(1)	Prospective cohort	★★★★	★★	★★	8
Carr 2008	Retrospective cohort	★★★★	★	★★	7
Kramer 2014	Prospective cohort	★★★★	★★	★★★	9
Vambergue 2007	Prospective cohort	★★★★	★★	★★★	9
Corrado 2007	Cross-sectional study	★★★	★	★★★	7
Bardugo 2023	Retrospective cohort	★★★★	★★	★★★	9
Yefet 2020	Retrospective cohort	★★★	★★	★★★	8
Hakkarainen 2015	Retrospective cohort	★★★★	★★	★★★	9
Vinograd 2020	Retrospective cohort	★★★	★★	★★★	8
Hiersch 2021	Retrospective cohort	★★★	★★	★★★	8
Retnakaran 2023	Retrospective cohort	★★★★	★★	★★	8
Retnakaran 2009(2)	Prospective cohort	★★★★	★★	★★	8
Yoles 2017	Retrospective cohort	★★★	★★	★★★	8
Selen 2023	Retrospective cohort	★★★★	★★	★★★	9
Retnakaran 2009(3)	Prospective cohort	★★★★	★★	★★	8

Glucose values on 100-g OGTT and risk of diabetes

The pooled ORs for developing diabetes among women with two or more abnormal values on the 100-g OGTT, compared to women with normal GCT or OGTT, was 12.29 (95% CI, 8.36, 18.07) (I^2=83%, P<0.00001). For women with only one abnormal value on the 100-g OGTT, the pooled OR was 4.44 (95% CI 2.71, 7.28) (I^2=88%, P<0.00001) (Figures 4.1 & 4.2).

The Pooled OR for risk of diabetes among women with normal 100-g OGTT abnormal GCT was 2.29 (95% CI, 1.41, 3.71), as compared to those with normal GCT (Supplement 5) (I^2=77%, P=0.01).

Figure 4.1 Pooled odds ratio of diabetes for two abnormal values and above on 100-g OGTT versus normal OGTT/normal GCT

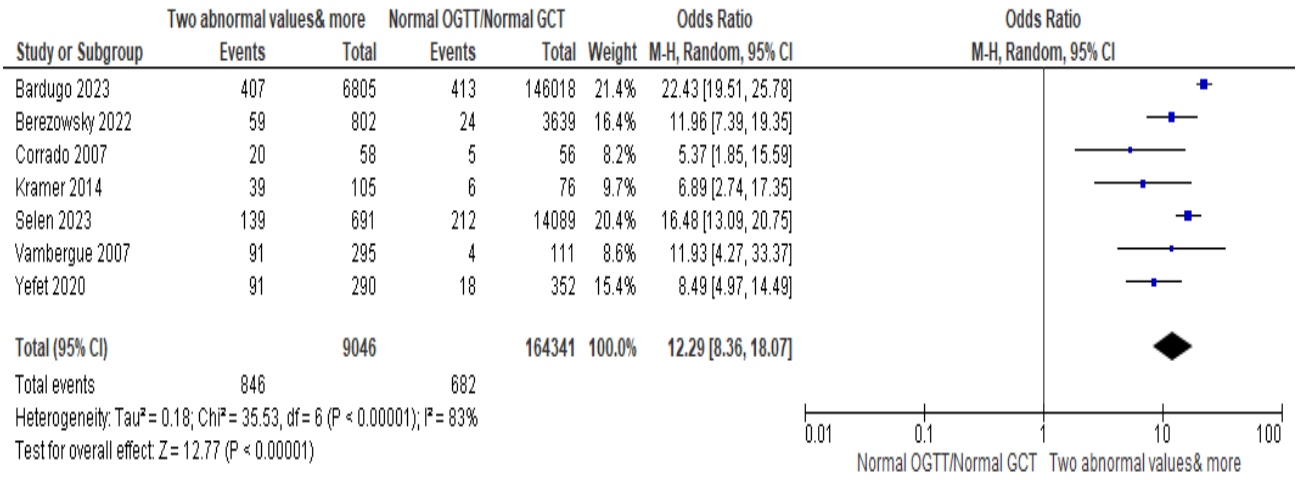
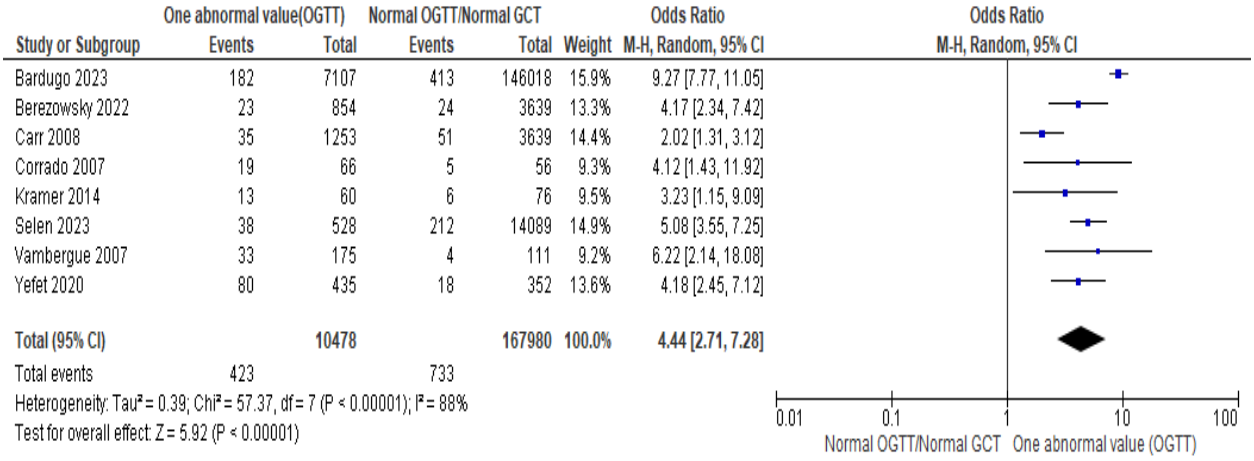


Figure 4.2 Pooled odds ratio of diabetes for one abnormal value on 100-g OGTT versus normal OGTT/normal GCT



Glucose values on 75-g OGTT and risk of diabetes

Although four studies used 75-g OGTT for GDM diagnosis, only qualitative summaries are presented due to inconsistencies in glucose measurement methods (capillary plasma glucose vs plasma glucose), glucose categorization, and diagnostic criteria for GDM. One study reported increasing risk of T2DM among women with one, two, and three abnormal values on the 75-g OGTT diagnosed by the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria, with HRs of 3.60 (95% CI, 3.09, 4.18), 8.86 (95% CI, 7.72, 10.18), and 24.24 (95% CI, 21.01, 27.97), respectively, compared to women with normal OGTT.[30] Another study

from the same Canadian cohort found that, among women with normal 75-g OGTT, the risk of T2DM increased per 1mmol/L increase in fasting, 1-h, and 2-h post-load glucose levels, with HRs of 3.79 (95% CI, 2.96, 4.84), 1.43 (95% CI, 1.33, 1.54), and 1.33 (95% CI, 1.23, 1.44), respectively.[17] Two other studies from Finland and Denmark, which used capillary plasma glucose and different diagnostic criteria, reported similar associations between elevated glucose values on the 75-g OGTT and increased risk of future diabetes.[19,28]

Subgroup analyses and Sensitivity analyses

Only one study provided results on risk of diabetes based on both the number and type of abnormal glucose values on the 100-g OGTT (Supplement 6).[26] Among women with single abnormal value, the ORs for risk of diabetes among these with isolated abnormal fasting, 1-h post-load, and 2- or 3-h post-load were 16.04 (95% CI, 11.60, 22.18), 8.72 (95% CI, 6.92, 10.99), and 7.49 (95% CI, 5.58, 10.04), respectively, as compared to those with normal GCT. The risk of diabetes increased significantly with the number of abnormal values on 100-g OGTT: ORs were 14.67 (95% CI, 12.36, 17.42) for two abnormal values, 40.63 (95% CI, 33.66, 49.05) for three, and 79.96 (95% CI, 56.84, 112.48) for four, all compared to women with normal GCT. Among women with two or more abnormal values, those with abnormal fasting glucose had substantially higher risk of diabetes (OR:54.99, 95% CI, 46.73, 64.72) than those without fasting abnormalities (OR:11.07, 95% CI, 9.16, 13.38). Specifically, the OR of diabetes among women with two abnormal values (with fasting), two abnormal values (without fasting), three abnormal values (with fasting), three anormal values (without fasting), four abnormal values, as compared to those with normal GCT was 40.75 (95% CI, 31.84, 52.17), 10.07 (95% CI, 8.17,12.40), 54.99 (95% CI, 46.73, 64.72) and 13.05 (95% CI, 9.65,19.87) respectively.

Subgroup analyses stratified by specific outcome, GDM diagnostic criteria, reference group, follow-up period, study design, and geographic region, produced less stable estimates due to smaller subgroup sample sizes. However, the findings were generally consistent with the main analysis (Supplement 7). Sensitivity analyses indicated that the study by Bardugo et al.[26] was the primary contributor to between-study heterogeneity in the meta-analyses across different comparison groups (Supplement 8). Although all included studies were observational, the overall rating of the evidence is considered high due to the magnitude of the observed effects, the presence of a clear dose–response relationship, and a low risk of bias.

111 Discussion

This systematic review and meta-analysis found that women with one abnormal value on the 100-g OGTT during pregnancy had more than four times greater risk of diabetes as compared to women with normal glucose tests, although this risk was much smaller than the twelve times increased risk in women with at least two abnormal values on the OGTT—a widely accepted diagnostic threshold for GDM. A four-fold increase in future risk of diabetes in women with one abnormal value on 100-g OGTT is an important issue that should not be taken lightly by any means.

The argument for a more flexible and inclusive definition of GDM, using one abnormal value as a threshold instead two, can be supported by analyses of association between maternal glucose measures with other long-term adverse outcomes such as cardiovascular diseases (CVDs). Elevated glucose levels on GCT, alone or combined with OGTT, have been suggested as a predictor of future risk of diabetes.[21,29,31,33] In a follow-up study of 259,164 pregnant women, Retnakaran & Shah found that each 1 mmol/L increment in GCT result was associated with a 13% higher risk of CVDs after adjusting for age, ethnicity, income, and rurality, with adjusted

HR of 1.13 (95%CI, 1.04-1.22). This relationship persisted even after excluding women with diagnosed with GDM.[36] Given that there may be a gradience between maternal hyperglycemia and long-term risks of diabetes and cardiovascular disease, it seems reasonable to use a more liberal criterion (e.g., at least one abnormal value of 100-g OGTT during pregnancy) to define GDM. Although women with "mild" forms of GDM may not require immediate medical intervention, healthcare providers should encourage them make proactive lifestyle changes to lower their future risk of diabetes and CVDs. To support future data collection and analysis, it may be time to call for change in disease coding system to differentiate 'severe' GDM which requires immediate medical attention is needed from 'mild' GDM, which although not requiring urgent intervention, still warrants early lifestyle modifications to reduce long-term health risks. Notably, women with abnormal fasting glucose levels had a significantly higher risk of developing diabetes compared to those without fasting abnormalities.[26] Therefore, particular attention should be given to fasting glucose levels when differentiating between severe and mild forms of GDM. Our study found that among women with normal OGTT, but abnormal GCT was more than twice as likely to develop diabetes than those with normal GCT. There are about 8% of pregnant women with normal 100-g OGTT but abnormal GCT.[26] Since some women may not undergo OGTT measured after an abnormal GCT, relying on GCT results combined with other risk factors could provide valuable insight into their future diabetes risk.

This review has several notable strengths. First, it is the first to quantify the risk of diabetes among women with one abnormal value on the 100-g OGTT and to make comparison with the risk of diabetes among these with at least two abnormal values on OGTT during pregnancy, the universally accepted criterion for the diagnosis of GDM. Second, we used women with normal GCT or normal OGTT as the reference group, representing true normoglycemia. In contrast,

prior reviews often included women with one abnormal value on the 100-g OGTT in the non-GDM group.[9,37] As a result, an underestimation of risk of diabetes for women with a history of GDM would be expected, as the control group included some pregnant women with mild hyperglycemia whose glucose levels were not truly normal. Third, this is the first review to quantify the risk of diabetes among women with normal 100-g OGTT but abnormal GCT, as compared to women with normal GCT.

Several limitations of this review should be acknowledged. First, some studies did not specify whether the follow-up outcome was T2DM and instead reported outcomes as diabetes or pre-diabetes (Table 1). However, given that most of the diabetes in the adult population are T2DM,[2] and that subgroup analyses restricted to T2DM yielded similar results, the inclusion of "soft" diagnosis on diabetes in some original studies is unlikely to alter the conclusions of this review. Second, the follow-up time varied considerably across the included studies (Table 1). Nonetheless, after excluding four studies that originated from the same cohort but had shorter follow-up periods, most of the remaining studies had over three years of follow-up. Moreover, subgroup analyses stratified by follow-up duration—less than five years, five to ten years, and over ten years—produced consistent results, supporting the robustness of our conclusions. Third, only one study examined the risk of diabetes based on the number of abnormal glucose values on the 75-g OGTT. Nevertheless, the direction and magnitude of the association between the 75-g OGTT results and future diabetes risk were similar to those observed with the 100-g OGTT. Fourth, few studies assessed the risk of diabetes among women with normal 100-g OGTT but abnormal GCT, which limits the strength of evidence for this group. Fifth, due to the limited number of original studies reporting time-to-event data, ORs were used for meta-analyses instead of HRs.

4.5 Conclusion and Implications

Our systematic review and meta-analysis found that women with one abnormal value on 100-g OGTT during pregnancy had more than a fourfold increased risk of developing diabetes compared to women with normoglycemia during pregnancy. Although this risk is substantially lower than the twelve times of risk of diabetes among women with at least two abnormal values on 100-g OGTT during pregnancy, a condition that has been universally accepted as a diagnosis of GDM. Women with a single abnormal value should be considered as having GDM, warranting appropriate clinical attention and recommendations to adopt intensified lifestyle changes aimed at reducing their future risk of developing diabetes.

4.6 References

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4.7 Supplements

Supplement 1. Search strategies

Search Strategy - Ovid MEDLINE(R) <1946 to July 12, 2023>

#	Searches	
1	Blood glucose/	183078
2	glucose intolerance/	9893
3	glucose tolerance test/	36858
4	(Glucose adj3 (tolera* or intolera* or fast* or value* or level* or test)).ti,ab,kf.	166203
5	(blood adj3 (sugar or glucose)).ti,ab,kf.	111579
6	OGTT.ti,ab,kf.	10292
7	(dysglyc?emi* or hyperglyc?emi*).ti,ab,kf.	77107
8	1 or 2 or 3 or 4 or 5 or 6 or 7	357225
9	pregnancy/	987308
10	(pregnan* or gestation* or trimester or antepartum or gravidity).ti,ab,kf.	735751
11	9 or 10	1191358
12	8 and 11	24612
13	Case-Control Studies/ or Control Groups/ or Matched-Pair Analysis/ or ((case* adj5 control*) or (case adj3 comparison*) or control group*).ti,ab.	947171
14	cohort studies/ or longitudinal studies/ or follow-up studies/ or prospective studies/ or retrospective studies/ or cohort.ti,ab. or longitudinal.ti,ab. or prospective.ti,ab. or retrospective.ti,ab.	3320102

15	**Cross-Sectional Studies/ or Prevalence/ or (cross-sectional or prevalence or transversal).ti,ab,kw.**	**1386671**
16	**13 or 14 or 15**	**5021771**
17	**12 and 16**	**9304**
18	**exp animals/ not humans.sh.**	**5137253**
19	**17 not 18**	**8814**
20	**(exp infant/ or exp child/ or adolescent/) not exp adult/**	**2139504**
21	**19 not 20**	**7428**
22	**exp Diabetes Mellitus, Type 2/**	**171005**
23	**(MODY or NIDDM or T2D*).tw.**	**56655**
24	**(non insulin* depend* or noninsulin* depend* or noninsulin?depend* or non insulin?depend*).tw.**	**12441**
25	**((typ? 2 or typ? II or typ?2 or typ?II) adj3 diabet*).tw.**	**186452**
26	**(((late or adult* or matur* or slow or stabl*) adj3 onset) and diabet*).tw.**	**5416**
27	**22 or 23 or 24 or 25 or 26**	**243816**
28	**21 and 27**	**1468**

Search Strategy - Embase <1974 to 2023 July 12>

#	Searches	
1	**Blood glucose/**	**251967**

2	**glucose intolerance/**	**21892**
3	**glucose tolerance test/**	**26487**
4	**(Glucose adj3 (tolera* or intolera* or fast* or value* or level* or test)).ti,ab,kw.**	**249796**
5	**(blood adj3 (sugar or glucose)).ti,ab,kw.**	**165341**
6	**OGTT.ti,ab,kw.**	**19562**
7	**(dysglyc?emi* or hyperglyc?emi*).ti,ab,kw.**	**115769**
8	**1 or 2 or 3 or 4 or 5 or 6 or 7**	**508957**
9	**pregnancy/**	**687769**
10	**(pregnan* or gestation* or trimester or antepartum or gravidity).ti,ab,kw.**	**948763**
11	**9 or 10**	**1172596**
12	**8 and 11**	**32997**
13	**exp cohort analysis/**	**1058435**
14	**exp longitudinal study/**	**196407**
15	**exp prospective study/**	**888101**
16	**exp follow up/**	**2074954**
17	**cohort$.tw.**	**1490067**
18	**exp case control study/**	**226372**

19	**(case$ and control$).tw.**	**896611**
20	**(cross sectional or questionnaire or questionnaires or survey or epidemiological study).ab,ti.**	**2103326**
21	**13 or 14 or 15 or 16 or 17 or 18 or 19 or 20**	**6351178**
22	**12 and 21**	**11393**
23	**(exp animal/ or animal experiment/ or nonhuman/) not (exp human/ or human experiment/)**	**7293819**
24	**22 not 23**	**11188**
25	**exp juvenile/ not exp adult/**	**2590066**
26	**24 not 25**	**9377**
27	**non insulin dependent diabetes mellitus/**	**336511**
28	**(MODY or NIDDM or T2D*).tw.**	**96489**
29	**(non insulin* depend* or noninsulin* depend* or noninsulin?depend* or non insulin?depend*).tw.**	**14734**
30	**((typ? 2 or typ? II or typ?2 or typ?II) adj3 diabet*).tw.**	**294643**
31	**(((late or adult* or matur* or slow or stabl*) adj3 onset) and diabet*).tw.**	**8202**
32	**27 or 28 or 29 or 30 or 31**	**407562**
33	**26 and 32**	**1998**

Search Strategy - CINAHL

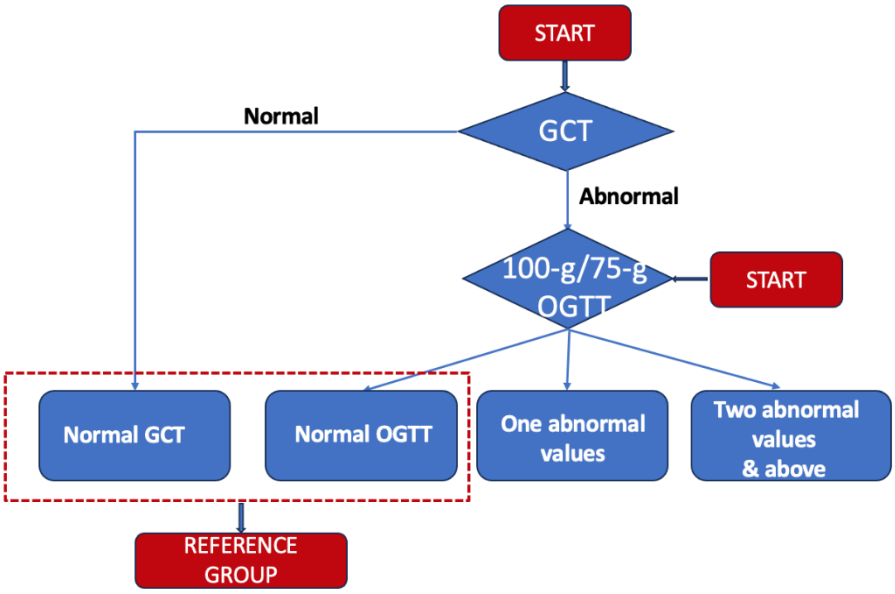
#	Searches

S1	**MH "Blood glucose"**	**42633**
S2	**MH "glucose intolerance"**	**3,513**
S3	**MH "glucose tolerance test"**	**7,943**
S4	**TI (Glucose N3 (tolera* or intolera* or fast* or value* or level* or test)) OR AB (Glucose N3 (tolera* or intolera* or fast* or value* or level* or test))**	**37,439**
S5	**TI blood N3 (sugar or glucose)) OR AB blood N3 (sugar or glucose))**	**27,649**
S6	**TI OGTT OR AB OGTT**	**2,478**
S7	**TI (dysglyc?emi* or hyperglyc?emi*) OR AB (dysglyc?emi* or hyperglyc?emi*)**	**2,978**
S8	**S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7**	**76,274**
S9	**MH "pregnancy"**	**226,560**
S10	**TI (pregnan* or gestation* or trimester or antepartum or gravidity) or AB (pregnan* or gestation* or trimester or antepartum or gravidity)**	**203,140**
S11	**S9 OR S10**	**302,069**
S12	**S8 AND S11**	**6,271**
S13	**TI ((animal or animals or canine* or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep*)**	**124,029**
S14	**S12 NOT S13**	**5,968**
S15	**((MH "Child+") OR (MH "Adolescence"))**	**1,098,776**

S16	**S14 NOT S15**	**4,220**
S17	**(MH "Case Control Studies+") or (MH "Control Group") or (MH "Matched-Pair Analysis") or (TI (case or cases) n5 TI (control or controls)) OR (AB (case or cases) n5 AB (control or controls)) OR (TI (case or cases) n3 TI (matched)) OR (AB (case or cases) n3 AB (matched)) OR TI (control group*)**	**108,773**
S18	**cohort[TIAB] OR Cohort Studies[Mesh] OR longitudinal[TIAB] OR prospective[TIAB] OR retrospective[TIAB]**	**43**
S19	**Cross-Sectional Studies[Mesh] OR "cross-sectional"[TIAB] OR "prevalence study"[tiab]**	**3**
S20	**S17 OR S18 OR S19**	**108,818**
S21	**S19 AND S20**	**275**
S22	**(MH "Diabetes Mellitus, Type 2")**	**69,638**
S23	**TX (NIDDM or MODY or T2DM or T2D)**	**66,965**
S24	**TX (non insulin* depend* or noninsulin* depend* or non insulin?depend* or noninsulin?depend*)**	**1,662**
S25	**TX ((typ* II or typ* 2) N6 diabet*)**	**91,883**
S26	**S22 OR S23 OR S24 OR S25**	**93,876**
S27	**S21 AND S26**	**49**

Supplement 2. Formation of comparison groups

GDM screening procedure by 50-g GCT and/or 100-g/75-g OGTT



Comparison groups

100-g OGTT

- One abnormal OGTT vs normal OGTT/normal GCT (*Primary* comparison)
- Two abnormal OGTT vs normal OGTT/normal GCT (*Primary* comparison)
- Normal OGTT vs normal GCT (*Secondary* comparison)

75-g OGTT

- One abnormal OGTT vs normal OGTT (*Primary* comparison)
- Two abnormal OGTT vs normal OGTT (*Primary* comparison)

Supplement 3. Sequential and Combinatorial Algorithm

Sequential Algorithm

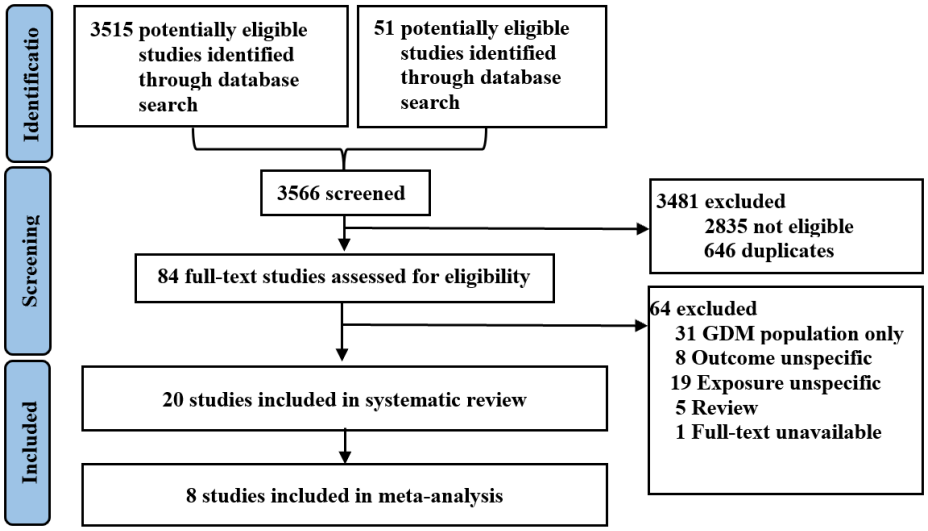
In this approach, for a meta-analysis of n studies, we perform n new meta-analyses, where one study is excluded from the calculations each time. The study that is responsible for the largest decrease in I^2 is dropped and a new set of n-1 studies is created. When two or more studies cause the same decrease in I^2 by their exclusion, we drop the study with the largest decrease in Q. We continue by successively re-analysing reduced sets of studies and applying the same rule one step before I^2 decreases below 50%. In the last step there is a chance more than one omitted study can result in I^2 dropping below 50%, we omit the study that will result in the minimum possible I^2.

Combinatorial algorithm

The combinatorial algorithm aims to identify clusters of studies whose exclusion can reduce the between study heterogeneity below 50%. When the exclusion of any single study does not suffice to drop the heterogeneity below 50%, the decrease in the I^2 is examined by the exclusion

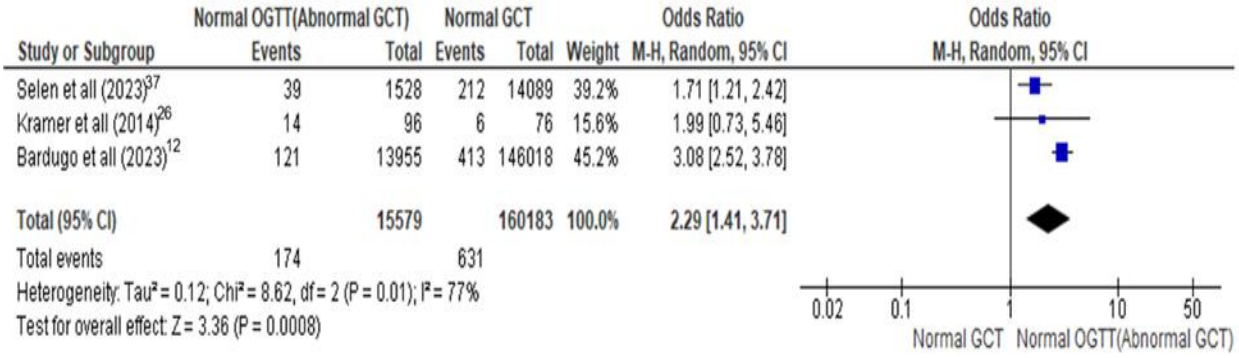
of all possible pairs of studies. If no pair exclusion achieves the goal of below of below 50%, we examine the decrease in the I^2 by the exclusion of all possible triplets of studies, and so forth. At the last step, If there are more than one set with equal number of excluded studies that decrease I^2 below 50%, we choose to omit the set that results in the minimum I^2.

Supplement 4. Study selection



Supplement 5

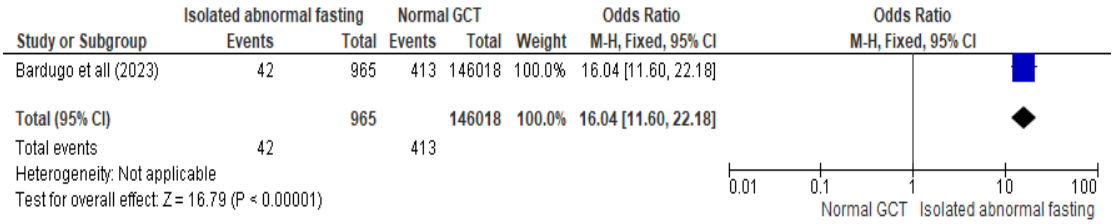
Pooled odds ratio for risk of diabetes/ between women with normal 100-g OGTT abnormal GCT and normal GCT



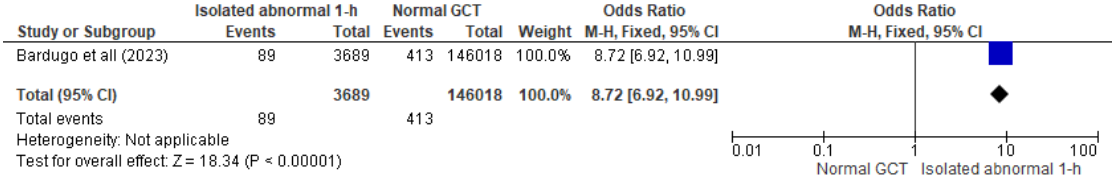
Supplement 6. Re-analysis of Israel study data (source: Bardugo A, Bendor CD, Rotem RS, et al. Lancet Diabetes Endocrinol. 2023;11(5):333-344. 8587(23)00062-1)

1.Among women with one abnormal value

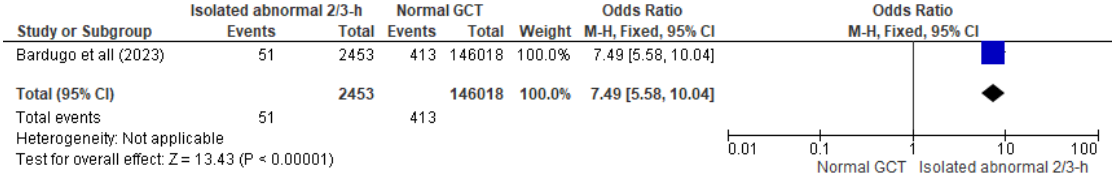
1.1 Odds ratio for type 2 Diabetes among women with one abnormal value (fasting) compared to normal GCT



1.2 Odds ratio for type 2 diabetes among women with one abnormal value (1-h post-load) compared to normal GCT

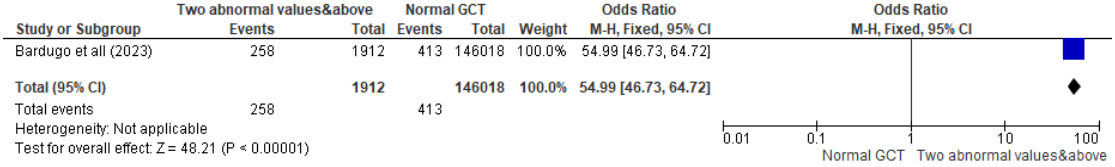


1.3 Odds ratio for type 2 diabetes among women with one abnormal value (2/3-h post-load) compared to normal GCT

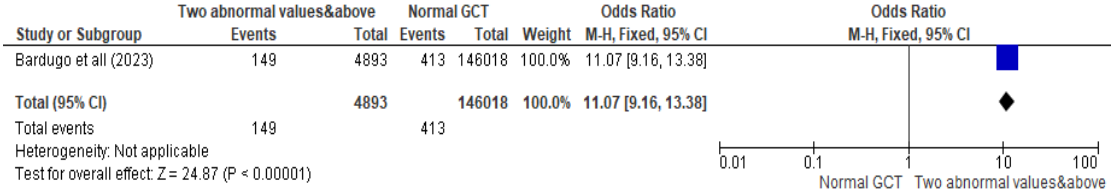


2. Among women with two abnormal values & above (Based on fasting status)

2.1 Odds ratio for type 2 diabetes among women with two abnormal values & above (with fasting) compared to normal GCT

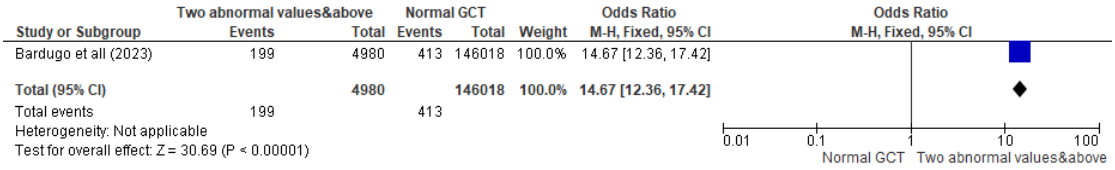


2.2 Odds ratio for type 2 diabetes among women with two abnormal OGTT & above (without fasting) compared to normal GCT



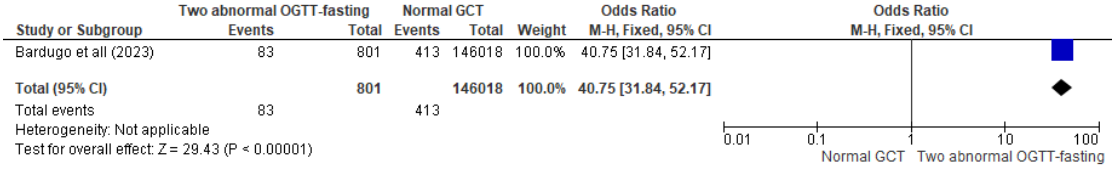
3. Among women with two abnormal values (overall, with fasting, without fasting)

3.1 Odds ratio for type 2 diabetes among women with two abnormal values on OGTT compared to normal GCT

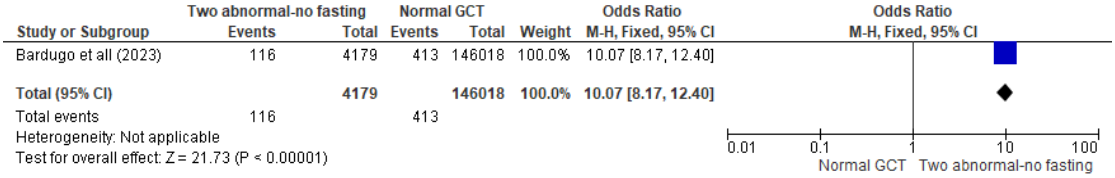


3.2 Odds ratio for type 2 diabetes among women with two abnormal values on OGTT (with

fasting) compared to normal GCT

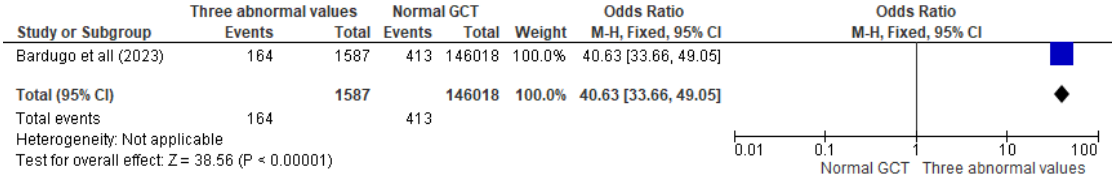


3.3 Odds ratio for type 2 diabetes among women with two abnormal values on OGTT (without fasting) compared to normal GCT

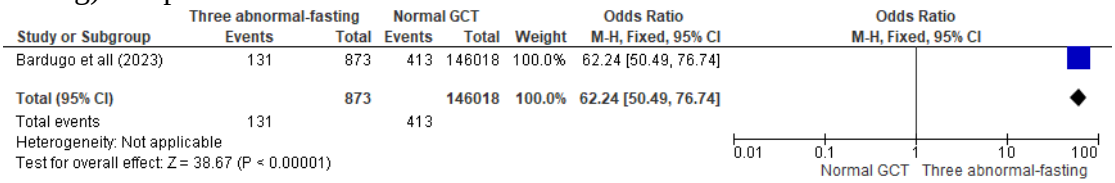


<u>4.Among women with three abnormal values (overall, with fasting, without fasting)</u>

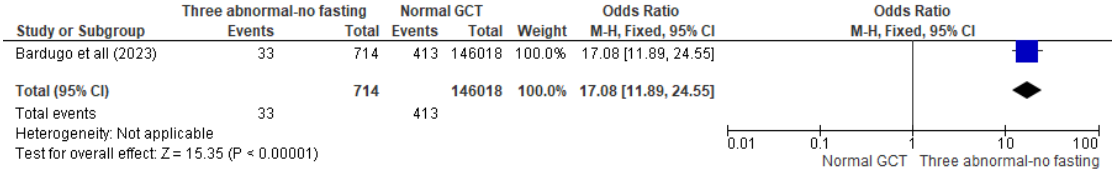
4.1 Odds ratio for type 2 diabetes among women with three abnormal values on OGTT compared to normal GCT



4.2 Odds ratio for type 2 diabetes among women with three abnormal values on OGTT (with fasting) compared to normal GCT

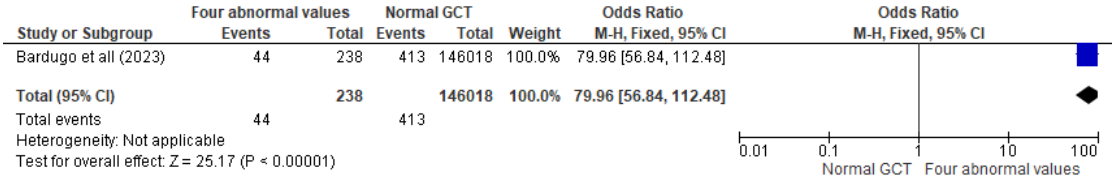


4.3 Odds ratio for type 2 diabetes among women with three abnormal values on OGTT (without fasting) compared to normal GCT



<u>5. Among women with four abnormal values</u>

5.1 Odds ratio for type 2 diabetes among women with four abnormal values on OGTT compared to normal GCT

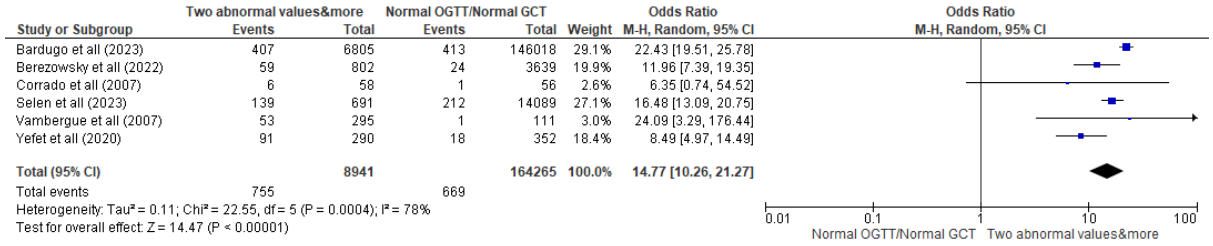


Supplement 7. Subgroup analysis

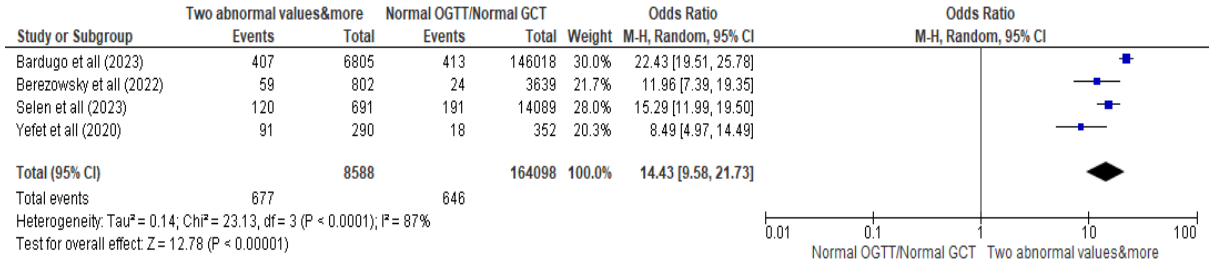
1.Comparison of two abnormal values & above versus normal OGTT/GCT

1.1 Based on outcome (Type 2 diabetes, Diabetes)

1.1.1 Odds ratio for diabetes among women with two abnormal values & above compared to normal OGTT/GCT

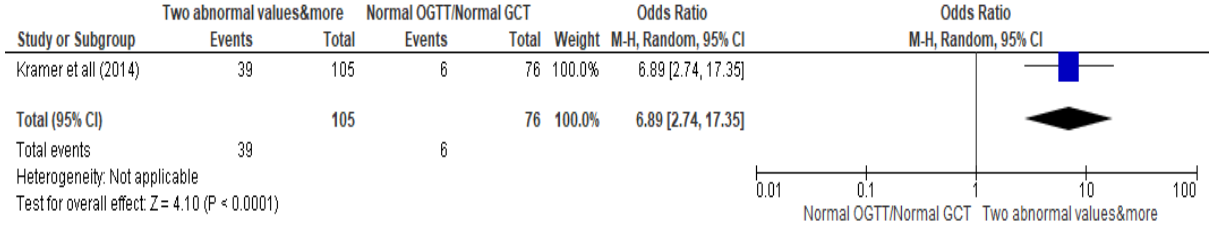


1.1.2 Odds ratio for type 2 diabetes among women with two abnormal values & above compared to normal OGTT/GCT

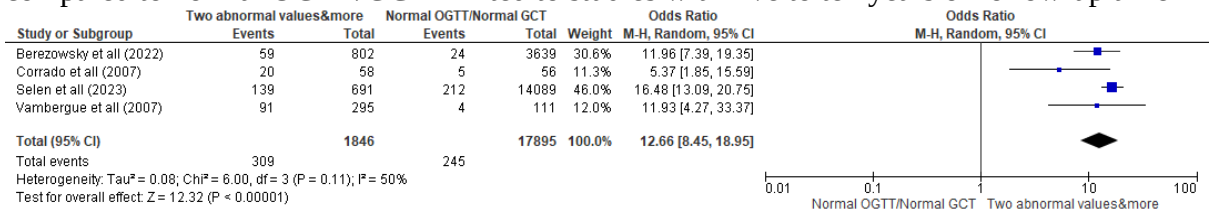


1.2 Follow-up period (less than five year, five to ten years, ten years and above)

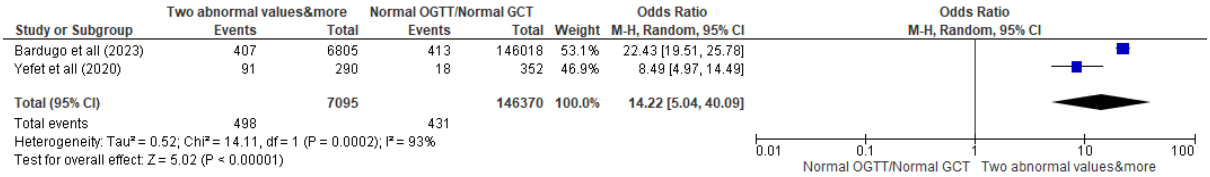
1.2.1 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to studies with five years of follow-up time



1.2.2 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to studies with five to ten years of follow-up time

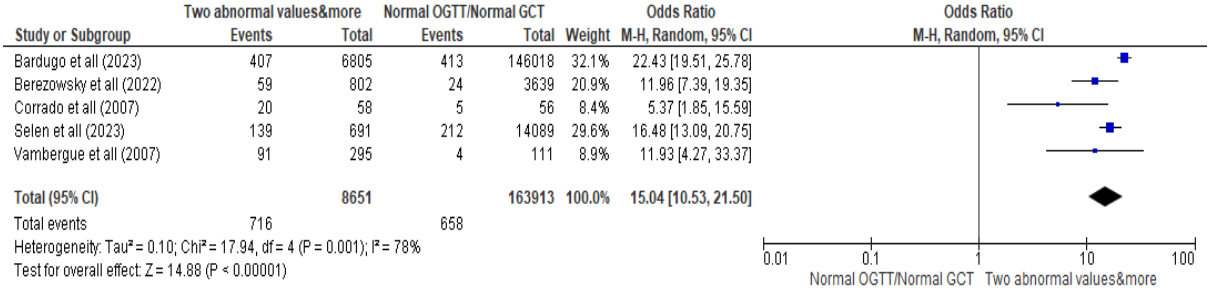


1.2.3 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to studies with ten years of follow-up time & above

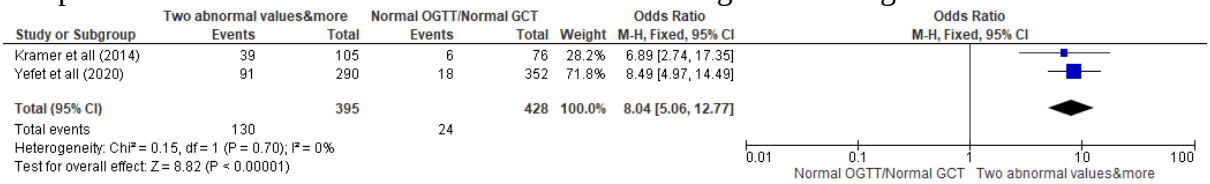


1.3 Based on diagnosis criteria of GDM (CC, NDDG)

1.3.1 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to studies using CC diagnosis criteria for GDM

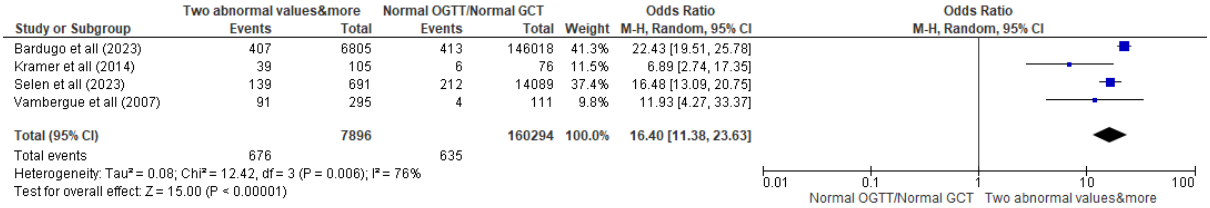


1.3.2 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to studies using NDDG diagnosis criteria for GDM

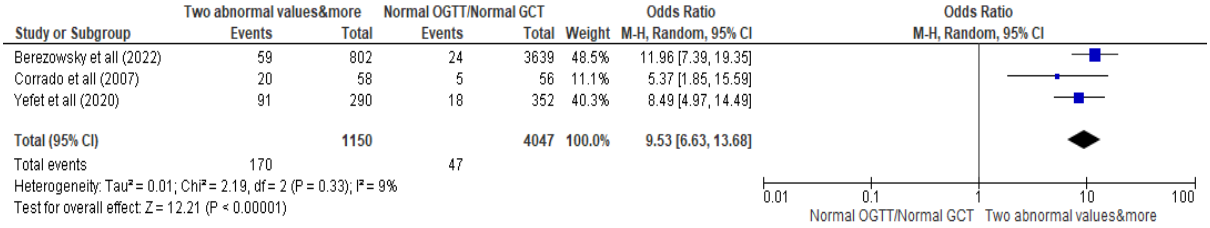


1.4 Based on the reference group(normal GCT, normal OGTT)

1.4.1 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal GCT limited to studies with normal GCT as the reference group

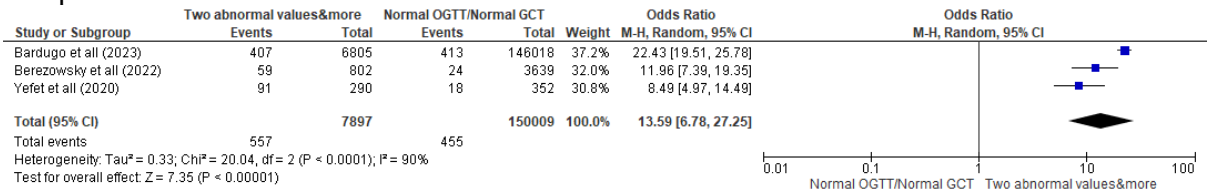


1.4.2 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT limited to studies with normal OGTT as the reference group

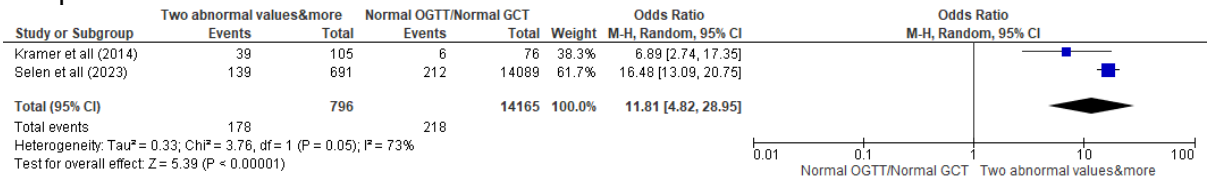


1.5 Based on country of study (Asian countries, North American countries, European countries)

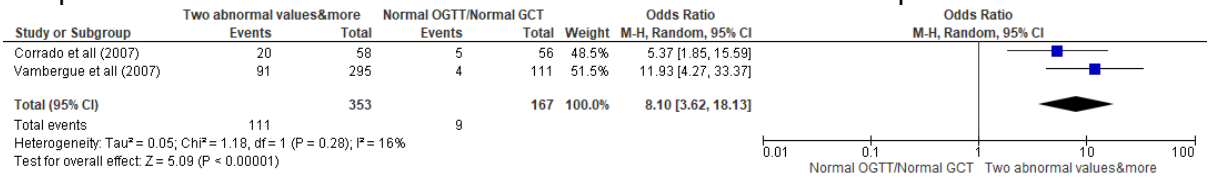
1.5.1 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to studies conducted in Asian countries



1.5.2 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to studies conducted in North American countries

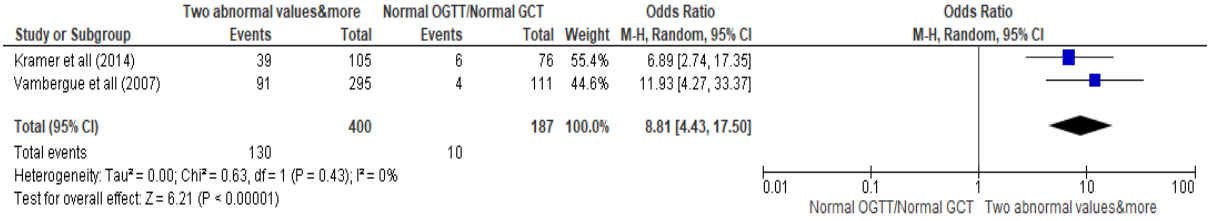


1.5.3 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to studies conducted in European countries

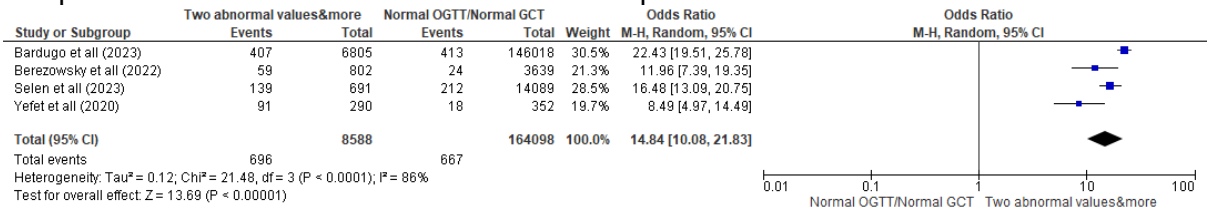


1.6 Based on study design (Prospective cohort study, Retrospective cohort study, Cross-sectional study)

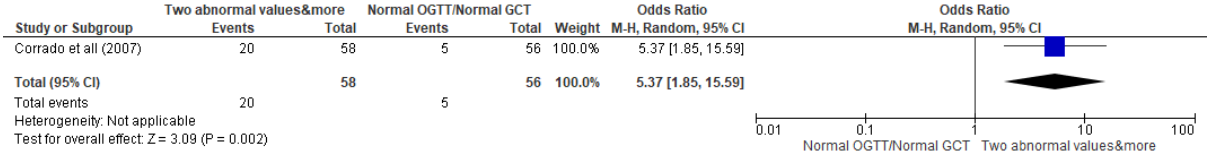
1.6.1 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to prospective cohort studies



1.6.2 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to retrospective cohort studies



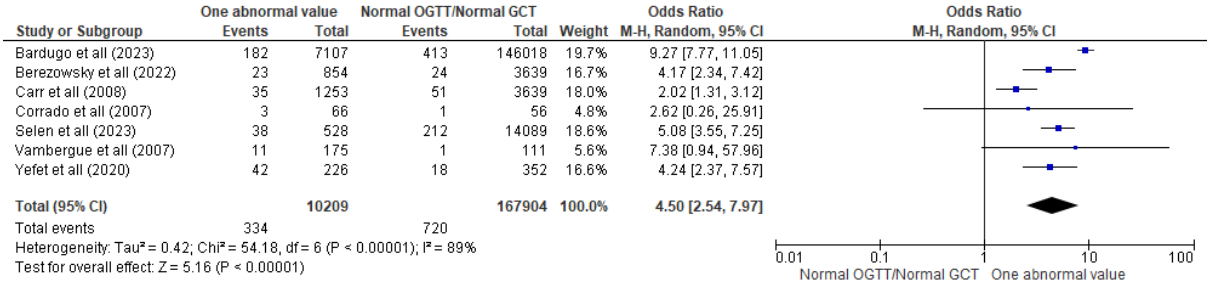
1.6.3 Odds ratio for diabetes/pre-diabetes among women with two abnormal values & above compared to normal OGTT/GCT limited to cross-sectional studies



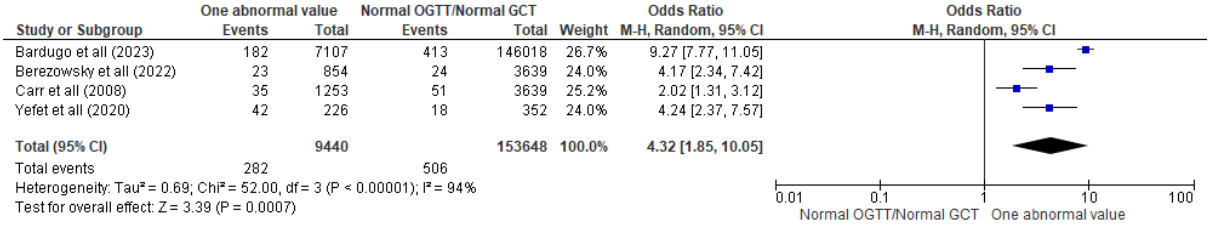
2.Comparison of one abnormal value versus normal OGTT/GCT

2.1 Based on outcome (Type 2 diabetes, Diabetes)

2.1.1 Odds ratio for diabetes among women with one abnormal value compared to normal OGTT/GCT

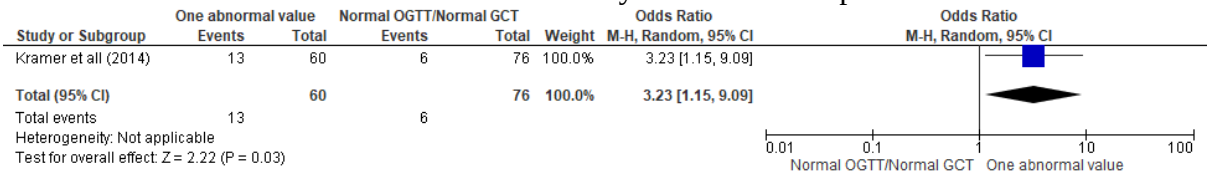


1.1.2 Odds ratio for type 2 diabetes among women with one abnormal value compared to normal OGTT/GCT

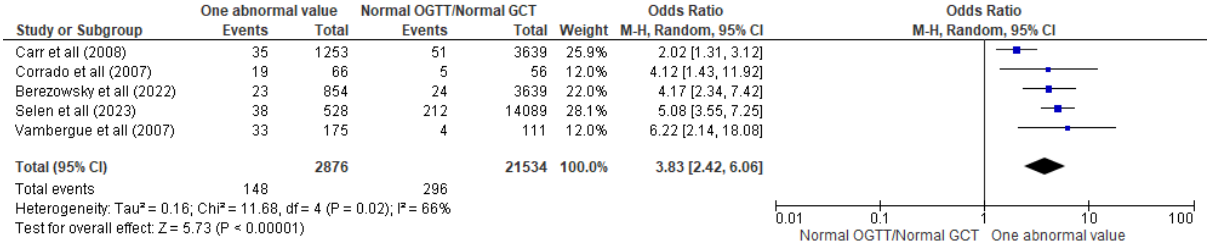


2.2 Follow-up period (less than five year, five to ten years, ten years and above)

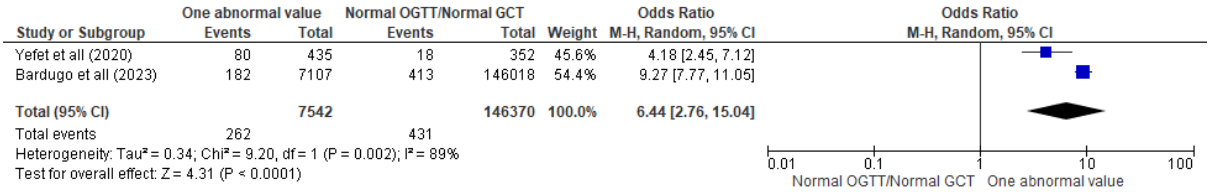
2.2.1 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies with five years of follow-up time



2.2.2 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies with five to ten years of follow-up time

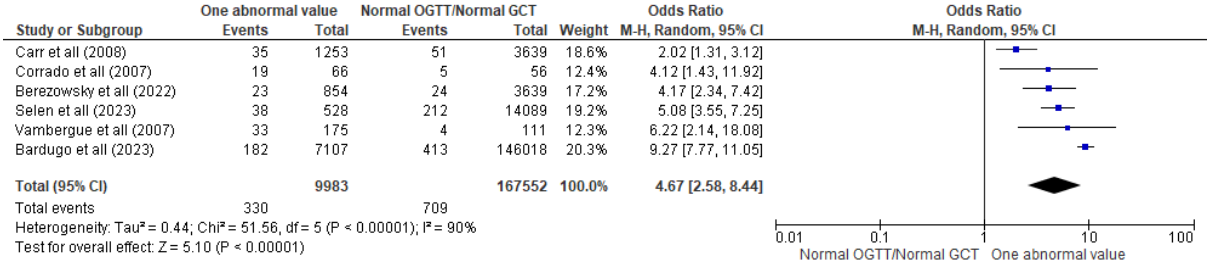


2.2.3 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies with ten years of follow-up time & above

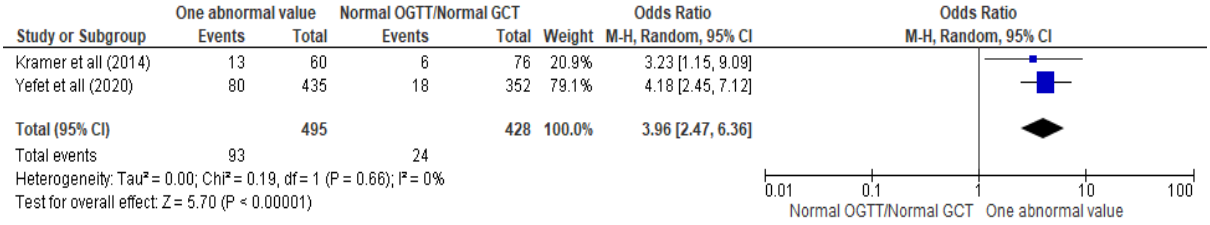


2.3 Based on diagnosis criteria of GDM (C&C, NDDG)

2.3.1 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies using C&C diagnosis criteria for GDM

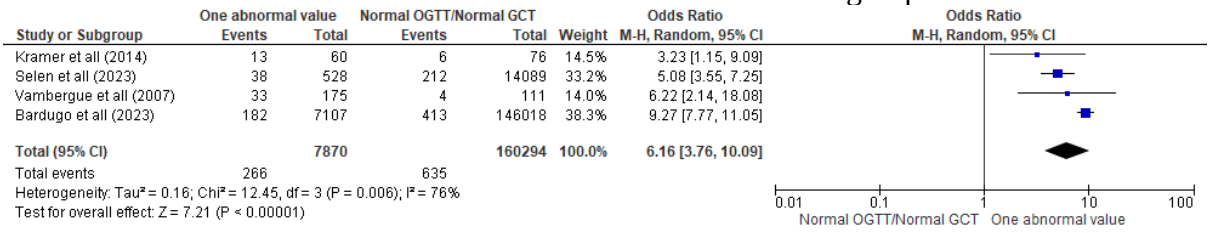


2.3.2 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies using NDDG diagnosis criteria for GDM

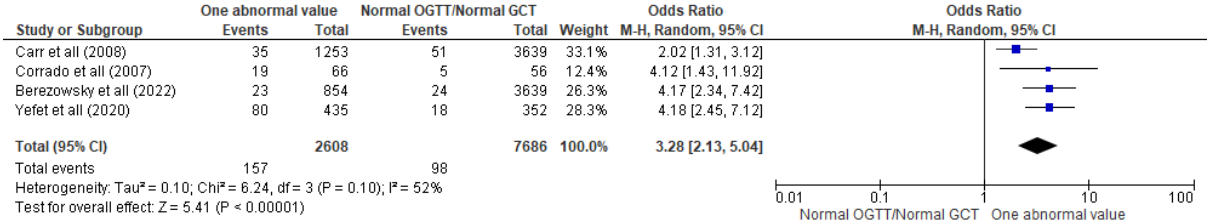


2.4 Based on the reference group(normal GCT, normal OGTT)

2.4.1 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal GCT limited to studies with normal GCT as the reference group



2.4.2 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT limited to studies with normal OGTT as the reference group

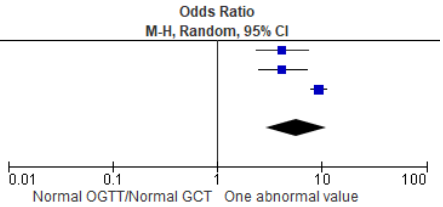


2.5 Based on study's country of study region (Asian countries, North American countries, European countries)

2.5.1 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies conducted in Asian countries

	One abnormal value		Normal OGTT/Normal GCT			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Berezowsky et all (2022)	23	854	24	3639	30.4%	4.17 [2.34, 7.42]
Yefet et all (2020)	80	435	18	352	31.5%	4.18 [2.45, 7.12]
Bardugo et all (2023)	182	7107	413	146018	38.1%	9.27 [7.77, 11.05]
Total (95% CI)		**8396**		**150009**	**100.0%**	**5.66 [2.88, 11.10]**
Total events	285		455			

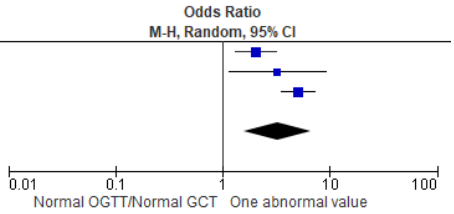
Heterogeneity: Tau² = 0.30; Chi² = 15.24, df = 2 (P = 0.0005); I² = 87%
Test for overall effect: Z = 5.04 (P < 0.00001)



2.5.2 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies conducted in North American countries

	One abnormal value		Normal OGTT/Normal GCT			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Carr et all (2008)	35	1253	51	3639	37.7%	2.02 [1.31, 3.12]
Kramer et all (2014)	13	60	6	76	22.8%	3.23 [1.15, 9.09]
Selen et all (2023)	38	528	212	14089	39.5%	5.08 [3.55, 7.25]
Total (95% CI)		**1841**		**17804**	**100.0%**	**3.24 [1.58, 6.62]**
Total events	86		269			

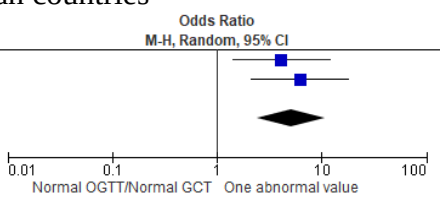
Heterogeneity: Tau² = 0.30; Chi² = 10.94, df = 2 (P = 0.004); I² = 82%
Test for overall effect: Z = 3.22 (P = 0.001)



2.5.3 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies conducted in European countries

	One abnormal value		Normal OGTT/Normal GCT			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Corrado et all (2007)	19	66	5	56	50.3%	4.12 [1.43, 11.92]
Vambergue et all (2007)	33	175	4	111	49.7%	6.22 [2.14, 18.08]
Total (95% CI)		**241**		**167**	**100.0%**	**5.06 [2.38, 10.74]**
Total events	52		9			

Heterogeneity: Tau² = 0.00; Chi² = 0.29, df = 1 (P = 0.59); I² = 0%
Test for overall effect: Z = 4.22 (P < 0.0001)

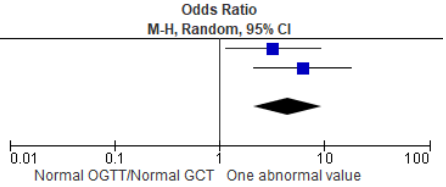


2.6 Based on study design (Prospective cohort study, Retrospective cohort study, Cross-sectional study)

2.6.1 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to prospective cohort studies

	One abnormal value		Normal OGTT/Normal GCT			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Kramer et all (2014)	13	60	6	76	51.5%	3.23 [1.15, 9.09]
Vambergue et all (2007)	33	175	4	111	48.5%	6.22 [2.14, 18.08]
Total (95% CI)		**235**		**187**	**100.0%**	**4.43 [2.11, 9.32]**
Total events	46		10			

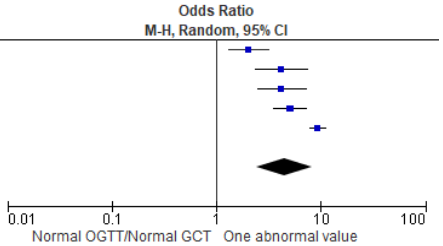
Heterogeneity: Tau² = 0.00; Chi² = 0.77, df = 1 (P = 0.38); I² = 0%
Test for overall effect: Z = 3.93 (P < 0.0001)



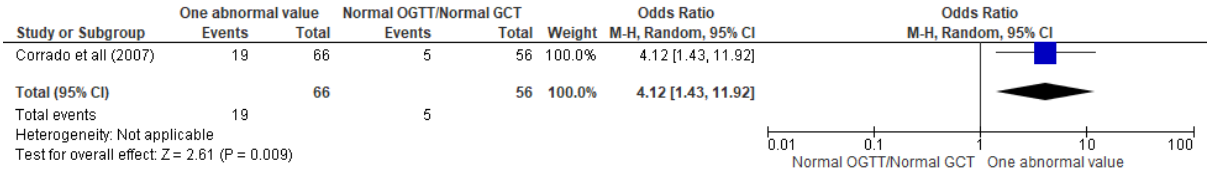
2.6.2 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to retrospective cohort studies

	One abnormal value		Normal OGTT/Normal GCT			Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI
Carr et all (2008)	35	1253	51	3639	20.0%	2.02 [1.31, 3.12]
Berezowsky et all (2022)	23	854	24	3639	18.6%	4.17 [2.34, 7.42]
Yefet et all (2020)	80	435	18	352	19.0%	4.18 [2.45, 7.12]
Selen et all (2023)	38	528	212	14089	20.6%	5.08 [3.55, 7.25]
Bardugo et all (2023)	182	7107	413	146018	21.8%	9.27 [7.77, 11.05]
Total (95% CI)		**10177**		**167737**	**100.0%**	**4.47 [2.42, 8.27]**
Total events	358		718			

Heterogeneity: Tau² = 0.44; Chi² = 54.64, df = 4 (P < 0.00001); I² = 93%
Test for overall effect: Z = 4.78 (P < 0.00001)



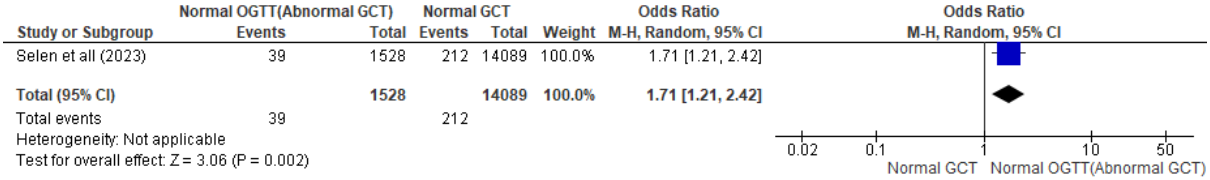
2.6.3 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to cross-sectional studies



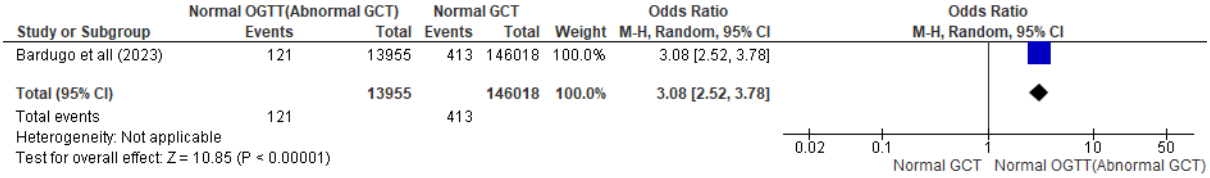
3.Comparison of normal OGTT abnormal GCT versus normal GCT

3.1 Based on outcome (Type 2 diabetes, Diabetes)

3.1.1 Odds ratio for diabetes among women with normal OGTT abnormal GCT compared to normal GCT

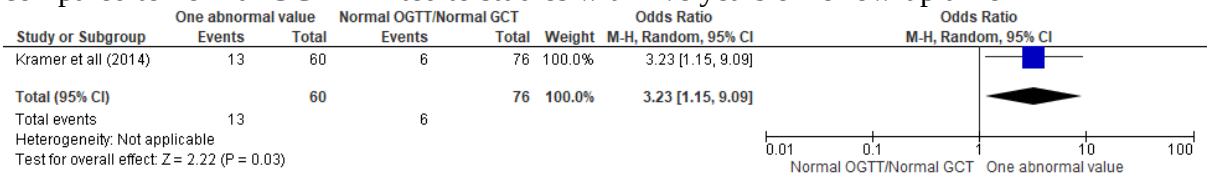


3.1.2 Odds ratio for type 2 diabetes among women with normal OGTT abnormal GCT compared to normal GCT

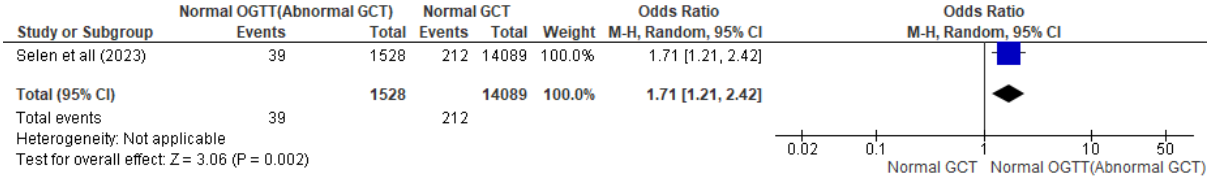


3.2 Follow-up period (less than five year, five to ten years, ten years and above)

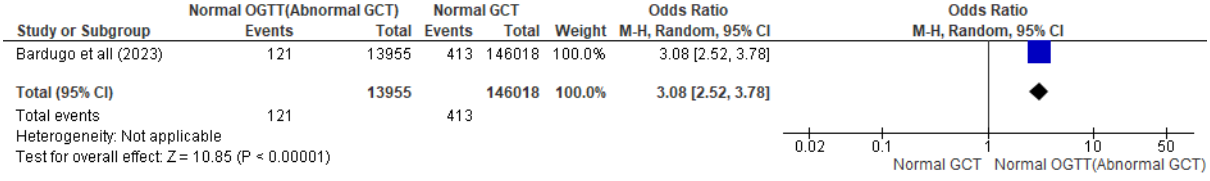
3.2.1 Odds ratio for diabetes/pre-diabetes among women with normal OGTT abnormal GCT compared to normal GCT limited to studies with five years of follow-up time



3.2.2 Odds ratio for diabetes/pre-diabetes among women with normal OGTT abnormal GCT compared to normal GCT limited to studies with five to ten years of follow-up time

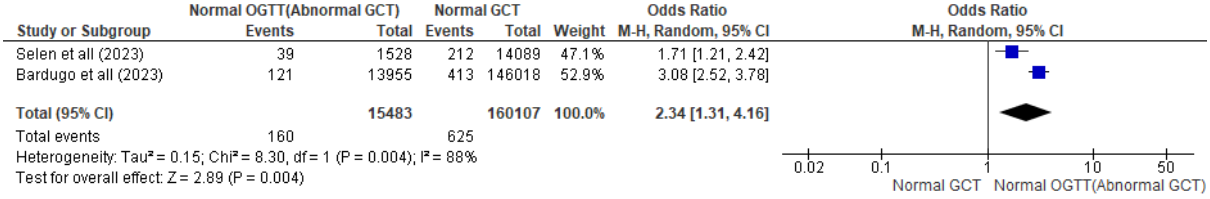


3.2.3 Odds ratio for diabetes/pre-diabetes among women with normal OGTT abnormal GCT compared to normal GCT limited to studies with ten years of follow-up time & above

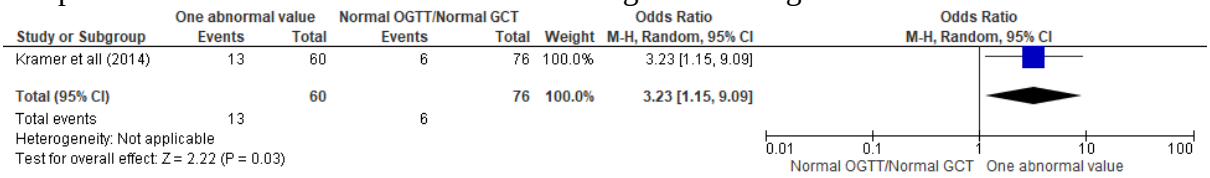


3.3 Based on diagnosis criteria of GDM (CC, NDDG)

3.3.1 Odds ratio for diabetes/pre-diabetes among women with normal OGTT abnormal GCT compared to normal GCT limited to studies using CC diagnosis criteria for GDM

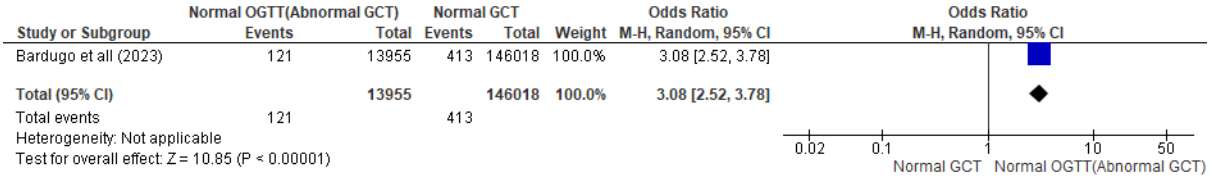


3.3.2 Odds ratio for diabetes/pre-diabetes among women with normal OGTT abnormal GCT compared to normal GCT limited to studies using NDDG diagnosis criteria for GDM

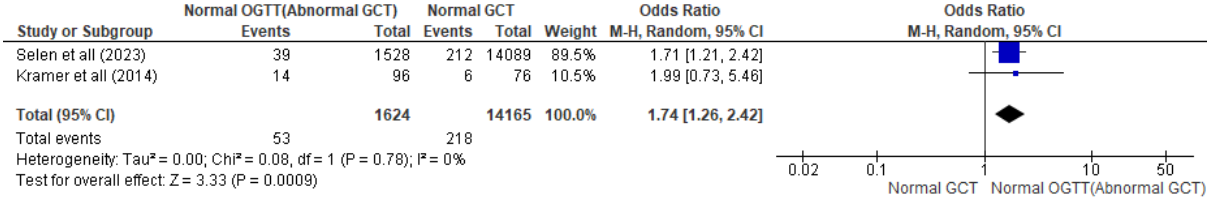


3.4 Based on study's country of study region (Asian countries, North American countries, European countries)

3.4.1 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies conducted in Asian countries

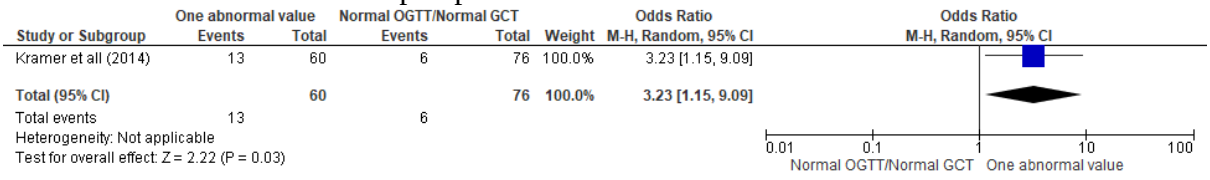


3.4.2 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to studies conducted in North American countries

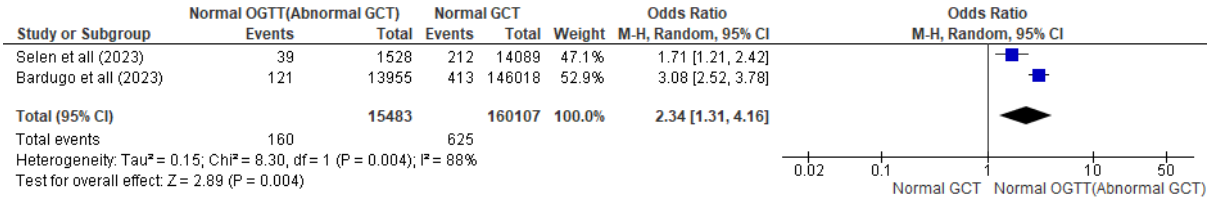


3.5 Based on study design (Prospective cohort study, Retrospective cohort study, Cross-sectional study)

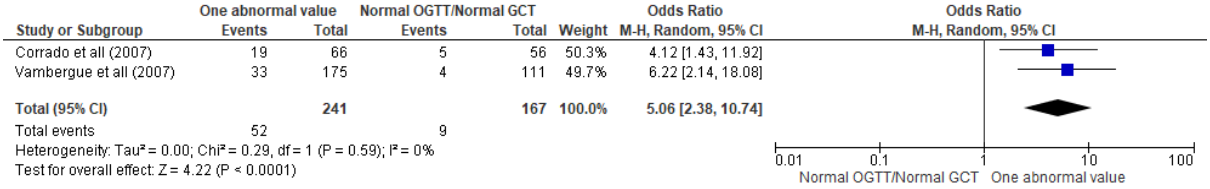
3.5.1 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to prospective cohort studies



3.5.2 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to retrospective cohort studies



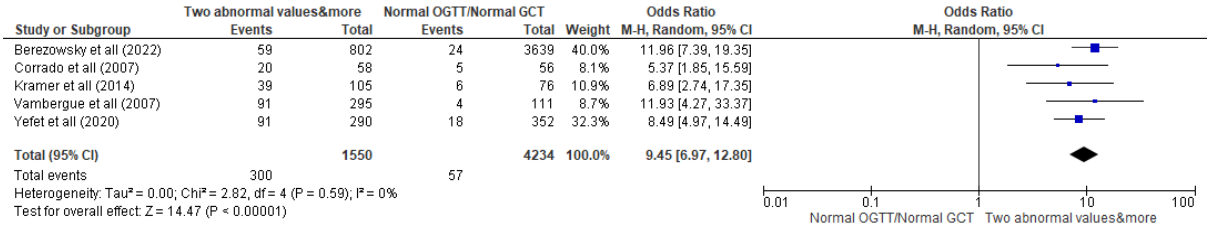
3.5.3 Odds ratio for diabetes/pre-diabetes among women with one abnormal value compared to normal OGTT/GCT limited to cross-sectional studies



Supplement 8 Sensitivity analysis – different comparisons

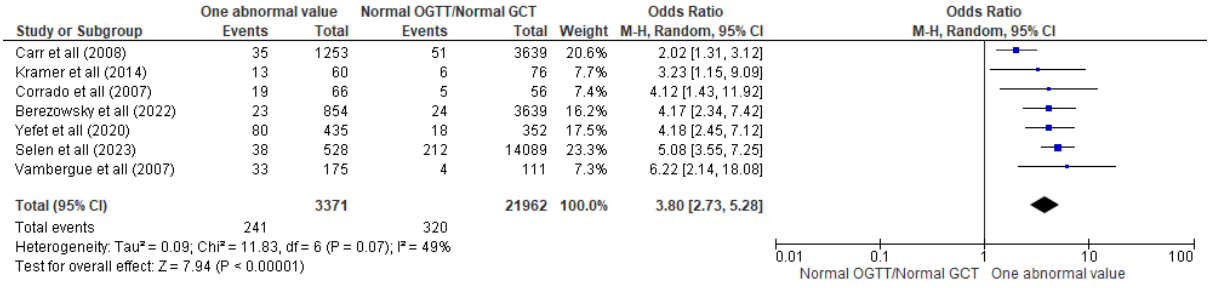
1.Comparison of two abnormal values & above versus normal OGTT/GCT

<u>The pair of Bardugo et all (2023) and Selen et all (2023) were excluded:</u>



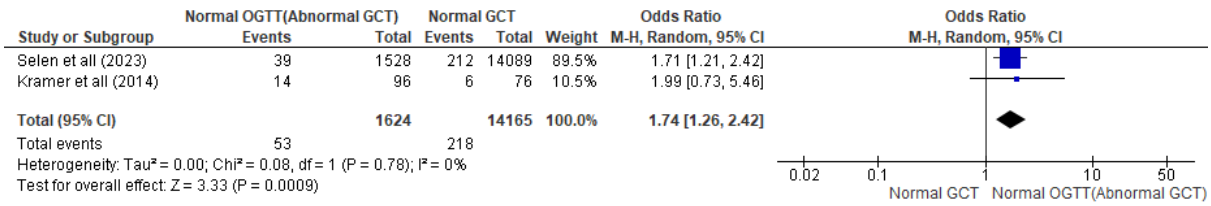
2.Comparison of one abnormal value versus normal OGTT/GCT

<u>The study of Bardugo et all (2023) was excluded:</u>



3.Comparison of normal OGTT abnormal GCT versus normal GCT

<u>The study of Bardugo et all (2023) was excluded:</u>


Normal OGTT(Abnormal GCT)
Normal GCT
Odds Ratio
Odds Ratio
Study or Subgroup
Events
Total
Events
Total
Weight
M-H, Random, 95% CI
M-H, Random, 95% CI
Selen et all (2023) 39 1528 212 14089 89.5% 1.71 [1.21, 2.42]
Kramer et all (2014) 14 96 6 76 10.5% 1.99 [0.73, 5.46]
Total (95% CI) 1624 14165 100.0% 1.74 [1.26, 2.42]
Total events 53 218
Heterogeneity: Tau² = 0.00; Chi² = 0.08, df = 1 (P = 0.78); I² = 0%
Test for overall effect: Z = 3.33 (P = 0.0009)
0.02
0.1
1
10
50
Normal GCT
Normal OGTT(Abnormal GCT)

Chapter 5

Association of hyperglycemia during pregnancy with future risk of type 2 diabetes among women: a nested case-control study using a US health administrative database

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Abstract

Background Recent studies suggest that women with hyperglycemia not reaching a diagnosis of gestational diabetes mellitus (GDM), such as these with one abnormal value on 100-gram(g) oral glucose tolerance test (OGTT), may have higher risk of adverse perinatal outcomes and long-term outcomes. There is no universally accepted screening criteria for GDM. Relying solely on a clinical diagnosis of GDM provides limited insight into the risk of developing diabetes later in life, particularly in the context that diagnostic criteria for GDM vary across jurisdictions. The objective of this study was to quantify the risk of type 2 diabetes mellitus (T2DM) among women based on the number of abnormal values on 100-g OGTT and fasting glucose status during pregnancy.

Methods We conducted a nested case-control study in a cohort of pregnant women with glucose challenge test (GCT) or OGTT results. A case was defined as having T2DM and a control was defined as no diagnosis of T2DM after the index delivery until the end of follow-up. Optimal variable matching approach was used for matching controls to cases (5:1) without replacement under the matching variables of maternal age at delivery, birth year, and gestational age at delivery. Patients were grouped into four groups: (1) normal GCT, (2) normal OGTT but abnormal GCT, (3) one abnormal value on OGTT, and (4) two or more abnormal values on OGTT. Among groups (3) and (4), further categorization was performed based on whether the fasting glucose value was abnormal. Sociodemographic and clinical characteristics between cases and controls were compared. Glucose value categorization was based on the National Diabetes Database Group (NDDG) and Carpenter & Coustan (C&C) criteria, respectively. Conditional logistic regression was employed to derive the best estimate of the association between glucose levels and risk of developing T2DM.

Results A total of 130,172 eligible women were identified from the pregnancy cohort. Of these, 1,390 cases and 6,950 matched controls were included in the analysis. Adjusted Odds Ratios (aOR) for the risk of T2DM among women with normal OGTT but abnormal GCT, one abnormal value, and at least two abnormal values on OGTT diagnosed by the NDDG criteria were 1.66 (95% CI,1.35, 2.04), 5.69 (95% CI, 4.34, 7.47), and 13.20 (95% CI, 10.52, 16.57), respectively. Whereas the aORs for the risk of T2DM among women with normal OGTT but abnormal GCT, one abnormal value, and these with at least two abnormal values diagnosed by the Carpenter & Coustan (C&C) criteria were 1.21 (95% CI, 0.94, 1.55), 2.62 (95% CI, 1.95, 3.51), and 11.69 (95% CI, 9.61, 14.23), respectively.

Conclusion Women with one abnormal value on the 100-g OGTT, particularly according to the NDDG criteria, had a significantly higher risk of developing T2DM compared to women with normal glucose value, although the magnitude of the association was not as strong as women with at least two abnormal values on 100-g OGTT. It may be prudent to put a "flag" of GDM on women with only one abnormal value on OGTT so that intensified lifestyle changes and regular glucose monitoring could be implemented to mitigate their risk of T2DM.

5.1 Background

Approximately 11.6% of Americans were living with diabetes in 2021.[1] Gestational diabetes mellitus (GDM) is one of the most significant risk factors for the subsequent development of type 2 diabetes mellitus (T2DM). Women diagnosed with GDM have an approximately eightfold increased risk of developing T2DM compared to those without GDM.[2] Furthermore, women with diabetes face a higher risk of cardiovascular disease and mortality than their male counterparts.[3] These findings underscore the importance of identifying women at elevated risk for T2DM, enabling timely surveillance and early interventions to prevent disease progression.

The diagnostic criteria for GDM were originally developed in 1964 to identify pregnant women at higher risk of diabetes and have undergone minor modifications since then.[4] Pregnant women are recommended to screen for GDM between 24 and 28 weeks of gestation using a two-step approach. This involves an initial 50-gram (g) glucose challenge test (GCT) as a screening test, followed by a 100-g oral glucose tolerance test (OGTT) as the diagnostic test if the GCT is abnormal.[5] A diagnosis of GDM is made when at least two abnormal values on the OGTT, including fasting and post-load glucose (1-hour(h), 2-h and 3-h), meeting the thresholds. The two-step approach remains the predominant method for GDM diagnosis in the United States, whereas most other countries have adopted newer criteria—characterized by lower glucose thresholds and a diagnosis of GDM based on a single abnormal value.[6-7]

Concerns have been raised regarding the two-step approach, as emerging evidence indicates that women with a single abnormal value on the 100-g OGTT are at elevated risk of developing T2DM. Our recent systematic review found that these women had approximately a fourfold increased risk of T2DM compared to those with normal glucose levels during pregnancy. A recent study from Israel has shown that the risk of women with isolated abnormal fasting glucose

on the 100-g OGTT had a higher risk of developing T2DM compared to those with two abnormal values on OGTT but normal fasting, and a comparable risk to women with three abnormal values but normal fasting.[8] This study was the first to quantify diabetes risk based on both the number of abnormal values on the 100-g OGTT and fasting glucose status. However, the findings warrant confirmation through larger studies conducted in other countries. The objective of our study was to quantify the risk of T2DM among women based on both the number of abnormal values and fasting glucose status on the 100-g OGTT during pregnancy using a large United States health administrative database.

5.2 Methods

Study design and Data source This nested case-control study utilized a large multicenter electronic health records (EHR) database-Cerner Real-World Data™ (CRWD) between 2000 and 2016, which accounted for 21.6% of overall inpatient EHRs in the United States.[9] It is a de-identified database that complies with the Health Insurance Portability and Accountability Act (HIPAA).[10-11] The database comprises data from nearly 69 million patients treated at 664 facilities affiliated with 117 health systems across all United States geographic regions since 2000.[10,12] The CRWD contains longitudinal patient data, including demographics, facility characteristics, diagnoses, procedures, laboratory results, and clinical events from both inpatient and outpatient settings.[13] This study received ethical approval from the Ottawa Health Science Network Research Ethics Board (20220430-01H).

Study Population Women who had at least one delivery (live births or stillbirth) of which the pregnancy period beginning from January 1st, 2000, and ending before December 31st, 2016, were eligible for inclusion. Live births and stillbirths, along with their corresponding pregnancy periods were estimated based on validated algorithms(Supplement 1.1).[14-15] To include women

registered in hospitals within the CRWD during the study period, the study population was restricted to those with at least one encounter during pregnancy and at least one encounter after delivery. Deliveries with records of 50-g GCT or 100-g OGTT during pregnancy were included. Deliveries were excluded if the laboratory modules of the hospitals where the delivery occurred were not part of the CRWD during the exposure window (24 weeks of gestation to delivery) (Supplement 1.2). Multifetal pregnancies identified during the pregnancy period were excluded (Supplement 1.3). Deliveries were also excluded if the mother had pre-existing diabetes, identified 180 days before conception until 90 days after conception (Supplement 1.4).[16-17] Deliveries lacking glucose records or involving irrelevant glucose measures, such as random plasma glucose, whole blood glucose, capillary glucose, urine glucose, or unspecified glucose measure, or those measured by 75-g OGTT were excluded (Supplement 1.5). Deliveries were excluded in case that abnormal GCT (≥140 mg/dL) was recorded without a follow-up 100-g OGTT. Additionally, OGTTs conducted before 24 weeks of gestation or those with missing values were excluded. If a woman had multiple eligible pregnancies, the first one was included. The delivery for selected pregnancy in the final cohort is index delivery.

Cases and controls A case was defined as a woman who developed T2DM after the index delivery and before the end of the follow-up period. T2DM was identified based on a combination of records of International Classification of Diseases (ICD)-9 and ICD-10 diagnosis codes.[18-19] A control was defined as a woman not being identified as having T2DM after index delivery until the end of follow-up period.

Exposures GDM screening was traditionally recommended for pregnant women with risk factors for GDM. According to the American Diabetes Association (ADA) statement in 2008, screening may be omitted in low-risk women, in whom all the following factors were present: age younger

than 25 years; BMI less than 25 before pregnancy; not of Hispanic, African American, American Indian, South or East Asian, or Pacific Islander descent; no first-degree relative with diabetes; no history of abnormal glucose tolerance; and no history of poor obstetric outcome.[20-22] In 2013, the American College of Obstetricians and Gynecologists (ACOG) recommended that every pregnant woman should be screened for GDM whether by the patient's medical history, clinical risk factors, or laboratory screening test results to determine blood glucose levels.[23] The two-step approach has been commonly recommended in the United State, which include a 50-g 1-hour (h) GCT and a 100-g 3-h diagnostic OGTT followed if the GCT is 140 mg/dL and above, [23] typically performed between 24 and 28 weeks of gestation. The Carpenter and Coustan (C&C) criteria or the National Diabetes Data Group (NDDG) criteria were recommended for the diagnosis of GDM.[23] Based on the C&C criteria, the diagnostic thresholds for the 100-g OGTT are as follows: fasting ≥5.3 mmol/L, 1-hour ≥10.0 mmol/L, 2-hour ≥8.6 mmol/L, and 3-hour ≥7.8 mmol/L.[4] According to the NDDG criteria, the corresponding thresholds are: fasting ≥5.8 mmol/L, 1-hour ≥10.6 mmol/L, 2-hour ≥9.2 mmol/L, and 3-hour ≥8.0 mmol/L.[4] Patients were categorized into primary exposure groups: normal GCT, normal OGTT but abnormal GCT, one abnormal value on OGTT, and two or more abnormal values on OGTT. Patients with one abnormal value, and two or more abnormal values on OGTT were further stratified based on fasting glucose status into two subgroups: abnormal fasting and normal fasting.

Covariates Covariates considered in this study included maternal age at delivery, gestational age at delivery, maternal race (Caucasian, Black, Hispanic, Asian/Pacific Islander, Native American), marital status (single, widowed/divorced, married/life partner), census region (Midwest, Northeast, South, West), birth year (2000-2004, 2005-2008, 2009-2012, 2013-2016), type of health insurance (private, self-insured, Medicaid, government/other), and hospital bed

size at the index delivery (<100, 100-199, 200-299, 300-499, ≥500). Maternal health behaviour variables included self-reported substance use during pregnancy: alcohol use (yes/no), tobacco smoking (yes/no), and drug use (yes/no). Pre-existing physical health conditions included pre-pregnancy obesity (yes/no)[24], pre-existing hypertension (yes/no)[25], and polycystic ovary syndrome (yes/no)[26]. Pre-existing mental health conditions include anxiety[27], depression[27], bipolar disorder[28], schizophrenia[29]. Pre-existing health conditions were identified by validated algorithms.

Matching method Optimal variable matching approach was used to match controls to cases without replacement.[30] This approach minimizes the overall "distance" between cases and controls across selected matching variables by using mathematical algorithms to identify the best matches while considering all cases and controls simultaneously.[31] Each case was matched to five controls based on the matching variables, which were weighted equally. For continuous variables, a difference (calliper) was applied. Matching variables and their callipers included maternal age at delivery (±1 year), gestational age at delivery (±1 completed weeks), birth year (±1 year). To ensure consistency in the control matching process, deliveries with missing data on any of the matching variables were excluded.

Statistical analysis Baseline characteristics, including the matching variables, were reported, and compared between cases and controls. Categorical variables were summarized as frequencies and percentages and compared using the chi-square tests. Continuous variables were displayed as means ± standard deviations (SD) and compared using t-test. Maternal age at delivery and gestational age at delivery were reported and compared both as continuous and categorical variable. Exposure groups were reported as counts and percentages according to the C&C criteria and the NDDG criteria separately. Conditional logistic regression was used to derive the best

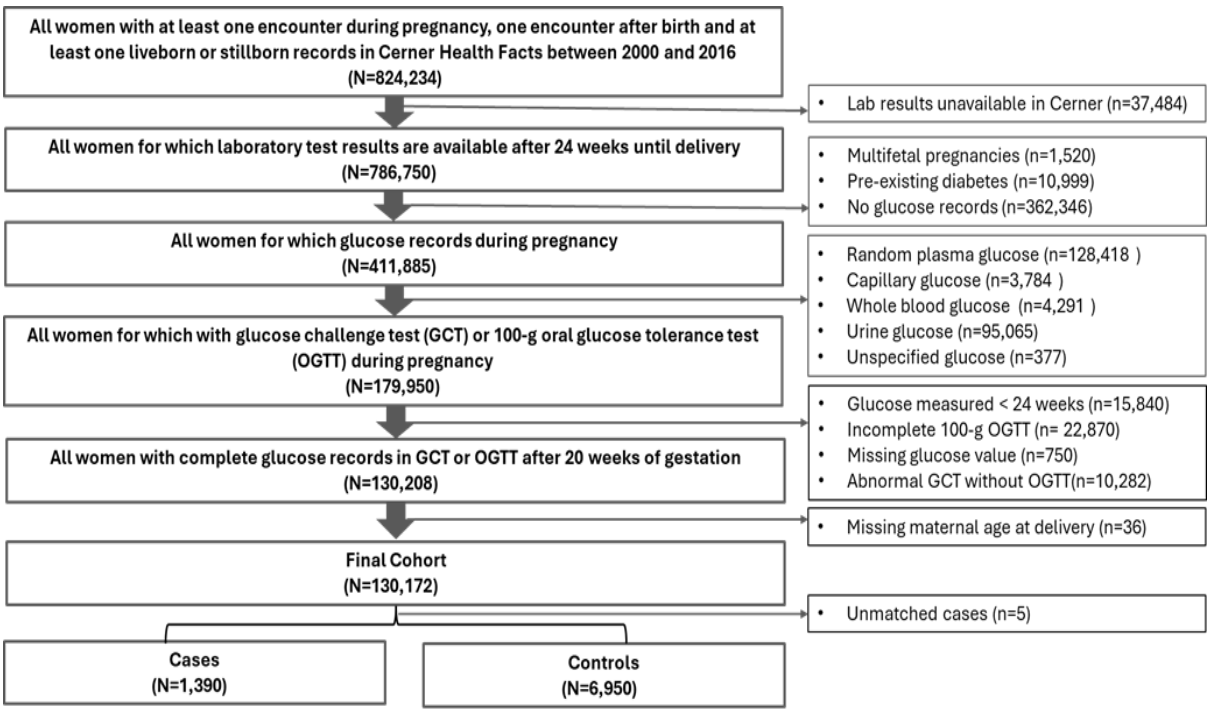
estimate of the association between glucose levels and risk of T2DM. Using normal GCT as the reference group, a base model was fitted on the matched dataset for each exposure group. Subsequently, a maximally adjusted model was fitted by adding all potential confounders to the base model. Potential confounding variables included in the multivariable models were maternal race, marital status, census region, type of health insurance, hospital bed size of index delivery, self-reported substance use during pregnancy, pre-existing health conditions (obesity, hypertension, polycystic ovary syndrome, pre-pregnancy anxiety), and mental health conditions (anxiety, depression, bipolar disorder, and schizophrenia). Odds ratios (OR), adjusted ORs (aOR) and 95% confidence intervals (CI) were reported separately for the C&C and the NDDG criteria. Confounders were carefully selected to avoid the occurrence of overadjustment.[32-33] All confounders included in multivariate regression analysis were selected based on their independent associations with both glucose levels in our source population and T2DM, using a significance cutoff of 0.05.[34] Multiple imputations was used to address missing data in the regression analyses. Five datasets were imputed by using the fully conditional specification logistic regression method, incorporating the matching variables in model method.[35-37] Statistical Analysis System (SAS) for windows, version 9.4 (SAS Institute, Cary, NC) was used to perform all analyses in this study.

5.3 Results

We initially identified a cohort of 823,234 pregnant women registered in the CRWD between 2000 and 2016. After excluding those with multifetal pregnancies, pre-existing diabetes, or without glucose measures, the study population was reduced to 411,885. Additional exclusions were made for pregnancies with irrelevant or incomplete glucose measures, missing glucose results, or glucose measurements taken before 24 weeks of gestation, resulting in a final cohort

of 130,208 pregnant women. Finally, pregnancies with missing data on the matching variable—maternal age at delivery— were excluded, yielding a final analytic cohort of 130,172. A detailed flowchart of the cohort formation was presented in Figure 5.1.

Figure 5.1 Study Population Selection



The mean maternal age of the cohort was 27.0 years (with SD of 5.7) (Table 5.1). About 68% of the cohort were Caucasian, and 51.8% resided in the Northeast of the United States. Approximately 40% were married or living with a life partner, and 42% had commercial insurances. Forty one percent of the included participants gave birth between 2013 and 2016, and 91.4% of deliveries were at term. Based on the C&C criteria, 4.3% of the cohort had at least two abnormal values, while 3.1% had one abnormal value. According to the NDDG criteria, 2.6% had at least two abnormal values, and 2.5% had one abnormal value.

We identified 1,395 women with T2DM in the cohort. However, five cases were excluded as five

controls could not be matched for these cases. As a result, the final matched study sample included 1,390 cases and 6,950 controls. Among pregnant women included in the matched analysis, there were no significant differences between cases and controls in maternal age categories, mean maternal age, gestational age at delivery, or birth year (Table 5.2). However, cases were more likely to smoke during pregnancy (13.4% vs. 7.9%) and to be obese prior to pregnancy (18.5% vs. 5.0%). Cases also had higher rates of pre-existing hypertension (7.4 vs. 1.8%) polycystic ovary syndrome (2.3% vs. 0.6%), depression (7.1% vs. 3.8%), and anxiety (4.4% vs. 3.0%) compared to controls. Additionally, cases were more likely to be non-Caucasian and single, less likely to have commercial insurance, and less likely to deliver at hospitals with fewer than 100 beds.

Table 5.1. Characteristics of study cohort (N=130,172)

Characteristics	**Cohorts**	
	n	**%**
	130,172	100
Maternal Age at delivery (years) *	27.10±5.74	
<20	12,721	9.77
20-24	33,018	25.36
25-29	39,195	30.11
30-34	32,122	24.68
≥35	13,116	10.08
Gestational age at delivery*		
<28	158	0.12
28-31	1,024	0.79
32-36	8,277	6.36
37-41	119,026	91.44
≥42	1,687	1.30
Birth Year*		
2000-2004	12,441	9.56
2005-2008	30,892	23.73
2009-2012	33,972	26.10
2013-2016	52,867	40.61
Race		
Caucasian	88,652	68.10

Black	19,900	15.29
Hispanic	5,078	3.90
Asian/Pacific Islander	3,856	2.96
Native American	1,105	0.85
Unknown	11,581	8.90
Marital Status		
Single	46,296	35.57
Widowed/Divorced	2,797	2.15
Married/Life Partner	56,347	43.29
Unknown	24,732	19.00
Health Insurance		
Commercial	54,979	42.24
Self-insured	2,540	1.95
Medicaid	32,083	24.65
Government (Others)	1,734	1.33
Unknown	38,836	29.83
Preexisting health conditions		
Obesity	8,402	6.45
Hypertension	2,241	1.72
Polycystic Ovary Syndrome	917	0.70
Mental health conditions		
Anxiety	4,147	3.19
Bipolar disorder	1,353	1.04
Depression	5,579	4.29
Schizophrenia	160	0.12
Substance use		
Tobacco smoking	13,441	10.33
Alcohol use	541	0.42
Drug use	4,631	3.56
Census Region		
Midwest	29,154	22.40
Northeast	67,513	51.86
South	17,269	13.27
West	16,236	12.47
Hospital's urbanization		
Urban	117,659	90.39
Rural	12,513	9.61
Hospital Bed-size		
<100	31,719	24.37
100-199	11,750	9.03
200-299	40,223	30.90
300-499	27,781	21.34
500+	18,699	14.36
C&C Criteria		
Normal GCT	109,248	83.93

Normal OGTT(abnormal GCT)	11,339	8.71
One abnormal value		
Abnormal Fasting	875	0.67
Normal Fasting	3,130	2.40
Two abnormal values & above		
Abnormal Fasting	2,332	1.79
Normal Fasting	3,248	2.50
NDDG Criteria		
Normal GCT	109,248	83.93
Normal OGTT(abnormal GCT)	14,275	10.97
One abnormal value		
Abnormal Fasting	247	0.19
Normal Fasting	2,984	2.29
Two abnormal values & above		
Abnormal Fasting	752	0.58
Normal Fasting	2,666	2.05

*Matching variables

Table 5.2 Characteristics of cases and matched controls (N=8,340)

Characteristics	Cases		Controls		P value
	n	%	n	%	
	1,390		6,950		
Maternal age at delivery* (years)	27.93±5.92		27.93±5.92		0.9901
<20	118	8.49	592	8.52	1.0000
20-24	308	22.16	1,538	22.13	
25-29	400	28.78	2,000	28.78	
30-34	369	26.55	1,845	26.55	
≥42	195	14.03	975	14.03	
Gestational age at delivery*(week)					0.9029
<28	0	0.00	5	0.07	
28-31	21	1.51	101	1.45	
32-36	115	8.27	573	8.24	
37-41	1236	88.92	6184	88.98	
≥42	18	1.29	87	1.25	
Birth year*					0.9998
2000-2004	222	15.97	1112	16.00	
2005-2008	441	31.73	2205	31.73	
2009-2012	415	29.86	2080	29.93	
2013-2016	312	22.45	1553	22.35	
Race					<.0001
Caucasian	784	56.40	4768	68.60	
Black	310	22.30	928	13.35	
Hispanic	97	6.98	371	5.34	
Asian/Pacific Islander	45	3.24	228	3.28	
Native American	10	0.72	35	0.50	
Unknown	144	10.36	620	8.92	

Marital status					<.0001
Single	558	40.14	1946	28.00	
Widowed/Divorced	50	3.60	163	2.35	
Married/Life Partner	537	38.63	2906	41.81	
Unknown	245	17.63	1935	27.84	
Health insurance					<.0001
Commercial	384	27.63	2920	42.01	
Self-insured	21	1.51	120	1.73	
Medicaid	388	27.91	1759	25.31	
Government (Others)	26	1.87	73	1.05	
Unknown	571	41.08	2078	29.90	
Preexisting health conditions					
Obesity	257	18.49	345	4.96	<.0001
Hypertension	103	7.41	128	1.84	<.0001
Polycystic Ovary Syndrome	32	2.30	39	0.56	<.0001
Mental health conditions					
Anxiety	61	4.39	164	2.36	<.0001
Bipolar disorder	31	2.23	48	0.69	<.0001
Depression	99	7.12	265	3.81	<.0001
Schizophrenia	7	0.50	5	0.07	<.0001
Substance use					
Tobacco smoking	186	13.38	550	7.91	<.0001
Alcohol use	7	0.50	28	0.40	0.5959
Drug use	54	3.88	155	2.23	0.0003
Census region					
Midwest	290	20.86	1136	16.35	<.0001
Northeast	702	50.50	4218	60.69	
South	207	14.89	817	11.76	
West	191	13.74	779	11.21	
Hospital's urbanization					0.7338
Urban	1252	90.07	6239	89.77	
Rural	138	9.93	711	10.23	
Hospital bed-size					<.0001
<100	222	15.97	1757	25.28	
100-199	72	5.18	445	6.40	
200-299	587	42.23	2563	36.88	
300-499	295	21.22	1156	16.63	
500+	214	15.40	1029	14.81	

*Matching variables

Conditional logistic regression was performed on the matched T2DM cases and controls in the primary analyses, adjusting for all covariates except the matching variables, marital status, mental health conditions other than depression, or substance use other than smoking. The study results were presented separately for the NDDG criteria and the C&C criteria (Tables 5.3, 5.4).

Table 5.3. Comparison of risks of T2DM across different glucose level based on the number of abnormal values by C&C criteria and NDDG criteria.

Maternal Outcomes	Cases		Controls		Base OR (95% CI)	Adjusted OR (95%CI)
	n	%	n	%		
C&C criteria						
Normal GCT	804	57.84	5,776	83.11	Reference	Reference
Normal OGTT(abnormal GCT)	87	6.26	609	8.76	1.11 [0.87, 1.41]	1.21 [0.94, 1.55]
One abnormal value	77	5.54	248	3.57	2.44 [1.85, 3.20]	2.62 [1.95, 3.51]
Two abnormal values & above	422	30.36	317	4.56	10.44 [8.73,12.48]	11.69 [9.61, 14.23]
NDDG criteria						
Normal GCT	804	57.84	5,776	83.11	Reference	Reference
Normal OGTT(abnormal GCT)	87	6.26	609	8.76	1.52 [1.25, 1.84]	1.66 [1.35, 2.04]
One abnormal value	77	5.54	248	3.57	5.28 [4.11, 6.79]	5.69 [4.34, 7.47]
Two abnormal values & above	422	30.36	317	4.56	11.87[9.66,14.60]	13.20 [10.52, 16.57]

Table 5.4. Comparison of risks of T2DM on a combination of number of abnormal values and fasting glucose status by C&C criteria and NDDG criteria

Maternal Outcomes	Cases		Controls		Base OR (95% CI)	Adjusted OR (95%CI)
	n	%	n	%		
C&C criteria						
Normal GCT	804	57.84	5,776	83.11	Reference	Reference
Normal OGTT(abnormal GCT)	87	6.26	609	8.76	1.11 [0.87, 1.41]	1.21 [0.94, 1.57)
One abnormal value						
Abnormal Fasting	21	1.51	59	0.85	2.85 [1.70, 4.79]	2.94 [1.67, 5.19]
Normal Fasting	56	4.03	189	2.72	2.32 [1.69, 3.18]	2.51 [1.80, 3.52]
Two abnormal values & above						
Abnormal Fasting	263	18.92	116	1.67	18.23 [14.15, 23.49]	19.45 [14.78, 25.58]

Normal Fasting	159	11.44	201	2.89	6.16 [4.88, 7.78]	7.30 [5.67, 9.40]
NDDG criteria						
Normal GCT	804	57.84	5,776	83.11	Reference	Reference
Normal OGTT(abnormal GCT)	155	11.15	790	11.37	1.52 [1.26, 1.85]	1.67 [1.36, 2.05)
One abnormal value						
Abnormal Fasting	12	0.86	18	0.26	5.03 [2.34, 10.81]	4.68 [2.05, 10.70]
Normal Fasting	114	8.20	172	2.47	5.22 [4.02, 6.78]	5.70 [4.30, 7.57]
Two abnormal values & above						
Abnormal Fasting	130	9.35	37	0.53	23.95 [16.36, 35.06]	24.56 [16.24, 37.15]
Normal Fasting	175	12.59	157	2.26	8.61 [6.76, 10.96]	10.05 [7.72, 13.07]

C&C criteria For the association between the number of abnormal values on the OGTT by C&C criteria and the risk of developing T2DM, the aOR among pregnant women with at least two abnormal values was 11.69 (95% CI, 9.61, 14.23) compared to women with normal GCT. For those with one abnormal value, the aOR was 2.62 (95% CI, 1.95, 3.51), and for women with normal OGTT but abnormal GCT, the aOR was 1.21 (95% CI, 0.94, 1.55). When stratifying by fasting glucose status, the risk of T2DM was slightly higher among women with isolated abnormal fasting value compared to those whose single abnormal value but with normal fasting: aORs were 2.94 (95% CI, 1.67, 5.19) and 2.51 (95% CI, 1.80, 3.52), respectively. Among women with at least two abnormal values, those with abnormal fasting had a significantly higher risk of T2DM (aOR:19.45, 95% CI: 14.78, 25.58) compared to those with normal fasting (aOR:7.30, 95% CI: 5.67, 9.40).

NDDG criteria Compared to women with normal GCT, aORs for the risk of developing T2DM among women with normal OGTT but abnormal GCT, one abnormal value, and two or more abnormal values on OGTT diagnosed by NDDG criteria were 1.66 (95% CI, 1.35, 2.04), 5.69 (95% CI, 4.34, 7.47), and 13.20 (95% CI, 10.52, 16.57), respectively. Among women with at least one abnormal value, the risk of T2DM among those with abnormal fasting was comparable

between those with abnormal fasting glucose (aOR:4.68, 95% CI, 2.05, 10.70), and those with normal fasting glucose(aOR:5.70, 95% CI, 4.30, 7.57). However, among women with two or more abnormal values, the risk of T2DM was significantly higher in those with abnormal fasting glucose (aOR:24.56, 95% CI, 16.24, 37.15) compared to those with normal fasting glucose (aOR:10.05, 95% CI, 7.72, 13.07).

5.4 Discussion

Our nested case-control study within a large pregnancy cohort found that women with one abnormal value on OGTT had a significantly elevated risk of developing T2DM compared to those with normal GCT. Specifically, the risk was approximately sixfold higher under the NDDG criteria and threefold higher under the C&C criteria. Among women with two or more abnormal values, regardless of the diagnostic criteria used, the risk of T2DM was more than tenfold higher compared to women with normal GCT. For women with one abnormal value on OGTT, the risk of T2DM was similar between those with abnormal fasting glucose and those with normal fasting glucose, irrespective of the criteria used. However, among women with two or more abnormal values, those with abnormal fasting glucose had more than twice the risk of T2DM compared to those with normal fasting glucose, under both diagnostic criteria. Additionally, women with normal OGTT but an abnormal GCT also had an increased risk of T2DM compared to those with normal GCT.

These findings are generally comparable with previous studies. According to a meta-analysis of eight studies, the pooled OR for developing diabetes or prediabetes among women with one abnormal value on the 100-g OGTT, compared to women with normal glucose levels, was 4.44 (95% CI, 2.71, 7.28). Sensitivity analyses revealed that this estimate remained consistent when

stratified by diagnostic criteria (NDDG vs. C&C). A cohort study in Israel conducted between 2001 and 2011 involving 787 pregnant women reported an adjusted hazard ratio (aHR) of 5.5 (95% CI, 2.6, 11.6) for T2DM among women with one abnormal value based on the NDDG criteria,[38] aligning closely with our findings. However, exceptions did occur. In a more recent study from Israel with large sample (n = 153,125; 2001–2020) found that women with one abnormal value on OGTT diagnosed by the C&C criteria had a markedly higher risk, with an aHR of 9.11 (95% CI, 7.64, 10.86) compared to women with normal GCT.[8] This recent Israeli study also reported that women with isolated fasting hyperglycemia had a nearly 16-fold increased risk compared to women with normal GCT, while those with isolated post-load glucose abnormalities had an eightfold increased risk. However, our study observed only a modest difference in T2DM risk between women with isolated abnormal fasting glucose and those with isolated abnormal post-load glucose. A key factor contributing to these differing results may be differences in screening strategies. Universal screening was implemented in Israel starting in 2010, achieving adherence rates over 90%.[39] In contrast, our study was conducted primarily in a high-risk screening context, with only 23% of pregnant women undergoing GCT or OGTT. This discrepancy in baseline screening practices likely led to lower baseline risk in the Israeli cohort, resulting in higher relative risks among those identified with glucose abnormalities—regardless of diagnostic criteria or fasting status.[8]

In terms of the risk of T2DM among women with at least two abnormal values on OGTT, the findings of our study are also consistent with those of previous studies. We found that women with at least two abnormal values diagnosed using the C&C criteria had approximately 12 times higher risk of developing T2DM compared to women with normal GCT. Similarly, women with at least two abnormal values under the NDDG criteria had about a 13-fold increased risk. An

Israeli study reported an adjusted aOR of 11.38 (95% CI, 6.89, 18.97) for the risk of T2DM among women with at least two abnormal values by the C&C criteria, compared to those with normal OGTT.[40] Additionally, a United States study found that such women had a higher risk of diabetes compared to those with normal GCT, with an aHR of 8.26 (95% CI, 6.49, 10.51).[41] Similar study results have also been reported in studies from Canada, Italy, and France,[42-44] although effect estimates varied somewhat, likely due to differences in GDM screening practices during the study periods. For instance, a study by Bardugo et al in Israel found an aHR of 24.84 (95% CI, 21.78, 28.34) for T2DM in women with at least two abnormal values on OGTT, remarkably higher than other studies including our study.[8] Again, the Israel study was conducted during the period of universal screening in that country, with lower baseline risk profile in the overall study population, resulting in higher relative risks among those identified with glucose abnormalities.[8]

Our study results would support the inclusion of women with one abnormal value as having GDM, especially for those identified using the NDDG. Furthermore, among women with at least two abnormal values on the OGTT, those with abnormal fasting glucose demonstrated more than twice the risk of developing T2DM compared to those with normal fasting values. This suggests that women with abnormal fasting glucose warrant close follow-up and early intervention to mitigate the risk of progression to T2DM. In resource-limited settings, targeted surveillance and preventive efforts should prioritize women with both abnormal fasting and abnormal post-load glucose values, as they represent the subgroup with the highest risk of T2DM following pregnancy.

There are several strengths to this study. It is the first study in the United States to quantify the risk of T2DM based both on the number of abnormal values on the OGTT and fasting glucose

status during pregnancy. In addition to rigorous confounding control through multivariable regression, the use of optimal matching further enhanced comparability between cases and controls. Specifically, matching on maternal age helped control for one of the most important risk factors for T2DM, matching on gestational age minimized potential variation in glucose values across different stages of pregnancy, while matching on birth year reduced residual confounding related to temporal changes in GDM screening practices, diagnostic criteria, and shifts in population-level risk.

Our study has some limitations. Although we made substantial efforts to control for confounding, some important risk factors—such as physical activity and dietary habits—were not available in the CRWD, which may have led to residual confounding. A 2014-2025 survey indicated that approximately 90% of obstetricians routinely screen for GDM using a two-step approach, following the Unites States Preventive Services Task Force recommendation that screening after 24 weeks of gestation offers a moderate net benefit for maternal and fetal outcomes.[45-46] Since our study included data from a period preceding the widespread adoption of this screening strategy, and most participants were already at elevated risk for diabetes, future studies using more recent United States data are needed to evaluate T2DM risk under current screening practices. In addition, the CRWD is an encounter-based database, which limits the ability to determine whether participants remained in the system throughout the full follow-up period. For those who exited earlier, the timing of exit is often unknown. To reduce the impact of this limitation, we included only women who had at least one encounter during pregnancy and one encounter after delivery, and we tightly matched cases and controls by the birth year of the index pregnancy. As demonstrated in prior research, substantial loss to follow-up in longitudinal studies—if non-differential between comparison groups—mainly affects statistical power rather

than the validity of point estimates for associations.[47]

5.5 Conclusion and Implications

Women with one abnormal value on the 100-g OGTT, particularly according to the NDDG criteria, had a significantly higher risk of developing T2DM compared to women with normal glucose value, although the magnitude of the association was not as strong as women with at least two abnormal values on 100-g OGTT. It may be prudent to put a "flag" of GDM on women with only one abnormal value on OGTT so that intensified lifestyle changes and regular glucose monitoring can be implemented to mitigate their risk of T2DM among them.

5.6 References

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5.7 Supplements

Supplement 1

Supplement 1.1 Algorithm used to identify live birth

The algorithm used to identify live birth was based on Moll et all'(2021) and Matcho et all's (2018) study. Since the transition from International Classification of Diseases, Ninth Revision (ICD-9) coding system to International Classification of Diseases, Tenth Revision (ICD-10) in 2015, our study period (2000-2016) therefore covers both International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) for diagnosis and procedures and International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM) for diagnosis and International Classification of Diseases, Tenth Revision, Procedure Coding System(ICD-10-PCS). The algorithm based on the ICD-10 coding system has been validated by Moll K's research team. The algorithm based on ICD-9 coding system has been validated by Matcho A's research team. Using the algorithm regarding ICD-10 coding from Moll's research team to identify live birth have 100% agreement with those determined through obstetrician-gynaecologist adjudication. The algorithm based on the ICD-9 coding system for classifying the pregnancy outcomes has been shown with 99–100% agreement across different databases.

A combination of diagnosis code(ICD-9-CM, ICD-10-CM, and Diagnosis-related groups(DRG)) and procedure code(ICD-9-CM for procedures codes, ICD-10-PCS, Healthcare Common Procedure Coding System (HCPCS) level II Current Procedural Terminology, Fourth Edition (CPT-4), and lab procedure observations identified by Logical Observation Identifiers Names and Codes (LOINC) concepts were used to develop hierarchical algorithms to classify live birth from other pregnancy outcomes. Firstly, those codes will be used to identify all possible pregnancy endpoints: live births(LB), stillbirths(SB), preterm births(PRE), elective abortion(AB), spontaneous abortion(SA), ectopic pregnancy(ECT), Trophoblastic and other abnormal products of conception, and(TRO) and deliveries of unknown outcome(DELIV) from this database. Although we focused on live births, any pregnancy endpoints can be relevant to determine possible clinical pregnancy outcomes. Deliveries of unknown outcomes without other pregnancy endpoints(except live birth) before or during the delivery record will be considered as live birth. Given that multiple records might represent the same delivery, the live birth records will be assessed on the timeline of the same women. The first live birth records will be identified, and next records will be identified and determined as a valid live birth if it is more than 182 days from the first record.

Supplement 1.2 The period of the lab module of the delivery hospital was defined as the period of the earliest date of the lab records and the latest lab records in Cerner Health Facts. Deliveries would be excluded if pregnancy start date plus 168 days (24 weeks) or delivery date is beyond the period of the lab module, which will be considered as lab results unavailable.

Supplement 1.3 Diagnosis code for identifying multi fetus pregnancy

ICD type	**Codes**

ICD-9-CM	"V31", "V32", "V33", "V34", "V35", "V36", "V37"
ICD-10-CM	"Z38.3","Z38.4","Z38.5","Z38.6","Z38.7", "Z38.8", "Z37.2","Z37.3","Z37.4","Z37.5","Z37.6","Z37.7"

Supplement 1.4 Algorithms for identifying diabetes.

Type	**Codes**
ICD-9-CM	"250","249","648.0","V58.67"
ICD-10-CM	"E10","E11","E08","E09","E13", "O24.0","O24.1","O24.3","O24.8","O99.81","Z79.4"
Medication	"insulin", "insulan", "pramlintide", "glimepiride", "glipizide", "pioglitazone", "hydrochloride", "glyburide", "acetohexamide", "chlorpropamide", "tolazamide", "tolbutamide", "rosiglitazone", "canagliflozin", "metformin hci", "dapagliflozin", "acarbose", "miglitol", "empagliflozin", "linagliptin", "ertugliflozin", "alogliptin", "saxagliptin", "sitagliptin", "simvastatin", "exenatide", "liraglutide", "albiglutide", "dulaglutide", "semaglutide", "nateglinide", "repaglinide"

Supplement 1.5 Methods to identify glucose measures

A combination of loinc codes, lab procedure name, lab procedure id is used to identify glucose measures; first, among these without Loinc codes mapping, below are these Loinc codes used to identify different glucose measures; lab procedure id and lab procedure name were used to identify these glucose which is not specified. Lab procedure ID and lab procedure name were used to identify glucose for these without Loinc code mapping or unspecific.

Fasting glucose in plasma	'10450-5' '12651-6' '14768-6' '14769-4' '14770-2' '14771-0' '1493-6' '14996-3' '1547-9' '1548-7' '1549-5' '1550-3' '1552-9' '1554-5' '1556-0' '1557-8' '1558-6' '17865-7' '25680-0' '35184-1' '39997-2' '39998-0' '39999-8' '40000-2' '40148-9' '40149-7' '40150-5' '40151-3' '40152-1' '40193-5' '40194-3' '40195-0' '40196-8' '40197-6' '40261-0' '40262-8' '40318-8' '41604-0' '47622-6' '48109-3' '48983-1' '51426-5' '53049-3' '62850-3' '66179-3' '70208-4' '76629-5' '77145-1' '87421-4' '88365-2' '21004-7'

One-hour post-load after 50-g OGTT	'12652-4' '12653-2' '12654-0' '12655-7' '12656-5' '12657-3' '12658-1' '12659-9' '14754-6' '1504-0' '16914-4' '20441-2' '21308-2' '21309-0' '21310-8' '25665-1' '25668-5' '25674-3' '25679-2' '26695-7' '26817-7' '26853-2' '26854-0' '27432-4' '29329-0' '29330-8' '29331-6' '29332-4' '29412-4' '40001-0' '40002-8' '40003-6' '40004-4' '40005-1' '40006-9' '40007-7' '40008-5' '40009-3' '40010-1' '40011-9' '40012-7' '40013-5' '40014-3' '40015-0' '40016-8' '40017-6' '40018-4' '40019-2' '40020-0' '40167-9' '40168-7' '40169-5' '40170-3' '40171-1' '40172-9' '40173-7' '40174-5' '40175-2' '40176-0' '40177-8' '40178-6' '40179-4' '40180-2' '40181-0' '40182-8' '40183-6' '40184-4' '40021-8' '40022-6' '40023-4' '40024-2' '40025-9' '40026-7' '40027-5' '40028-3' '40029-1' '40185-1' '40186-9' '40187-7' '40188-5' '40189-3' '40190-1' '40191-9' '40192-7' '40198-4' '40199-2' '40200-8' '40201-6' '40202-4' '40203-2' '40204-0' '40205-7' '40206-5' '40207-3' '40208-1' '40209-9' '40210-7' '40211-5' '40212-3' '40213-1' '40214-9' '40215-6' '40216-4' '40030-9' '40031-7' '40032-5' '40033-3' '40034-1' '40035-8' '40036-6' '40037-4' '40038-2' '40039-0' '40040-8' '40041-6' '40042-4' '40043-2' '40044-0' '40045-7' '40153-9' '40154-7' '40155-4' '40156-2' '40157-0' '40158-8' '40159-6' '40160-4' '40161-2' '40162-0' '40163-8' '40164-6' '40165-3' '40166-1' '40217-2' '40218-0' '40219-8' '40220-6' '40221-4' '40222-2' '40259-4' '40260-2' '40263-6' '41024-1' '48810-6' '50583-4' '53093-1' '54392-6' '54393-4' '54394-2' '54395-9' '54396-7' '54397-5' '54398-3' '54399-1' '54496-5' '54497-3' '54498-1' '54499-9' '56751-1' '57350-1' '59791-4' '59792-2' '59793-0' '59794-8' '59795-5' '12621-9' '12622-7' '12623-5' '12624-3' '12625-0' '12626-8' '12627-6' '12639-1' '12640-9' '59796-3' '59797-1' '77681-5' '12615-1' '12616-9' '12617-7' '12618-5' '12619-3' '12620-1' '12641-7' '12642-5' '12643-3' '12644-1' '12645-8' '12646-6' '12647-4'
One-hour post-load (100-g OGTT)	'11142-7' '11143-5' '14753-8' '1501-6' '1502-4' '1525-5' '1501-6' '14753-8'
Two-hour post-load (100-g OGTT)	'14757-9' '1494-4' '1514-9' '1515-6'
Three-hour post-load (100-g OGTT)	'14764-5' '1531-3' '1537-0' '1540-4' '1544-6' '1545-3' '9375-7' '9376-5' '9377-3' '9378-1' '1530-5'
One-hour post-load (75-g OGTT)	'1507-3' '1508-1' '1527-1' '32319-6' '51597-3' '55352-9'
Two-hour post-load (75-g	'14137-4' '1496-9' '14995-5' '1518-0' '1519-8' '1533-9' '1539-6' '1542-0'

OGTT)	'32320-4' '32321-2"32322-0' '55351-1' '55381-8' '6749-6' '6752-0' '6756-1'
Unspecified post-load OGTT	'49689-3' () '49688-5' (75-g OGTT post-load) One-hour post-load(Test unspecific) '10449-7' '14756-1' '14763-7' '1498-5' '1499-3' '1500-8' '1512-3' '1513-1' '1522-2' '1523-0' '1524-8' '1534-7' '1535-4' '1543-8' '19104-9' '20438-8' '20439-6' '25663-6' '25671-9' '25673-5' '26539-7' '30263-8' '30264-6' '30265-3' '32359-2' '40285-9' '40287-5' '40319-6' '40320-4' '40321-2' '40322-0' '47859-4' '48984-9' '48985-6' '54249-8' '54251-4' '95078-2' '95105-3' Two-hour post-load(Test unspecific):'12610-2' '14752-0' '14759-5' '14760-3' '14761-1' '1521-4' '20436-2' '20440-4' '24353-5' '25667-7' '30266-1' '30267-9' '40286-7' '40323-8' '43278-1' '44919-9' '49134-0' '6689-4' '72171-2' '77677-3' '80959-0' '81324-6' '1492-8' Three-hour post-load (Test unspecific):'12611-0' '12614-4' '14765-2' '14766-0' '14767-8' '1530-5' '18342-6' '19105-6' '20437-0' '25666-9' '25669-3' '25672-7' '25676-8' '25677-6' '26541-3' '26543-9' '26544-7' '26554-6' '26555-3' '30251-3' '30252-1' '30253-9' '40324-6' '50586-7' '50587-5' '50588-3' '50589-1' '50608-9' '95103-8' '95104-6' '95106-1' '95107-9' '95108-7' '95109-5'
Urine glucose	'2349-9' '35212-0' '53328-1' '22705-8' '50555-2' '5792-7' '63382-6' '25428-4'
Capillary glucose	'32016-8"41653-7' '51596-5' '40858-3' '59813-6' '1556-0'

Chapter 6

Discussion

This thesis aimed to clarify the association between varying degrees of hyperglycemia during pregnancy and two major adverse outcomes—stillbirth and T2DM—both of which have been prominently associated with gestational glucose intolerance. The evidence of mild hyperglycemia and these outcomes has not been well established. My thesis work attempts to fill this gap. I hypothesized that 1) GDM is associated with an increased risk of stillbirth when appropriate methodologies are applied; 2) borderline maternal hyperglycemia that does not meet current diagnostic criteria for GDM (i.e., one abnormal value on OGTT in some jurisdictions) is associated with elevated risks of stillbirth and (especially) diabetes, although the associations are not as strong a definitive diagnosis of GDM (i.e., two or more abnormal values on OGTT). This thesis, including both systematic reviews and analyses of large population health data, had largely confirmed these hypotheses.

6.1 Summary of Research Findings

The systematic review of the association between maternal hyperglycemia and stillbirth found that women with at least two abnormal values on 100-g OGTT by the C&C criteria (i.e., a definitive diagnosis of GDM) had about four times higher risk of stillbirth compared to women with normal GCT. Among women with one abnormal value or women with normal OGTT but abnormal GCT (i.e., borderline hyperglycemia), the risk of stillbirth was slightly higher compared to women with normal GCT, although not statistically significant. The risk of stillbirth was also slightly higher among women with at least one abnormal value diagnosed by the IADPSG criteria compared to women with normal OGTT, but with high probability due to chance. To avoid immortal time bias, our systematic review included only studies in which

women with GCT or OGTT measured during pregnancy, so that both the exposed and non-exposed groups survived until the time of glucose measurement (24 to 28 weeks of gestation). As a result, the sample size for the systematic review was limited. For example, only 48 events were reported across all studies included in the meta-analysis comparing women with at least two abnormal values to those with normal GCT under C&C criteria, leading to some instability in the effect estimate. Therefore, a retrospective cohort study was conducted by using a large electronic health record (EHR) database from the United States to confirm this finding. We found that women with GDM (defined by at least two abnormal values by the C&C criteria or NDDG criteria) had more than three times the risk of late stillbirth compared to women without GDM. This large retrospective cohort study, involving nearly one million pregnant women, partly confirmed the finding of the meta-analysis. Furthermore, women with both early GDM (before 24 weeks) and late GDM (after 24 weeks) had a similar risk of late stillbirth compared to women without GDM.

For the association between maternal hyperglycemia and T2DM, our systematic review found that women with one abnormal value on the 100-g OGTT (i.e., borderline hyperglycemia without a definitive diagnosis of GDM) had about four times greater risk of diabetes or pre-diabetes. Similar findings were observed when stratifying by the C&C and the NDDG criteria. Among women with at least two abnormal values on the 100-g OGTT (i.e., a definitive diagnosis of GDM), the risk of developing diabetes or pre-diabetes was approximately twelve times higher compared to women with normal GCT or OGTT. Additionally, women with normal OGTT but abnormal GCT had about twice the risk of developing diabetes or prediabetes compared to those with normal GCT. One large retrospective cohort study from Israel using the C&C criteria assessed the risk of T2DM based on the number and type of abnormal values on the 100-g

OGTT. Among women with the same number of abnormal values on OGTT, those with abnormal fasting glucose had more than twice the risk of developing T2DM compared to those with normal fasting glucose. This systematic review suggested that borderline hyperglycemia – which is not universally accepted as a diagnosis of GDM–was still associated with substantially increased future risk of diabetes, although the magnitude of this risk was not as high as that observed for GDM. Moreover, fasting glucose status during pregnancy plays a significant role in predicting the risk of T2DM. To confirm the findings from the systematic review, we conducted a nested case-control study within a pregnancy cohort derived from Cerner Real World Data™ (CRWD). This study found that women with one abnormal value on the 100-g OGTT under the C&C criteria had about three times the risk of T2DM compared to women with normal GCT; the risk of T2DM among women with one abnormal value under the NDDG criteria was about six times higher than that in women with normal GCT. Among women with at least two abnormal values on the OGTT, the risk of T2DM was about twelve times higher under the C&C criteria and approximately thirteen times higher under the NDDG criteria, compared to women with normal GCT. These findings were consistent with the results of the systematic review. Our nested case-control study on the association between borderline hyperglycemia and T2DM was one of the largest conducted to date, providing both stable estimates and valuable additional evidence on this association. Among women with one abnormal value on the 100-g OGTT in our case-control study, the risk of T2DM did not differ substantially between those with abnormal fasting glucose and those with normal fasting glucose, under either the NDDG or C&C criteria. However, among women with at least two abnormal values under either criteria, those with abnormal fasting glucose had approximately three times the risk of developing T2DM compared to those with normal fasting glucose. The magnitude of the increased risk of T2DM associated

with the number of abnormal values on the OGTT, whether fasting glucose status was considered, was smaller than that reported in the Israeli study.[27] One key factor is the GDM screening strategy: Israel implemented universal screening in 2009, covering most of the study period, while in the United States, universal screening was only recommended in 2014—just two years before the end of follow-up in our cohort.[14,18] Differences in GDM screening strategies lead to substantial variations in the baseline risk of T2DM between study populations. Under Israel's universal screening policy, the baseline risk would be lower than that under a high-risk screening policy as it includes many otherwise young and healthy women, leading to an artificially increased estimated relative risk (or OR) of diabetes in those with abnormal values on OGTT. For example, assuming a fixed risk of 10% in the exposed group, a study conducted in a high-risk population with a baseline risk of 5% would yield a relative risk (or OR) of 2, whereas in a low-risk population with a baseline risk of 2%, the relative risk (or OR) would increase to 5. This example highlights the importance of considering the background risk profile of the study population when interpreting and comparing results across studies. Differences in race and ethnicity between the Israeli study and ours may partly explain the discrepancies in findings. Prior research has shown significant variation in the GDM–T2DM association by race and ethnicity; for example, Asian women with GDM have been found to be at higher risk of developing T2DM compared to Caucasian women.[29]

6.2. Strengths and limitations

There are several strengths in this thesis. Regarding the association between GDM and stillbirth, a recent systematic review did not find an increased risk of stillbirth among women with GDM compared to those without. However, that review exhibited a high degree of heterogeneity (I^2 > 80%) and included studies with substantial biases, notably immortal time bias.[30-32] Our

systematic review found that women with at least two abnormal values on OGTT by the C&C criteria (i.e., a definitive diagnosis of GDM) had over three times higher risk of stillbirth compared to women with normal GCT, in the absence of heterogeneity across studies (I^2=0). Combining women diagnosed with GDM by different criteria resulted in a high degree of heterogeneity among studies, raising concerns about the appropriateness of the meta-analysis conducted in the previous review. More importantly, some studies in the previous systematic review introduced significant immortal time bias because the exposed group was needed to survive until glucose measurement for GDM screening at 24–28 weeks of gestation, whereas the unscreened non-exposed group did not need to survive until that time. As a result, early stillbirths (20–28 weeks) were much more likely to occur in the non-exposed group, leading to biased effect estimates. Our systematic review addressed this issue by including only studies in which GCT or OGTT measured during pregnancy to ascertain exposure status. Under this scenario, both the exposed and non-exposed groups needed to survive until glucose measurement at 24 to 28 weeks of gestation. As a result, our pooled effect measures are unlikely to be affected by immortal time bias. Although our review had a much smaller sample size due to the exclusion of studies relying solely on clinical diagnosis of GDM to ascertain exposure status, the pooled OR is expected to provide an unbiased estimate of the true association between maternal hyperglycemia and risk of stillbirth. Importantly, the findings from the systematic review were replicated in our large retrospective cohort study. The samples size of our cohort study was particularly large (about one million pregnancies), exceeding the total number of participants across all studies in our systematic review. In this cohort study, eligibility criteria were carefully defined by excluding pregnancies that ended before 28 weeks of gestation and including only women diagnosed with GDM between 24 and 28 weeks of gestation, thereby minimizing the

immortal time bias. Additionally, the ability to evaluate the association of early versus late GDM with stillbirth within the same population provided valuable insights into the potential mechanisms underlying these associations.

For the association between hyperglycemia during pregnancy and future risk of diabetes, our systematic review is the first to quantify the risk of diabetes among women with one abnormal value on 100-g OGTT and make comparison with the risk among women with at least two abnormal values on OGTT, the universally accepted criterion for diagnosing GDM. Importantly, we used women with normal GCT or normal OGTT as the reference group, representing a truly normoglycemic population. In previous systematic reviews, the non-GDM reference group often included women with one abnormal value on the 100-g OGTT.[33-34] As a result, the risk of diabetes among women with a history of GDM may have been underestimated, since the control group included individuals with elevated glucose levels who did not meet the diagnostic criteria for GDM. Our systematic review is also the first to quantify the risk of diabetes among women with normal 100-g OGTT, but abnormal GCT as compared to those with normal GCT. The findings of our systematic review were confirmed by our nested case-control study, which was based on a cohort of 130,172 pregnant women. We found that women with one abnormal value diagnosed by the C&C criteria had about three times higher risk of T2DM compared to women with normal GCT, and women with one abnormal value diagnosed by the NDDG criteria had about six times the risk of T2DM compared to women with normal GCT. This is the first study in the United States to quantify the risk of T2DM based on both the number of abnormal values and fasting glucose status during pregnancy. In addition to rigorous adjustment for confounders using logistic regression analysis, tight matching further balanced the distribution of these factors between cases and controls. There are some discrepancies between our nested case-control study

and the Israel study.[27]

Limitations of this thesis should be acknowledged. The systematic review for stillbirth had much smaller sample size than the previous review as we applied strict exclusion criteria requiring the included study population to have glucose screening/diagnostic tests during pregnancy, and not solely a clinical diagnosis of GDM, to avoid immortal time bias. Another limitation of the systematic review was that none of the included studies assessed the association of maternal hyperglycaemia with risk of stillbirth based on both number and type of abnormal values on OGTT. As a result, our analysis could only quantify the risk of stillbirth according to the number of abnormal values, without accounting for the specific types (e.g., fasting vs. post-load glucose) of abnormal values. For the cohort study on stillbirth, some early GDM cases may have been misclassified as late GDM as some high-risk women who should have been undergone diabetes screening during the first trimester may have experienced delayed testing into the second trimester. Although we applied validated algorithms to differentiate pre-gestational diabetes from GDM, there could still be potential erroneous records in the administrative health datasets used in this study. As a result, some cases identified as early GDM may have actually represented undiagnosed pre-existing diabetes. To minimize this misclassification, we utilized all available clinical data from 180 days prior to conception through 90 days post-conception for the index pregnancy to ascertain pre-existing diabetes. To ensure comparability between early and late GDM and to avoid immortal time bias in the cohort study, we focused on late stillbirth occurring after 28 weeks of gestation. Consequently, we were unable to assess the outcomes of early stillbirth occurring before 28 weeks for women with early GDM. Future studies should be conducted comparing women with early GDM and non-GDM regarding the risk of overall stillbirth. For the systematic review on maternal hyperglycemia and the risk of subsequent

diabetes, we were unable to restrict the primary analysis to T2DM as the outcome because about half of the included studies reported outcome of diabetes and/or pre-diabetes, without specifying whether it was T2DM. However, given most diabetes cases in the adult population are T2DM, and subgroup analysis limited to studies specifying T2DM yielded similar results, the inclusion of a 'soft' diagnosis of diabetes in some studies is not expected to alter the conclusions. Follow-up time varied substantially across included studies. While follow-up duration varied substantially across studies, subgroup analyses stratified by follow-up time also produced consistent findings, further supporting the robustness of our results. Only one study evaluated the risk of future diabetes based on the number of abnormal values on the 75-g OGTT, precluding a meta-analysis similar to that conducted for the 100-g OGTT. As few studies assessed risk of diabetes among women with normal 100-g OGTT but abnormal GCT, evidence in this subgroup population was relatively weak. Furthermore, due to the scarcity of original studies reporting time-to-event data, we were unable to estimate hazard ratios in meta-analyses.

For the nested case-control study examining the association between hyperglycemia during pregnancy and T2DM, some important risk factors such as physical activity and dietary habits were not available in the CRWD, so that residual confounding may exist. A 2014-2025 survey revealed that 90% of obstetricians reported routinely screening for GDM using a 2-step approach, following the recommendation from the United States Preventive Services Task Force to screen all pregnant women for GDM at 24 weeks of gestation to improve maternal and fetal outcomes.[35-36] However, since the majority of our study period preceded the widespread adoption of universal screening, most participants in our cohort and nested case-control studies were likely subject to high-risk screening protocols. This implies that the study population may have had a higher baseline risk for GDM and T2DM. Future studies using more recent United States

database are warranted to evaluate these associations in the context of universal GDM screening. Since the CRWD is an encounter based EHR database, it is unclear whether original cohort members remained in the system through the end of the follow-up; if some of them exited earlier, the exact timing of their exit could not be determined. To minimize the impact of uncertainty regarding cohort exit time, we included women with at least one encounter during pregnancy and at least one encounter after delivery, and tightly matched the cases and controls by year of birth of the index pregnancy. On the other hand, as demonstrated in previous studies, large losses to follow-up for longitudinal associations, if occurring randomly among the comparison groups, affect mostly statistical power, with limited impact on point estimates of the magnitude of the association.[37]

6.3 Conclusions and Implications

This thesis work found that women with borderline hyperglycemia during pregnancy that did not meet current diagnostic criteria for GDM in some countries/jurisdictions (e.g., one abnormal value on OGTT) may still be at increased risk of stillbirth and, more notably, T2DM, although the association was not strong as with universally accepted diagnosis ((e.g., two or more abnormal values on OGTT) of GDM. Although immediate medical intervention may not be necessary, it is important to consider developing guidelines that encourage lifestyle modifications to mitigate the potential risk of adverse outcomes in women with borderline hyperglycemia during pregnancy. For women with elevated fasting glucose values, intensified glucose management may be warranted, as they appear to be at higher risk of long-term outcomes compared to women with only abnormal values on post-load OGTT. Large scale studies assessing the association of both the number of abnormal values on OGTT and fasting glucose status with adverse short- and long-term outcome should be conducted in the future.

Since universal screening policies are in place in most countries, prenatal care offers a unique opportunity for glucose screening in young adult women at their first point of contact with the healthcare system. As a result, pregnancy is an excellent time to identify women at increased risk of adverse short- and long-term outcomes such as stillbirth, T2DM, and cardiovascular diseases CVDs, thereby allowing for targeted interventions to mitigate those risks in affected women. This thesis focused on stillbirth and T2DM, two of the most prominent adverse outcomes associated with hyperglycemia during pregnancy. As a next step, I propose to conduct a retrospective cohort study using large-scale population health databases in the province of Ontario to assess the association between maternal hyperglycemia and CVDs in women.

6.4 Reference

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Chapter 7

Future directions

My PhD thesis work focused on stillbirth and T2DM, two of the most prominent adverse outcomes associated with hyperglycemia during pregnancy. Another important adverse outcome that has been related to maternal hyperglycemia is CVD. I have proposed to conduct a retrospective cohort study using large population health databases in Canadian province of Ontario to assess the association between maternal hyperglycemia and CVDs in women. Chapter 7 included two documents: a protocol titled “Maternal glucose level and future risk of developing cardiovascular diseases: a systematic review and meta-analysis protocol” which was published by BMJ Open in 2023 (Zeng N, Wen W, Corsi DJ, Li W, Kibret T, Wen SW. Maternal glucose levels and future risk of developing cardiovascular disease: a systematic review and meta-analysis protocol. BMJ Open. 2023 May 2;13(5):e069251. doi: 10.1136/bmjopen-2022-069251. PMID: 37130662; PMCID: PMC10163547.) and a postdoctoral fellowship application that has been submitted to Heart and Stroke foundation in September 2024.

7.1 Protocol for a systematic review

Maternal glucose level and future risk of developing cardiovascular diseases: a systematic review and meta-analysis protocol

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Abstract

Introduction Hyperglycemia during pregnancy has been considered one of the risk factors for cardiovascular diseases(CVD) for women. Although the evidence regarding the association between gestational diabetes mellitus(GDM) and subsequent cardiovascular disease has been synthesised, there is a lack of systematic review covering the evidence of the association among the non-GDM population. This systematic review and meta-analysis therefore aims to summarise existing evidence on the association between maternal glucose level and the risk of future CVD in pregnant women with or without a diagnosis of GDM.

Methods and analysis This systematic review protocol was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P) guidelines. Comprehensive literature searches were performed in the following electronic database: MEDLINE, EMBASE and CINAHL to identify relevant papers from inception to December 31, 2022. All observational studies (case-control studies, cohort studies, cross-sectional studies) will be included. Two reviewers will perform the abstract and full-text screening based on the eligibility criteria through Covidence. The Newcastle-Ottawa Scale (NOS) will be used to assess the methodological quality of included studies. Statistical heterogeneity will be assessed by using the I^2 test and Cochrane's Q test. If the included studies are homogeneous, pooled estimates will be calculated and meta-analysis will be performed using Review Manager 5 (RevMan) software. Random effects will be used to determine weights for meta-analysis, if needed. Pre-specified subgroup analysis and sensitive analysis are needed. The study results will be reported in the sequence of main outcomes, secondary outcomes, and important subgroup analysis for each type of glucose level separately.

Ethics and dissemination Given no original data will be collected, so ethics approval is not

applicable for this review. The results of this review will be disseminated by publication and conference presentation.

PROSPERO registration number:

Strengths and limitations

- This review has no restriction to the language.
- The search strategy was developed with assistance of an experienced librarian.
- This is the first review focusing on the impact of specific maternal glucose level instead of a diagnosis of GDM on the future risk of cardiovascular disease.
- The certainty of the evidence might be influenced by the limited included high-quality studies.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death according to 2017 WHO report.[1] It is estimated that CVD had caused nearly 18 million deaths worldwide in 2019, accounting for 32% of all deaths.[1] An estimated 30% of female deaths are caused by CVDs.[2] Most CVDs can be prevented if individuals at high CVDs risk can be detected and appropriately managed in a timely manner. Thus, targeting women at increased risk for developing CVDs will be useful for early screening and prevention for CVDs incidence as well as lowering mortality rate of CVD.[3] A recent review found that women with pregnancy complications, including preterm birth, preeclampsia, gestational hypertension, still birth, and gestational diabetes etc., have higher risk of developing CVD.[4] Perinatal care might potentially provide the opportunity to identify future risk of CVD for the majority of women given that over 80% women will experience pregnancy at some point in their lifetime and hence likely undergo antepartum glucose screening.[5] Perinatal glucose screening for gestational diabetes mellitus (GDM) has been part of standard obstetrical care worldwide to identify women at increased risk of short- and long-term adverse outcomes in the context of hyperglycaemia during pregnancy to ensure treatment is given to reduce these complications. Universal screening for GDM in asymptomatic pregnant women after 24 weeks of gestation has been recommended since 2013 in the United States.[6,7] Meanwhile, the National Institutes of Health (NIH) and the American College of Obstetricians and Gynaecologists(ACOG) advocate that women with significant risk factors of GDM, such as body mass index >35 kg/m2 or maternal age ≥ 40 years, and previous macrosomia must be screened for unrecognized type 2 diabetes before 24 weeks of gestation.[8,9] Glucose screening may be an effective and efficient tool to identify women at-risk of developing CVD for prevention if there is an association between glucose level at screening with subsequent risk of CVD in the general

obstetric population.

Previous studies have shown that elevated maternal glycemia levels were associated with future risks of developing CVD. A recent systematic review and meta-analysis covering 5,390,591 women revealed that those with GDM had twice the risk of future CVD, which was apparent within the first decade after delivery.[10] However, the association between maternal hyperglycaemia and subsequent CVD risk may extend to the non-GDM range based on the finding of a few recent studies.[4,11] Although the association of borderline elevated maternal glycemia level with future risks of developing CVD in pregnant women may not be as strong as GDM, interventional targeting of this group of women may have a higher impact on the overall disease burden in the society as the size of affected women is larger.

Although the evidence regarding the association between GDM and subsequent cardiovascular disease has been synthesised, there is a lack of systematic review covering the evidence of the association among the non-GDM population. This systematic review and meta-analysis therefore aim to summarise existing evidence on the association between maternal glucose level and the risk of future CVD in pregnant women with or without a diagnosis of GDM.

Research Question

Does an elevated maternal glucose level increase women's risk of CVD regardless of having a GDM diagnosis?

Protocol

This systematic review protocol was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P) guidelines. Appendix 1 provides the details of the PRISMA-P checklist. The protocol has been registered on the PROSPERO International Prospective Register of Systematic Reviews. The date, rationale, and details of

changes for each section will be provided if protocol adjustments are required.

METHODS

Eligibility Criteria

Population This review focused on women who had a delivery history along with glucose level measured during the pregnancy. Studies included in this review were required to report results for either both the GDM and non-GDM population, or non-GDM population only**.**

Exposure Most criteria for diagnosing GDM or type 2 diabetes in clinical practice include 100-g 3-h OGTT or 75-g 2-h oral glucose tolerance test (OGTT).[12,13] This review will examine maternal glucose level measured by fasting plasma glucose (FPG), post plasma glucose (PLG) measured by the 50-gram glucose challenge test(50-g GCT), the 75-gram oral glucose tolerance test (75-g OGTT) or the 100-gram oral glucose tolerance test(100-g OGTT), and the glycated haemoglobin level (HbA1c). No specifications were made regarding the gestational age of the maternal glucose level.

Comparator For this review, the reference group referred to those women whose glucose levels were in the lowest range (as defined by each study). The index pregnancy refers to the eligible pregnancy of which the glucose measurements were included.

Outcome The primary outcome was incident of CVD following the delivery of the index pregnancy, which is a composite of heart failure, coronary heart disease (myocardial infarction, angina, myocardial ischemia, coronary artery disease, acute coronary syndrome, angina), stroke, transient ischemic attack, intracranial haemorrhage, and peripheral vascular disease. Studies that include any of the conditions above were included in this review. The secondary outcome was any CVD diagnosis.

Setting and language This review will not restrict settings, publication date and languages.

Study Design All observational studies (case-control studies, cohort studies, cross-sectional studies) will be included.

Exclusion This review will exclude case reports, case-series, unpublished abstracts, commentaries, editor letters, and reviews without original data presented. Studies without comparison of exact glucose level, but rather with a categorization of the population by a diagnosis of GDM or non-GDM will be excluded as well. Studies indexed as child only and animal studies will be excluded from this review.

Information Sources Comprehensive literature searches were performed in the following electronic database: MEDLINE, EMBASE and CINAHL to identify relevant papers. Since no restrictions were set for the date of publication, the search will be from inception to December 31, 2022. Information sources will be updated prior to submission of this review. To ensure all relevant studies were covered in this review, the information sources will be further supplemented by scanning the reference list of selected studies and systematic reviews of similar scope.

Search Strategy Our search strategy was created with the aid of a medical librarian based at the University of Ottawa who is experienced in systematic review searches. A combination of Medical Subject Headings [MeSH] and keyword terms relevant to both exposure and outcome of interest were used to develop the search strategy. No date restrictions or languages were set to the literature search. Search strategies for MEDLINE, EMBASE and CINAHL were presented in Appendixes 2 ,3 and 4 respectively.

STUDY RECORDS

Data Management

Results from the literature search are to be imported, screened, stored, and analysed by

professional software-based platforms, including Review Manager 5 (RevMan) and Covidence. Duplicates will be automatically removed by Covidence in addition to manually checking for study similarities (year of publication, author's name, volume, issue, etc.,) via authors.

Selection Process

Two reviewers (NZ, WW) will perform the abstract and full-text screening based on the eligibility criteria through Covidence. Any disagreements between the two reviewers will be resolved via a third reviewer (SWW). During the phase of full-text screening, a separate category will be created on excel to include those relevant studies through scanning the reference list of included studies and systematic reviews of the same scope. Meanwhile, the reasons for exclusion will be documented during the full-text screening. A final decision regarding the included studies could be made based on both the results from Covidence and the spreadsheet. PRISMA flow diagrams will be used to present the results from the study selection.

Data Collection

Data extraction form will be created as a standardised data collection tool. A pilot test using the form will be conducted among groups, and if necessary, the form will be modified based on the group feedback. Data obtained from included studies will be independently extracted by two reviewers (NZ, WW). A calibration exercise will be undertaken by reviewers to ensure consistent methods of assessment across reviewers. The principal investigator (SWW) will be involved in the event of any discrepancies that arise between reviewers.

Data Items

The following data items will be extracted from included studies: 1) Study data: title, author name, year of publication, country of study, journal, sample size, study period, study design, follow-up period, and limitations. 2) Population: characteristics of the participants, e.g., mean

age, social economic status, race/ethnicities, whether there was a diagnosis of GDM, whether there was a progression to type II diabetes. 3) Exposure: maternal glucose level measured using FPG, PPG from 50g-GCT or 75g-OGTT or 100g-OGTT, and HBA1C. If the maternal glucose level is categorised, then the cut-off points will be specified. 4) Comparison: reference category will be specified if the maternal glucose level is a categorical variable; 5) Outcome: composite of outcome events, start of follow-up period, length of follow-up for outcome variables; 6) Effect Measures: reported effect measures for the composite outcomes and separate outcomes if available, including p-values, standard deviation, and confidence intervals; 7) Sources of funding

Outcomes and prioritisation

The composite of cardiovascular diseases (as defined by each study) will be collected. Cardiovascular diseases include heart failure, coronary heart disease (myocardial infarction, angina, myocardial ischemia, coronary artery disease, acute coronary syndrome, angina), stroke, transient ischemic attack, intracranial haemorrhage, peripheral vascular disease as well as relevant procedures (coronary artery bypass grafting, percutaneous coronary intervention, or carotid endarterectomy etc.,). The composite of the cardiovascular diseases will be regarded as the main outcome, while each reported cardiovascular disease will be considered as the secondary outcome.

Risk of bias assessment

The Newcastle-Ottawa Scale (NOS) will be used to assess the methodological quality of included studies.[14] The major three domains (8 items) of bias to be assessed consist of selection, comparability, and ascertainment of outcome (cohort or cross-sectional studies) or exposure (case-control studies). Cohort studies and case-control studies will be assessed by NOS for cohort studies and NOS for case-control studies separately. Adapted NOS, which is adjusted

based on the NOS for cohort studies, will be used to assess the cross-sectional studies. A maximum of one star could be assigned for the numbered items under the selection as well as ascertainment of outcome or exposure domain. A maximum of two stars could be assigned for the numbered items under the section of comparability. A study with scores from 7-9, 4-6, 0-3 will be considered high quality, high risk, and high risk of bias respectively. Two reviewers (NZ, WW) will be assigned to assess the quality of each study, and a third reviewer (SWW) will be consulted whenever disagreements arise. The results of the risk of bias assessment will be presented in a table.

Data Synthesis

Patient characteristics (i.e., age, race/ethnicities, social economic status), follow-up periods for outcome, type of test to measure glucose level will be assessed. Statistical heterogeneity will be assessed by using the I^2 test and Cochrane's Q test. Based on the guidelines of Cochrane,[15] heterogeneity will be interpreted as "may not be important", "moderate heterogeneity", "substantial heterogeneity" and "considerable heterogeneity" when the I^2 is in the range of 0-40%, 30-60%, 50-90% and 75-100% respectively. If the included studies are homogeneous, pooled estimates will be calculated and meta-analysis will be performed using Review Manager 5 (RevMan) software. Random effects will be used to determine weights for meta-analysis, if needed. If heterogeneity is at a substantial level ($I^2 >= 50\%$ or $P<0.1$) or data is not present, a qualitative (narrative) synthesis or summary will be performed instead of meta-analysis. The study results will be reported in the sequence of main outcomes, secondary outcomes, and important subgroup analysis for each type of glucose level separately.

Subgroup analysis will be performed to investigate possible causes of between-study variability or to explore the robustness of meta-analysis. Considering that the gestational age of

measurement of glucose level greatly impacts the value of maternal glucose level, the study results will be stratified based on Gestational age at glucose measurement. Study results will be further stratified based on the study design to determine whether the study design influences the magnitude of association. Additionally, Study results will be stratified by age to determine whether age impacts the association between maternal glucose level and future risk of CVD. Given that the length of follow-up periods for CVD might affect the magnitude of the association between the maternal glycemia level and future risk of CVD, subgroup analysis will be conducted by stratifying the duration of follow-up periods for outcomes. Finally, since the race/ethnicities might modify the association of interest, subgroup analysis will be performed based on the race/ethnicities of most of the study population. Meta-regression might be considered to deal with potential heterogeneity if more than ten studies cover the covariates of interest. The following comparisons will be performed by each factor below if the data is available.

Stratification by gestational age of glucose measurement:

- Gestational age less than 24 weeks
- Gestational age between 24-28 weeks
- Gestational age more than 28 weeks
- Anytime during pregnancy

Stratification by study type:

- Cohort studies
- Case-control studies
- Cross-sectional studies

Stratification by age:

- < 35 years old
- ≥ 35 years old

Stratification by length of follow-up periods for outcomes:

- < 5 years
- ≥ 5 years

Stratification by race/ethnicities of most of the study population:

- Asians
- Non-Hispanic Whites
- Hispanic Whites
- Black

Sensitivity analysis will be performed wherever there are small studies, studies with high or very high risk of bias, and industry funded studies.

Meta-bias(es)

Outcome reporting biases will be evaluated by comparing outcomes reported in protocols with outcomes presented in studies. If the protocol of the study is unavailable, outcomes presented in the methods will be compared to the outcomes presented in the results from the published paper**.** Sensitive analysis will be performed to evaluate the impact of selective reporting on meta-analyses results, if needed. If the number of included studies exceeds ten, ten funnel plots will be used to explore publication bias,[16] but if it is less than ten, publication bias will not be evaluated given the limited power.

Confidence in Cumulative Evidence

To assess the overall strength of the body of evidence, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach will be used. The quality of

evidence will be assessed from the following domains: risk of bias, imprecision, indirectness, inconsistency, and publication bias. Given that all studies in this review were observational studies, evidence will start with low ratings, with the potential of upgrading if the magnitude of the effect is large enough, or if there is a dose-response relationship or if all plausible biases could decrease the magnitude of an apparent effect.[17]

Ethics and dissemination: ethical and safety considerations and any dissemination plan (publications, data deposition and curation) should be covered here.

Authors Contribution:

NZ and SWW designed the study. NZ and WW developed the search strategy. All authors contributed to drafting the protocol. All authors read, revised, and approved the final version of the protocol. SWW is the guarantor of the review.

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Competing interest's statement: None declared.

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Appendix 2
Search Strategy - Ovid MEDLINE(R)

#	Searches
1	Blood glucose/
2	glucose intolerance/
3	glucose tolerance test/
4	(Glucose adj3 (tolera* or intolera* or fast* or value* or level* or test)).ti,ab,kf.
5	(blood adj3 (sugar or glucose)).ti,ab,kf.
6	OGTT.ti,ab,kf.
7	(dysglyc?emi* or hyperglyc?emi*).ti,ab,kf.
8	1 or 2 or 3 or 4 or 5 or 6 or 7
9	pregnancy/
10	(pregnan* or gestation* or trimester or antepartum or gravidity).ti,ab,kf.
11	9 or 10
12	8 and 11
13	Case-Control Studies/ or Control Groups/ or Matched-Pair Analysis/ or ((case* adj5 control*) or (case adj3 comparison*) or control group*).ti,ab.
14	cohort studies/ or longitudinal studies/ or follow-up studies/ or prospective studies/ or retrospective studies/ or cohort.ti,ab. or longitudinal.ti,ab. or prospective.ti,ab. or retrospective.ti,ab.
15	Cross-Sectional Studies/ or Prevalence/ or (cross-sectional or prevalence or transversal).ti,ab,kw.

16	13 or 14 or 15
17	12 and 16
18	exp animals/ not humans.sh.
19	17 not 18
20	(exp infant/ or exp child/ or adolescent/) not exp adult/
21	19 not 20
22	exp Cardiovascular Diseases/
23	cardiovasc*.ti,ab.
24	coronary.ti,ab.
25	heart.ti,ab.
26	myocardial.ti,ab.
27	circulat*.ti,ab.
28	CAD.ti,ab.
29	ISCHEMIA/
30	(atherosclero* or arteriosclero* or PVD or PAOD or PAD).ti,ab.
31	(peripheral adj3 dis*).ti,ab.
32	(isch* or CLI).ti,ab.

33	arteriopathic.ti,ab.
34	dysvascular*.ti,ab.
35	22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34
36	21 and 35

Appendix 3
Search Strategy - Embase

#	Searches
1	Blood glucose/
2	glucose intolerance/
3	glucose tolerance test/
4	(Glucose adj3 (tolera* or intolera* or fast* or value* or level* or test)).ti,ab,kw.
5	(blood adj3 (sugar or glucose)).ti,ab,kw.
6	OGTT.ti,ab,kw.
7	(dysglyc?emi* or hyperglyc?emi*).ti,ab,kw.
8	1 or 2 or 3 or 4 or 5 or 6 or 7
9	pregnancy/
10	(pregnan* or gestation* or trimester or antepartum or gravidity).ti,ab,kw.
11	9 or 10

12	8 and 11
13	exp cohort analysis/
14	exp longitudinal study/
15	exp prospective study/
16	exp follow up/
17	cohort$.tw.
18	exp case control study/
19	(case$ and control$).tw.
20	(cross sectional or questionnaire or questionnaires or survey or epidemiological study).ab,ti.
21	13 or 14 or 15 or 16 or 17 or 18 or 19 or 20
22	12 and 21
23	(exp animal/ or animal experiment/ or nonhuman/) not (exp human/ or human experiment/)
24	22 not 23
25	exp juvenile/ not exp adult/
26	24 not 25
27	exp cardiovascular disease/
28	cardiovasc*.ti,ab.

29	coronary.ti,ab.
30	heart.ti,ab.
31	myocardial.ti,ab.
32	circulat*.ti,ab.
33	CAD.ti,ab.
34	ischemia/
35	(atherosclero* or arteriosclero* or PVD or PAOD or PAD).ti,ab.
36	((arter* or vascular or vein* or veno* or peripher*) adj3 (occlus* or reocclus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab.
37	(peripheral adj3 dis*).ti,ab.
38	(claudic* or IC).ti,ab.
39	(isch* or CLI).ti,ab.
40	arteriopathic.ti,ab.
41	dysvascular*.ti,ab.
42	(leg adj3 (occlus* or reocclus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab.
43	(limb adj3 (occlus* or reocclus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab.
44	(lower adj3 extrem* adj3 (occlus* or reocclus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab.
45	((iliac or femoral or popliteal or femoro* or fempop* or crural) adj3 (occlus* or

	reocclus* or steno*or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab.
46	((femor* or iliac or popliteal or fempop* or crural or poplite* or infrapopliteal or inguinal or femdist* or inguinal or infrainquinal or tibial) adj3 (occlus* or reocclus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)).ti,ab.
47	27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46
48	26 and 47

Appendix 4
Search Strategy - CINAHL

#	Searches
S1	MH "Blood glucose"
S2	MH "glucose intolerance"
S3	MH "glucose tolerance test"
S4	TI (Glucose N3 (tolera* or intolera* or fast* or value* or level* or test)) OR AB (Glucose N3 (tolera* or intolera* or fast* or value* or level* or test))
S5	TI blood N3 (sugar or glucose)) OR AB blood N3 (sugar or glucose))
S6	TI OGTT OR AB OGTT
S7	TI (dysglyc?emi* or hyperglyc?emi*) OR AB (dysglyc?emi* or hyperglyc?emi*)
S8	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7
S9	MH "pregnancy"
S10	TI (pregnan* or gestation* or trimester or antepartum or gravidity) or AB (pregnan* or gestation* or trimester or antepartum or gravidity)

S11	S9 OR S10
S12	S8 AND S11
S13	TI ((animal or animals or canine* or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep*)
S14	S12 NOT S13
S15	((MH "Child+") OR (MH "Adolescence"))
S16	S14 NOT S15
S17	(MH "Case Control Studies+") or (MH "Control Group") or (MH "Matched-Pair Analysis") or (TI (case or cases) n5 TI (control or controls)) OR (AB (case or cases) n5 AB (control or controls)) OR (TI (case or cases) n3 TI (matched)) OR (AB (case or cases) n3 AB (matched)) OR TI (control group*)
S18	cohort[TIAB] OR Cohort Studies[Mesh] OR longitudinal[TIAB] OR prospective[TIAB] OR retrospective[TIAB]
S19	Cross-Sectional Studies[Mesh] OR “cross-sectional”[TIAB] OR “prevalence study”[tiab]
S20	S17 OR S18 OR S19
S21	S19 AND S20
S22	(MH "Cardiovascular Diseases+")
S23	TX cardiovasc*
S24	TX coronary
S25	TX heart

S26	TX myocardial
S27	TX circulat*
S28	TX CAD
S29	TX Ischemia
S30	TX atherosclero* or arteriosclero* or PVD or PAOD or PAD
S31	TX (arter* or vascular or vein* or veno* or peripher*) n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)
S32	TX peripheral n3 dis*
S33	TX claudic* or IC
S34	TX isch* or CLI
S35	TX arteriopathic
S36	TX dysvascular*
S37	TX leg n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)
S38	TX limb n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)
S39	TX (lower n3 extrem*) n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*
S40	TX (iliac or femoral or popliteal or femoro* or fempop* or crural) n3(occlus* or reocclus* or re-occlus* or steno*or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)
S41	(MH "Leg/BS”)

S42	(femor* or iliac or popliteal or fempop* or crural or poplite* or infrapopliteal or inguinal or femdist* or inguinal or infrainquinal or tibial) n3 (occlus* or reocclus* or re-occlus* or steno* or restenos* or obstruct* or lesio* or block* or harden* or stiffen* or obliter*)
S43	S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39 OR S40 OR S41 OR S42
S44	S21 AND S43

7.2 Fellowship application

Title: Maternal hyperglycemia and future risks of developing cardiovascular diseases in pregnant women

Research Summary

1.Rationale

With the obesity epidemic and lifestyle changes in modern society, it is essential to investigate whether and how elevated glucose levels during pregnancy increase the future risk of cardiovascular diseases (CVDs). The prevalence of gestational diabetes mellitus (GDM) in Ontario increased from 4.2% of pregnancies in 1999 to 12.0% in 2019. With the rising burden of GDM, there is growing concern about an associated increase in CVDs. However, only a few studies have investigated the link between GDM and the subsequent development of CVDs among women. To our knowledge, no study has assessed the association of interest by race. To this end, the proposed study will use large population health data from the Canadian province of Ontario to assess the association of elevated glucose levels in pregnant women with future risks of developing CVDs.

2. Hypothesis

Hyperglycemia in pregnancy, regardless of a diagnosis of GDM, leads to an increased risk of CVDs after pregnancy.

3. Objectives

1) To assess the association between maternal glycemia levels and future risks of developing CVDs based on the number and type of abnormal values on oral glucose tolerance test (OGTT) among women with and without GDM.

2) To assess racial differences in the association between maternal glycemia levels with future

risks of developing CVDs based on the number and type of abnormal values on OGTT among women with and without GDM.

4. Methodological approach (including sex and gender considerations in the research design)

4.1 Study design and study population

This will be a population-based retrospective cohort study in Ontario. I will conduct this study in Ontario's Institute for Clinical Evaluative Sciences (ICES). ICES hosts data from various provincial health and demographic data, which are linkable using unique identifiers. The study population will include women who gave birth (including live births and stillbirths) in an Ontario hospital from April 1st, 2012, to March 31st, 2023, with birth weight ≥500 grams or gestation ≥20 weeks, residing in Ontario. Pregnant individuals who had a history of diabetes before pregnancy will be excluded as hyperglycemia initiated during pregnancy is the exposure of interest.

4.2 Data sources/data linkage

4.2.1 The BORN Information System (BIS): BORN Ontario, a world-leading birth registry that collects data on every birth in Ontario through the BIS, started on April 1st, 2012. The BORN databases capture maternal demographics and health behaviours, obstetric history, maternal clinical information, labour and delivery, early neonatal care, and birth and neonatal outcomes.[1] BORN BIS also contains data collected by antenatal specialty (AS), prenatal screening follow-up (PSOF), and Genetics/MFM (GMFM) in Ontario.

4.2.2 Canadian Institute for Health Information (CIHI): CIHI runs and maintains the Discharge Abstract Database (DAD) and the National Ambulatory Care Reporting System (NACRS). Each year, ICES receives CIHI-DAD and CIHI-NACRS maternal, newborn, and up to one-year-old child records from acute care and emergency facilities in Ontario.

4.2.3 Ontario Laboratory Information Service database (OLIS): This includes data for laboratory test orders and results from community, hospital, and public health laboratories across Ontario. Laboratories have gradually enrolled in OLIS to contribute their data, starting in 2007. Enrolment generally occurred by region across the province.[2] Individuals are linked between data sources through a unique and reproducibly encrypted health card number.

4.2.4 Data linkage: The data linkage will start from the BIS datasets. We will first pull a base cohort from the aggregate infant data of birthdates within the required timeframe. Then, we will merge records with records during follow-up using a unique linking variable. After that, we will further link the study cohort with OLIS to obtain maternal prenatal glucose test results. The record linkage will occur at ICIS (Please see the data linkage flow chart in Appendix A).

4.3 Outcome measures

First occurrences of CVDs after childbirth for the index pregnancy will be the outcome measure. Hospitalizations for CVDs, including heart failure, coronary heart disease (myocardial infarction, angina, myocardial ischemia, coronary artery disease, acute coronary syndrome, angina), stroke/transient ischemic attack, intracranial hemorrhage, and peripheral vascular disease will be identified by ICD-9 diagnostic and procedure codes (Appendix B). We will use the methods developed by Birman-Deych et al to ascertain CVD events from hospital discharge data.[3] Ascertainment of outcomes will be censored at death or first occurrence of the outcome or the end of follow-up (March 31, 2023), whichever occurred first.

4.4 Exposure measure

Number and type (fasting blood, 1-hour(h) and 2-h post glucose) of abnormal values on OGTT, regardless of GDM status, will be the primary exposure measure. The normal cut-off values for 75-gram(g) OGTT were fasting blood, 1-hour(h) and 2-h post glucose loading of < 5.3 mmol/L

(95 mg/dL), < 10.6 mmol/L (190 mg/dL), and < 9.0mmol/L (162 mg/dL) respectively.

4.5 Covariates

Important covariables that will be considered in the analysis include calendar year of birth, maternal race, maternal age at childbirth, parity, assisted reproductive technology-derived pregnancy, substance use during pregnancy, maternal pre-existing health conditions, multiple gestation, gestational age at birth, type of birth (caesarean, vaginal, or assisted), infant sex, and birth weight. Since no individual records on household income and education exist, in this study, I will create two neighbourhood measures: the median household income and the percentage of the adult population aged 25 to 64 years with a university degree at a dissemination area (DA) level to estimate the maternal household income and education level using 2016 Canadian Census data, which will then be linked to the PCCF+ (Postal Code Conversion File Plus) data, and then each postal code will be assigned to a unique DA unit in Ontario.[4]

4.6 Sample size and study power

In Ontario, there are approximately 140,000 births per year.[5] Thus, we estimate there will be around 1,540,000 births in Ontario between 2012 and 2023 (the period that our study will be based). After considering up to 10% sample loss from a lack of glucose values in earlier years in prenatal screening lab results, the total available sample size will be estimated to be 1,386,000 births. Based on the literature review and assuming a 7.5-year follow-up on average, incidences of CVDs are around

1.8%.[6-7] Given a significance level (α) = 0.05, this sample size would allow greater than 80% power to detect a 20% increase in the rate of CVDs among women with at least one abnormal OGTT value compared to these with normal OGTT value. Although the sample size of women with at least two abnormal values would be smaller compared to the group with one abnormal

OGTT value, the association should be stronger so that the study power therefore would be similar. The power for glucose value treated as continuously distributed variable would be much higher, so no power estimation for those analyses is needed.

4.7 Limitations and measures to mitigate the limitations.

Administrative data is prone to errors, either at the data gathering or at the data entering stage. These errors likely have occurred randomly, which may reduce precision but could also cause bias.[8-9] Information on partners is not available. Preliminary analysis of ICES data found that some important variables, such as maternal race, had missing values of >3%. I will consider multiple imputation if the proportion of missing data exceeds 5%. In addition, I will perform sensitivity analysis using the actual dataset to assess the robustness of the study findings. Glucose measured in the first trimester will be excluded to assess the impact of them on the measures of association.

4.8 Sex and gender considerations in the research design

Across our datasets, we will have access to extensive information on gender-related factors and social determinants of health that shape the pregnancy and postpartum experience, including maternal self-reported ethnicity, age, income, education, and geography. All are factors that intersect with gender and the development of CVD among women.

5. Analysis and reporting

I will use multiple imputation methods to account for missing data on the covariates. The number of imputed datasets will depend on the percentage of missing data of covariates.[10-11]

Objective (1) In this analysis, all pregnant women with OGTT measurement will be included, with OGTT value as the primary exposure measure, and first occurrence of CVDs as the outcome measure. In the primary analysis, 4 cohorts will be formed: all OGTT values normal

(reference), 1 OGTT value abnormal (exposure 1), 2 OGTT abnormal (exposure 2), and 3 or more OGTT values abnormal (exposure 3). Cohort characteristics will be described for categorical variables by n (%) and for continuous variables using mean ± SD or median (25th–75th percentile) according to their distribution. Cumulative incidence curves for each outcome will be constructed as 1 minus the Kaplan-Meier survival curve. Cox proportional hazards regression will be used to model the independent association of maternal OGTT value with CVDs, after adjusting for potential confounding variables.

Objective (2) All these analyses will follow the procedures described in the analysis for Objective (1), after stratifying data according to maternal race: White, Black, Asian, and others.

6. Timeline

I plan to conduct this study over 2 years, starting from July 1, 2025, with 6 months for record linkage and data preparation, 1 year for statistical analysis of data, and 6 months for drafting report and manuscripts.

7. Applicant's role

Under the supervision of a team of faculty members, I will conduct a thorough review of relevant literature, develop a solid research plan, carry out the record linkage, create the working file, conduct the statistical analysis of the data, and draft report for HSF and manuscripts for scientific publication.

8.The expected contributions (i.e., impacts and benefits)

Since the universal screening policies are in place, prenatal care offers the opportunity for many adult women the first contact with health care providers and screening of glucose level. Maternal hyperglycemia is a modifiable risk. If my study demonstrates that elevated maternal glucose is a true risk factor for future CVDs, these finding will bear practical importance, in addition to the expected theoretical contribution to the scientific literature of the mechanisms of maternal

glucose and CVDs association. Most previous studies on the association of maternal glucose levels with future risks of developing CVDs in pregnant women have focused on GDM, and few studies have assessed the impact of borderline elevated maternal glucose level. Although the association of borderline elevated maternal glucose level with future risks of developing CVDs in pregnant women may not be as strong as GDM, intervention targeting on this group of women may have a higher impact to the overall disease burden in the society for the size of affected women is larger.

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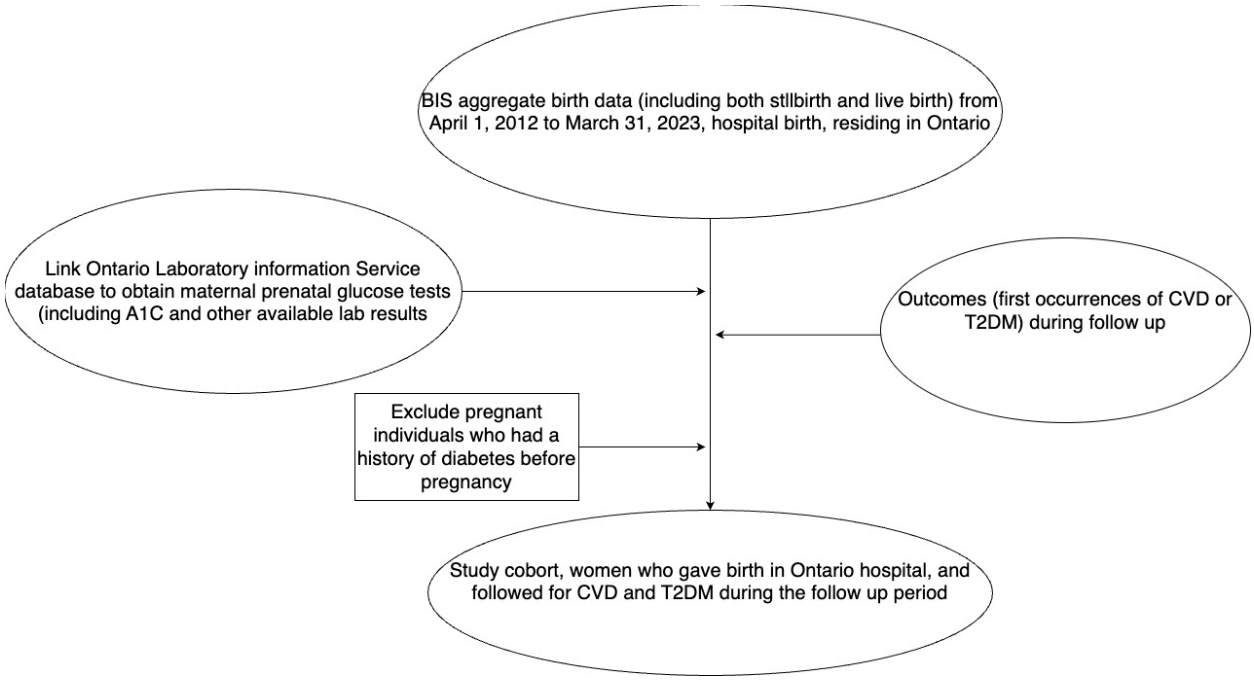
Appendix A. Flowchart of data linkage to identify the study cohort



Appendix B. ICD Codes to identify cardiovascular diseases

Stroke/transient ischemic attack (TIA): 433.x1, 434.x1, 435.x, 436, 437.1x, 437.9x, 438.x; 66
Coronary heart disease: 410.x–414.x, 429.2, V45.81

Intracranial hemorrhage 430, 431, 432.x, 800.2, 800.3, 800.7, 800.8, 801.2, 801.3, 801.7, 801.8,

803.2, 803.3, 803.7, 803.8, 804.2, 804.3, 804.7, 804.8, 852.x, 853.x;66