STUDY PROTOCOL

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Studying the Heterogeneity of Gestational Diabetes Mellitus: Cardio-Metabolic Alteration and Treatment Response in a Multi-Ethnic Population in Singapore (GDM-CARE)

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STUDY PROTOCOL

1. BACKGROUND AND RATIONALE

Pregnant women may experience transient hyperglycemia, also known as gestational diabetes mellitus (GDM). GDM increases risks of adverse pregnancy and neonatal outcomes, including pre-eclampsia, macrosomia, preterm birth, and offspring respiratory distress syndrome. The prevalence of GDM varies from 1% to over 30% in different populations and is particularly high (i.e. 2- to 3-fold greater) among pregnant women of Asian descent (i.e. Chinese and Indians) when compared with others (i.e. North American Europeans).

Emerging evidence shows that GDM phenotypic subtypes might have various underlying pathophysiology that could lead to different treatment outcomes. Current guidelines by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommend universal screening for GDM among pregnant women during 15-30 weeks of gestation. Since such guidelines define GDM based on any abnormal reading of fasting (≥5.1 mmol/L), 1-hr (≥10 mmol/L) and/or 2-hr (≥8.5 mmol/L) plasma glucose level cut-offs, pregnant women could be categorized into five different glycemic impairment phenotypes, namely: 1) fasting glucose impairment alone; 2) 1-hr post-challenge glucose intolerance alone; 3) 2-hr post-challenge glucose intolerance alone; 4) both 1-hr and 2-hr post-challenge glucose intolerance alone; 5) both fasting and post-challenge glucose abnormality. In a pilot study conducted in NUH, we have discovered that racial and ethnic differences exist in glycemic impairment phenotypes. For example, more than a third of Caucasian women (38%) diagnosed with GDM had fasting glucose impairment alone; while the majority of Singapore Chinese (84%), 2 thirds of Singapore Malay (65%) and more than half of Singapore Indian (57%) women diagnosed with GDM had 1-hr and/or 2-hr post-challenge glucose intolerance alone. In addition, a recent study showed that Indian women developed GDM at lower BMI and had better insulin sensitivity than Scandinavian women. This suggests a potential variation in both insulin sensitivity and fat deposition related β-cell activity across heterogeneous GDM phenotypes, independent of known risk factors of GDM (i.e. maternal BMI, family history of type 2 diabetes (T2D), history of macrosomia and ethnicity).

1.1. General Introduction

Ideally, all women with GDM should be given advice on dietary modification to better manage the condition. However, there is only 15% women with dietary advice provided in Singapore. In addition, evidence in treatment response to diet and/or medication (i.e. metformin and/or insulin) prescribed according to different GDM phenotypes is largely lacking in our local clinical setting. Emerging evidence suggests that maternal fasting glucose levels are strongly related to birth size, while post-challenge glycemic levels are strongly related to postpartum maternal T2D. That being said, the difference in GDM phenotypes defined by glycemic abnormality may result in different pregnancy outcomes. Therefore, we speculate that identification of these heterogeneous phenotypes offers better opportunities for more effective/precise treatments for the prevention of GDM complications. By studying the cardio-metabolic alteration and treatment response of heterogenous GDM phenotypes, we would improve our understanding of GDM pathophysiology and further tailor effective treatment strategies to individuals based on their GDM phenotype. Once such proof-of-concept is established, we may even further study the prediction model to GDM phenotype based on signatures of metabolites and biomarkers in early pregnancy, in order to provide more timely prevention in women at risk of GDM.

1.2. Rationale and justification for the Study

Therefore, it is necessary and clinically significant to address the heterogeneity in GDM in terms of cardio-metabolic alteration and treatment responses, especially among a higher risk group of overweight and obese pregnant women from different ethnic background in Singapore. In this proposed pregnancy cohort, we aim to conduct a longitudinal study among 800 overweight (23-24.9 kg/m^2) or obese (≥25 kg/m^2) singleton pregnant women—without a history of diabetes and composed of all ethnicities (mainly Chinese, Malay and Indian)—in a Singaporean tertiary hospital (National University Hospital, NUH) during their first trimester. We intend to recruit all pregnant women no later than 14 weeks of gestation (including 13W6D) and screen them for GDM during 15-30 weeks of gestation. We will further follow up the GDM women (~200) from 34-36weeks gestational to delivery. We will characterize heterogeneous GDM phenotypes using a comprehensive cardio-metabolic examination throughout pregnancy.

2. HYPOTHESIS AND OBJECTIVES

Our primary outcome will be unique glycemic profiling recorded via a continuous glucose monitoring device and distinctive cardio-metabolic biomarkers alteration from early to late pregnancy that determine different GDM phenotypes. Our secondary outcome will be specific treatment response according to different GDM phenotypes in late pregnancy. We hypothesize that GDM heterogeneity might not only be differentiated by patterns of glycemic profiles and signatures of cardio-metabolic biomarkers or metabolites throughout pregnancy, but also lead to phenotype-specific treatment response in late pregnancy. Furthermore, given the early discovery of unique glycemic profiles and cardio-metabolic biomarkers and metabolites among GDM subjects in the first trimester, we also plan to explore a prediction model using machine deep learning technique.

Specific Aim 1 (Primary Outcome): To characterize GDM phenotypes through trajectories of glycemic profiling and cardio-metabolic biomarkers or metabolites from early to late pregnancy.

Hypothesis: Different GDM phenotypes manifest specific patterns of glucose profiling (i.e. mean glucose level, TIR [% time in range between 3.5-7.8 mmol/L, glycemic variability indices]), altered levels of insulin sensitivity and secretion measures (i.e. homeostatic model assessment of insulin resistant [HOMA-IR], HOMA-β, Insulinogenic index/HOMA-IR, Matsuda index), fasting, 1-hour and 2-hour insulin, fasting c-peptide, HbA1c, fasting adipokines [e.g. leptin and adiponectin]), and different combination of targeted lipidomic and metabolomic metabolites (i.e. Acylcarnitines, fatty acids and amino acids).

Specific Aim 2 (Secondary Outcome): To study treatment response linked to different GDM phenotypes.

Hypothesis: GDM phenotypes such as fasting glucose impairment alone and both fasting and post-challenge glucose abnormality will require immediate attention of medication (metformin and/or insulin), while GDM phenotypes such as different pattern of post-challenge glucose intolerance alone will require more diet and life style modification other than medication.

Exploratory Aim 1 (Machine Learning prediction model): To use machine learning to develop a prediction model for GDM diagnosis in the first trimester.

Hypothesis: With comprehensive information on continuous glycemic profiling (i.e. greater glycemic variability), clinically established biomarkers (i.e. greater HOMA-IR) and targeted metabolites (i.e. greater Acylcarnitines) in early pregnancy, a prediction model for GDM might be applicable.

2.1. Potential Risks and benefits:

a. End Points - Efficacy

Diabetes begets diabetes. This vicious cycle needs to be interrupted if diabetes epidemic is to be combatted. We are addressing one of Singapore's major health challenges – the very high prevalence of GDM during pregnancy, and its future development of abnormal glucose metabolism in mothers, and accumulated adiposity in their offspring. Categorization of GDM phenotypes may have further implications on patient-related outcomes, such as guiding targeted pregnancy care, offspring outcomes (i.e. macrosomia), and maternal outcomes (i.e. postnatal glucose intolerance). The patient-related outcomes are trans-generational and studying the pathophysiological link between mother and child is critical for developing more efficient and precise screening approaches or targeted interventions to disrupt the vicious cycle.

b. End Points - Safety

All clinical visits are under the routine care of O&G specialists. The nature of the study is observational and we do not foresee any safety issue at the end of the study.

3. STUDY POPULATION

3.1. List the number of subjects to be enrolled.

800 pregnancy women

3.2. Criteria for Recruitment

This is a prospective longitudinal study based on NUH O&G outpatient clinic. A cohort of 800 pregnant women will be recruited at NUH over a period of 18 months.

3.3. Inclusion Criteria

1. Overweight and obese subjects with BMI 23 kg/m^2-24.9 kg/ m^2 and ≥ 25.0 kg/ m^2, respectively;
2. Aged 21-45 years with singleton pregnancy;
3. Plan to be followed up during the whole pregnancy and deliver at NUH;
4. All ethnicities mainly Chinese, Malay or Indian for pregnant subject;
5. Can complete questionnaires in English language;
6. Willing to wear CGM device at least for 7 days at each required clinic visit.

3.4. Exclusion Criteria

1. Participants who are not Singapore citizens or Singapore Permanent Residents, not intending to eventually deliver in NUH and to reside in Singapore for the next 2 years;
2. Have serious skin conditions (e.g. eczema) that precludes wearing the sensor for 14 days;
3. With history of Type 1 diabetes or Type 2 diabetes;
4. With chronic preexisting life-threatening conditions including pancreatic cancer, end-stage kidney dysfunction, and psychosis.
5. Unable to read or speak English.

3.5. Withdrawal Criteria

If there is a study closure due to DSMB review, the withdrawal will apply.

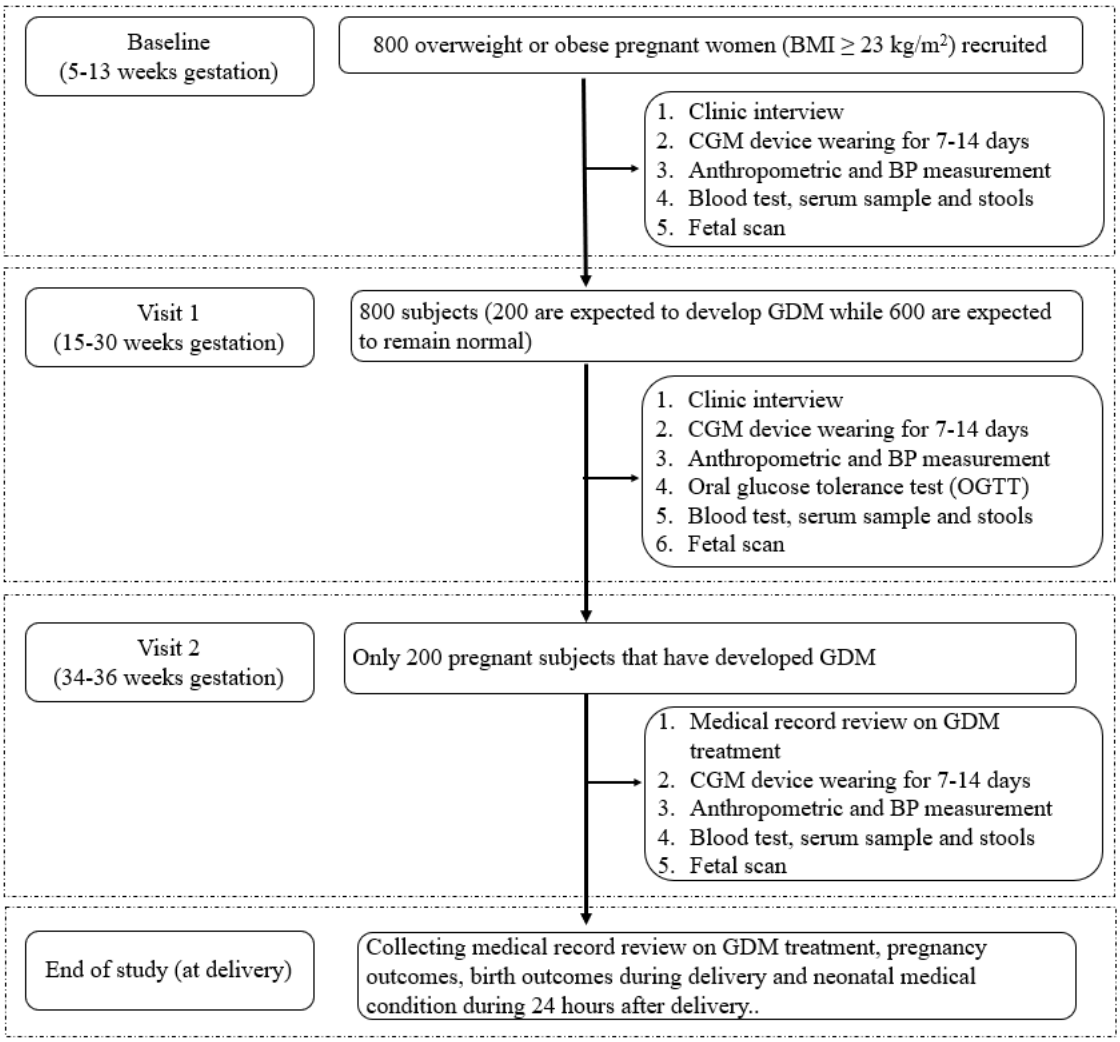
3.6. Subject Replacement

No, subject(s) will not be replaced once she (they) drops out.

4. TRIAL SCHEDULE

Not applicable since this is not a trial.

5. STUDY DESIGN



5.1. Summary of Study Design

As shown in the figure above, we will recruit participants attending NUH antenatal clinic during their first trimester. We will receive consents and collect blood samples at screening phase and/or baseline visit. Blood samples, CGM and fecal collection will align with the antenatal genetic visit. Then we will review them during 15-30 weeks and 34-36 week gestation. All three visits involve blood drawn and continuous glucose monitoring application.

6. METHODS AND ASSESSMENTS

6.1. Randomisation and Blinding

Not applicable.

6.2. Contraception and Pregnancy Testing

All our participants are confirmed pregnant cases by NUH specialists.

6.3. Study Visits and Procedures

At screening and baseline visit for all participants (5-13 weeks gestation): questionnaires will be performed by trained research coordinators. And then random blood sample will be collected during the research visit. Anthropometric and blood pressure measurement will be performed by the clinic nurse. The fecal collection kit will be handed over to the participants and it would be arranged for

courier service within one month. The continuous glucose monitoring device will be worn under the supervision of research coordinators, and the courier service will be arranged for collection same time while collecting the fecal kit. Medical record regarding fetal scan and routine blood test results will be collected.

At research visit 1 for all participants (15-30 weeks gestation): Fasting blood sample will be collected during the research visit. Anthropometric and blood pressure measurement will be performed by clinic nurse. The fecal collection kit will be handed over to the participants and it would be arranged for courier service within one month. The continuous glucose monitoring device will be worn under the supervision of research coordinators, and a courier service will be arranged together with the fecal kit collection within one month. Medical record regarding fetal growth, routine blood test and oral glucose tolerance test (OGTT) will be obtained at visit 1.

At research visit 2 only for patients diagnosed with gestational diabetes (34-36 weeks gestation): Random blood sample will be collected during the research visit. Anthropometric and blood pressure measurement will be performed by clinic nurse. The fecal collection kit will be handed over to the participants and it would be arranged for courier service within one month. The continuous glucose monitoring device will be worn under the supervision of research coordinators, and the courier service will be arranged together with the fecal kit collection within one month. Medical record regarding fetal growth and routine blood test will be obtained at visit 2.

At delivery: Pregnancy complications, gestational age at delivery, delivery mode, neonatal anthropometric biometry and medical condition during the 24 hours after delivery, treatment for GDM, Apgar score, and other pregnancy maternal and fetal outcomes information will be collected.

If a withdrawal from a subject occurs, the evaluation from her latest visit will sustain and valid for analysis. Subjects may withdraw voluntarily from participation in the study at any time. Subjects may also withdraw voluntarily from blood drawn, wearing continuous glucose monitoring and fecal collection in the subsequent visits. All participants will still be under standard antenatal care in NUH clinics, and no adverse event is expected after withdrawal.

7. TRIAL MATERIALS

Not applicable.

7.1. Trial Product (s)

Not applicable.

7.2. Storage and Drug Accountability

Not applicable.

8. TREATMENT

8.1. Rationale for Selection of Dose

This is a standard observational cohort, and no treatment will be given to the participants from the research team. All participants will be under the standard care under NUH clinics and treated routinely if they are diagnosed with any pregnancy complications such as gestational diabetes mellitus or hypertensive disorder during pregnancy.

8.2. Study Drug Formulations

Not applicable.

8.3. Study Drug Administration

Not applicable.

8.4. Specific Restrictions / Requirements

Not applicable.

8.5. Blinding

Not applicable.

8.6. Concomitant therapy

Not applicable.

9. SAFETY MEASUREMENTS

9.1. Definitions

Define terms e.g. what would be regarded as UPIRTSO events, Serious adverse events etc..
Include details of the protocol specific reporting, procedures, including the individual responsible for each step (e.g. the Investigator, the medical monitor, etc.), how decisions will be made regarding determining relatedness and grading severity, how reports will be distributed and what follow up are required. Include specific details of reporting procedures for:

- Deaths and life threatening events
- other SAEs
- Other adverse events

9.2. Collecting, Recording and Reporting of "Unanticipated Problems Involving Risk to Subjects or Others" – UPIRTSO events to the NHG Domain Specific Review Boards (DSRB)

UPIRTSO events refers to problems, in general, to include any incident, experience, or outcome (including adverse events) that meets ALL of the following criteria:

1. **Unexpected**
   In terms of nature, severity or frequency of the problem as described in the study documentation (eg: Protocol, Consent documents etc).

2. **Related or possibly related to participation in the research**
   Possibly related means there is a reasonable possibility that the problem may have been caused by the procedures involved in the research; and

3. **Risk of harm**
   Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Reporting Timeline for UPIRTSO Events to the NHG DSRB.

1. Urgent Reporting: All problems involving local deaths, whether related or not, should be reported immediately – within 24 hours after first knowledge by the NHG investigator.

2. Expedited Reporting: All other problems must be reported as soon as possible but not later than 7 calendar days after first knowledge by the NHG investigator.

9.3. Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Science Authority (HSA)

1. **For Industry sponsored Trials**
   All SAEs will be reported to HSA according to the HSA Guidance for Industry "Safety Reporting Requirements for Clinical Drug Trials."

2. **For Principal Investigator initiated Trials**
   All SAEs that are unexpected and related to the study drug must be reported to HSA.

"A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

- Results in death.
- Is life-threatening (immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in congenital anomaly/birth defect.
- Is a Medically important event.

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event should be reported as serious."

All SAEs that are unexpected and related to the study drug will be reported. The investigator is responsible for informing HSA no later than 15 calendar days after first knowledge that the case qualifies for expedited reporting. Follow-information will be actively sought and submitted as it becomes available. For fatal or life-threatening cases, HSA will be notified as soon as possible but no later than 7 calendar days after first knowledge that a case qualifies, followed by a complete report within 8 additional calendar days.

9.4. Safety Monitoring Plan

There will not be potential risk in the studied participants in this research.

9.5. Complaint Handling –

All complaints will be recorded by the ground staff and reported to the PI. Also, all participants can contact the PI directly with her contact listed in the consent form. The research PI will look into the issue being complained and try all means to solve it. All complains will be addressed and feedback will be given back to the participant.

10. DATA ANALYSIS

10.1. Data Quality Assurance

Study subjects will be identified on all study-related CRFs and databases only by the study ID number, which will

not include any personal identifying information. Collected data will be reviewed intermittently by PI and Co-PI to assure the accuracy of the data.

10.2. Data Entry and Storage

Data will be stored electronically after each subject encountered and will be stored in NUH. A set of de-identifying patients' data related to this study will be stored in NUS work station as well.

11. SAMPLE SIZE AND STATISTICAL METHODS

11.1. Determination of Sample Size

Based on our prior unpublished Asia-Pacific collaborative study on both Chinese and Indians overweight and obese pregnant subjects (BMI $\geq$ 23 kg/m^2), the effect size based on analysis of variance (ANOVA) for comparison of glucose across five groups will be more than 0.60 (large effect size). Considering a local setting in Singapore that might carry lower effect size in glycemic difference between GDM phenotypes, we are considering a moderate effect size (0.3) for the sample size calculation. As we will be interested in comparing 10 outcomes, the sample size of 200 participants with GDM and overweight/obese will be sufficient to detect a difference of 0.3 effect size across five GDM phenotype with 80% power and 0.5% type-I error (applying Bonferroni correction for 10 multiple comparisons to control type-I error rate to 5%) and 10% drop-out rate. The sample size assumes prevalence of GDM phenotypes at 20% for fasting glucose impairment alone (n = 40), 60% for post-challenge glucose abnormality alone (n = 120), and 20% for both fasting and post-challenge glucose abnormality (n = 40).

11.2. Statistical and Analytical Plans

a. **Primary outcome analysis:** The primary analysis will be performed on the changes of glycemic profiles, glycemic indexes and targeted metabolites from early to mid-pregnancy (baseline visit and visit 1 in this study) in relation to GDM phenotypes. Time-series analysis and linear regression will be applied for this analytical plan.

b. **Secondary outcome analysis:** The secondary analysis will be performed on the association between OGTT 0, 1-hr and 2-hr glycemic values (continuous variables) and GDM phenotypes (categorical variables) (as exposure) (visit 1 in this study) with treatment strategies (diet alone, diet and metformin, diet and metformin and/or insulin) received in late pregnancy (as outcomes) (visit 2 in this study). Ordinal logistic regression will be applied for this analytical plan.

c. **Exploratory outcomes analysis:** In order to build machine learning prediction models, both stepwise logistic regression and ensemble random forest methods will be applied for variable selection. (Mukkesh needs to nourish this).

12. ETHICAL CONSIDERATIONS

12.1. Informed Consent

All participants included in this study will need to give their consents. Since non-English speakers will be ruled out from our recruitment, language-speaking deprivation will not be a limitation for our study. The investigator and research coordinators will comply with the SGGCP guidelines and to the ethical principals that have their origin in the Declaration of Helsinki. The PIs, site PIs and research coordinators will take consents for the patients, and all research purposes including blood drawn, fecal collection, and continuous glucose monitoring device wearing will all be included in the consent form.

12.2. IRB review

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by the IRB / NHG DSRB.

12.3. Confidentiality of Data and Patient Records

To minimize the risk of loss of confidentiality all information related to study subjects will be confidential and kept in secure cabinets or password-protected computer files. Study subjects will be identified on all study-related CRFs and databases only by the study ID number, which will not include any personal identifying information. Research records, samples, and consent forms will be maintained by Department of O&G, both in NUH and NUS, to allow for retrospective analysis.

13. PUBLICATIONS

Publication policy for study findings will follow the international guideline published elsewhere.

14. RETENTION OF TRIAL DOCUMENTS

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as IRB records and other regulatory documentation will be retained by the PI in a secure storage facility. The records will be accessible for inspection and copying by authorized authorities within 5 years after trial completion.