

Research Title: " Prevention of pre-eclampsia using metformin: a randomized control trial (PREMET)"

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INTERNAL RESEARCH GRANT COMPETITION (IRGC) Cycle 4

RESEARCH PLAN

Study Title:

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Contents

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| 1. Background | 3 |
| 2. Study aims and goals | 5 |
| 3. Objectives of this study | 5 |
| 4. Preliminary data or studies | 5 |
| 5. Study design and methodology | 7 |
| 6. Sample size and calculation | 13 |
| 7. Data collection methods and procedure | 13 |
| 8. Data Management and Analysis plan | 14 |
| 9. Anticipated results and evaluation criteria | 14 |
| 10. Plans for disseminating research results | 15 |
| 11. Future plans | 15 |
| 12. Bibliography and References | 16 |

Research Plan

1. Background

Preeclampsia (PET) is one of the leading causes of maternal morbidity and mortality (1). Its pathophysiology is poorly understood. Consequently, there are no efficient preventive and treatment modality. PET is associated with significant perinatal morbidity and mortality including prolonged hospitalization and is a major contributor to a large proportion of iatrogenic preterm birth. Women who suffer from PET are at a greater risk of hypertensive and cardiovascular diseases in later life and more likely to suffer from premature death (2). Since the only treatment for PET is delivery, a logical approach to reducing the incidence and therefore consequences is prevention. For this to be effective those at risk have to be identified and any timely interventions introduced.

Amongst the risk factors are maternal age, obesity, medical disorders such as antiphospholipid syndrome, hypertensive disorders, renal diseases diabetes mellitus and previous PET. A history of PET increases the risk of recurrence 7 fold and this is compounded by GA at delivery for the affected pregnancy (3,4). Moreover, both chronic hypertension and pre-existing diabetes increase the risk of PET which is further enhanced by the degree of glycemic control (5,6). Interestingly maternal and paternal history of diabetes and hypertension have been associated with increased risk of PET (7). Additionally, maternal age > 40 years and pregnancy interval > 10 years increases the risk of PET by two to three folds respectively (8,9). A BMI > 35 increases the risk of PET by 4 folds in both multiparous and nulliparous women (3,10). Moreover, assisted reproduction techniques and multiple gestation have also been associated with increased risk of PET (11). A combination of diabetes and obesity, which has a high prevalence in Qatar, significantly increases the risk of PET. Despite these risk factors only a fraction of those with at high risk eventually develop PET as the current criteria for prediction is not specific.

Various measures to predict, prevent and treat pre-eclampsia have been investigated and tried by several groups/researchers (12). However, these have not been very successful, primarily because PET is a disease of theories with an unclear primary pathophysiology and thus no clear target for either these predictive tools or interventions. Evidence, however, does suggest that in patients at risk of developing PET there is inadequate trophoblastic invasion, placental hypo-perfusion, and endothelial cell activation (13–15). This the basis for the most widely non-invasive clinical tool of Doppler velocimetry of the uterine artery; but again, this has a poor sensitivity (16,17).

An imbalance in pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) (decreased) and antiangiogenic factors such as soluble FMS-like tyrosine kinase -1 (sFlt-1) (increased) has been implicated for the inadequate remodeling of spiral arteries (which is essential for the maintenance of a normal placental perfusion) in those who develop PET (18,19).



Vascular endothelial growth factor (VEGF) is an important signaling protein in health and diseases such as cancer, kidney diseases and PET (20). The VEGF family is composed of five members and three tyrosine kinase receptors. The signaling proteins are: VEGFA, VEGFB, VEGFC, VEGFD and placental growth factor (PIGF) (reviewed in (20)). The receptor tyrosine kinase consists of VEGFR1 (also known as Flt-1), VEGFR2 and VEGF3, with different binding capacity to VEGF proteins (20,21). A soluble Fms like tyrosine kinase-1 (sFlt-1) a truncated form of VEGFR1, has also been identified which lacks the VEGFR1 cytosolic domain (22). In addition to ligand binding, VEGFR has been shown to be activated through non-ligand binding and mechanical forces (23–25). VEGF proteins are upregulated under hypoxic conditions such as PET, growth factor signaling and by hormones such as estrogen (20).

PET is characterized by a hypoxic environment, resulting in considerable changes in maternal leukocyte gene expression, altered expression of the VEGF signaling pathway and AMP-activated protein kinase (AMPK) and secretion of sFlt-1 into the maternal circulation (26–31). VEGF including PIGF has been identified as a crucial in the signaling pathway for angiogenesis and vasculogenesis during placental development (32–35). Indeed, deletion of a single VEGF allele in mice resulted in embryonic lethality due to immature angiogenesis (36,37). The invasion of maternal spiral arteries by cytotrophoblasts is vital for adequate oxygen and nutrient supply (14,38–40). This process is believed to be mediated by binding of VEGF and PIGF to Flt-1 (40). Further, fetal DNA variants at the Flt-1 region has been associated with PET and reported recently (59). However, the pathophysiology of PET is still unclear and understanding of its molecular mechanism is warranted.

A decreased level of VEGF and PIGF free form has been observed that has been attributed to their blockade by the increased level of sFlt-1 in pre-eclamptic women (29,41,42). Indeed, the introduction of sFlt-1 to a pregnant rat led to hypertension and proteinuria similar to that seen in PET women (42). Furthermore, an altered ratio of serum PIGF/sFlt-1 has been found to be associated with PET diagnosis and disease severity (43–46). Indeed, there is now a drive to use this ratio in screening women at risk of PET but most of the data were generated in late pregnancy rather than in early pregnancy – a time when interventions have been shown to have maximum impact (46). It would seem from this greater understanding of the underlying physiological changes in women that develop PET that any interventions that have the potential to alter this milieu are more likely to be successful.

Very recently a double-blind, placebo-controlled trial, that randomly assigned pregnant women without diabetes who had a body-mass index of more than 35 to receive metformin, at a dose of 3.0 g per day, or placebo (225 women in each group) from 12 to 18 weeks of gestation until delivery showed a significant reduction in the incidence of PET in those who received metformin (47). Since metformin is now frequently prescribed to obese type II diabetic women and gestational diabetics with poor glycemic control on diet, it is hypothesized in those at risk of PET, metformin will not only reduce the incidence of PET but will modify the PIGF/s-Flt-1 ratio in favor of normal pregnancies.



2. Study aims and goals

Aim 1: To investigate whether the administration of metformin to women at high risk of pre-eclampsia will reduce the incidence.

Aim 2: To determine accuracy of the ratio of PIGF/sFlt-1 in predicting PET and the effect of metformin on this ratio

Aim 3: To study the molecular mechanisms and signaling pathways associated with increased levels of sFlt-1 and PET

3. Objectives of this study

Aim 1: To investigate whether the administration of metformin to women at high risk of pre-eclampsia will reduce the incidence.

The objectives under this aim will include

1. A comparison of the incidence of pre-eclampsia and its severity in women receiving metformin versus standard care,
2. A comparison of the GA at delivery, maternal and fetal outcomes in those receiving Metformin versus standard care,
3. A comparison of the timing of onset of PET between the two groups and

Aim 2: To determine accuracy of the ratio of PIGF/sFlt-1 in predicting PET and the effect of metformin on this ratio

The objectives under this aim will include,

1. A comparison of the serum levels of the PIGF/sFlt-1 ratio measured in early pregnancy between non-PET and PET patients.
2. A comparison of the PIGF/sFlt-1 ratio PET in patients who received metformin versus standard care group.

Aim 3: To study the molecular mechanisms and signaling pathways associated with increased levels of sFlt-1 and PET.

Regarding aim 3: in response to the reviewer's comments, the study team has agreed to collect the blood sample and placenta from the patients and store them for future analysis.

4. Preliminary data or studies

Metformin safety in pregnancy



substantial evidence on the benefits of metformin an antihyperglycemic agent when pregnant women with gestational and Type 2 diabetes. In polycystic ovary syndrome

1 Jan - Revised On May 2017

Page 5 of 22

(PCOS) patients, it is known to decrease the risk of first trimester miscarriages and the incidence of gestational diabetes (48,49). In diabetic pregnancies, metformin has been associated with favorable maternal and neonatal outcomes (50,51). Observed maternal outcomes include less weight gain during pregnancy, better glycemic control, and reduced fat distribution (52). Other benefits include a reduction in pregnancy induced hypertension and PET, lower risk of neonatal hypoglycemia and ICU admission. Neonatal outcomes include: reduced fat around organs, which could result in improved insulin resistance and prevention of diabetes later in life (52). Even though metformin is known to cross the placenta, its use in early pregnancy or late pregnancy has not been shown to be associated with an increased risk of congenital malformation or adverse neonatal outcomes except a lower neonatal birth weight (53–56).

Metformin has been safely administered at the dose we propose to use here to diabetic patients. It has become the first line treatment of type 2 DM in pregnancy and poorly controlled gestational diabetes on diet based on the NICE guideline and the national diabetes guideline in Qatar. However, anticipated side effects include: GI disturbances such as gas (4-12%), heartburn, stomach pain (3-4%), nausea (7-9%), and GI changes. Serious side effects associated with its use are hypoglycemia and lactic acidosis which are extremely rare (<1%) (57).

Metformin in PET:

Recently data are emerging on reduced incidence of gestational hypertension and pre-eclampsia with metformin use in pregnant patients with diabetes, PCOS or obese non-diabetic patients (58). In a prospective study of pregnant PCOS women, metformin was shown to be associated with a significant reduction in the incidence of gestational hypertension (54). A randomized double-blinded placebo controlled trial of obese pregnant women without diabetes on metformin, showed a 75% reduction in the incidence of pre-eclampsia (47). A recent systematic review and meta-analysis concluded that metformin is associated with a lower incidence of pre-eclampsia in women with gestational diabetes mellitus or Type 2 diabetes (59), emphasizing its potential role in the prevention of PET. However, it concluded that robust evidence is still lacking and randomized control trials (RCT) are needed. Furthermore, there have been no such studies in our local GCC population to the best of our knowledge.

Therefore, we would like to conduct this RCT to explore the role of metformin in the prevention of PET. Pre-eclampsia is considered a multisystem and complex disease with no definite preventive or treatment measures other than delivery or termination of pregnancy. The use of metformin if confirmed to be effective will therefore not only significantly decrease the maternal and fetal morbidity and mortality associated with pre-eclampsia but also a significant decrease in the health care expenditure. Indeed, there are no randomized control trials (RCT) that specifically investigated the use of Metformin for the prevention of PET. Since metformin reduces the gestational weight gain, it follows that it would most likely reduce incidence of PET

^{60, 75} Effect of metformin on maternal and fetal outcomes in obese pregnant women



(EMPOWaR) trial showed no effect but timing of the intervention (introduction of metformin was much later in pregnancy) (61).

Metformin has been in use since 1957 but its specific mechanism of action is unknown (62). Previously, AMPK-activation has been identified as one of the mechanisms by which it exerts its angiogenic activity (63). However, metformin does not activate AMPK directly. Instead, AMPK activation is a result of metformin inhibition of the mitochondrial respiratory chain complex 1 (reviewed in (64,65)). Apart from diabetes, it has also been implicated in the prevention of cancer, adverse outcomes in women with PCOS and most recently in the prevention of PET (reviewed in (66)). In an ex-vivo study using primary placental tissue from PET placentas, metformin treatment resulted in reduced endothelial dysfunction, enhanced vasodilatation and induced angiogenesis by sFlt-1 and endoglin reduction possibly at the mitochondrial level (30). Despite these, the exact mechanism behind its role in the prevention of PET remains poorly understood. Further clarification of how metformin decreases the incidence of pre-eclampsia needs to be examined. Here we plan to explore altered maternal serum levels of sFlt-1, PIGF and VEGF in pregnant women using metformin.

5. Study design and methodology

This is an open label, randomized control trial (RCT) in which high risk for pre-eclampsia pregnant subjects will be randomly assigned to either an intervention group (metformin 1 gm twice daily plus aspirin 100 mg per day and standard of care) versus control group (aspirin 100 mg per day and standard of care) that will be administered between 11^{+0} to 13^{+6} weeks of gestation until delivery (appendix 1). Only women at high risk of pre-eclampsia as defined by the ACOG practice bulletin will be included (see inclusion criteria). Patient assignment will not be blinded as control group will not be given a placebo; the data will be analyzed on an intention to treat basis. Enrolled subjects will be followed throughout pregnancy and up to 30 days post-delivery (as per hospital practice).

Study definitions:

High risk of preeclampsia:

defined as pregnant women with at least one of the following; History of preeclampsia, especially when accompanied by an adverse outcome, Multifetal gestation, chronic hypertension, Type 1 or 2 diabetes, Renal disease, Autoimmune disease (ie, systemic lupus erythematosus, the antiphospholipid syndrome), OR more than one of the following: Nulliparity, Obesity (body mass index greater than 30), Family history of preeclampsia (mother or sister), Sociodemographic characteristics (African American race, low socioeconomic status), Age 35 years or older, Personal history factors (eg, low birth weight or small for gestational age, previous adverse pregnancy outcome, more than 10-year pregnancy interval)

Preeclampsia:

defined by the presence of **Blood pressure** defined as systolic blood pressure of 140 mm Hg or more or diastolic blood pressure of 90 mm Hg or more on two occasions at least 4 weeks apart. Ian - Revised On May 2017



hours apart after 20 weeks of gestation in a woman with a previously normal blood pressure or systolic blood pressure of 160 mm Hg or more or diastolic blood pressure of 110 mm Hg or more. (Severe hypertension can be confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy). and

Proteinuria defined as 300 mg or more per 24 hour urine collection (or this amount extrapolated from a timed collection), or protein/creatinine ratio of 0.3 mg/dL or more or dipstick reading of 2+ (used only if other quantitative methods not available).

OR in the absence of proteinuria, new-onset hypertension with the new onset of any of the following:

Thrombocytopenia defined as platelet count less than $100,000 * 10^9/L$

Renal insufficiency defined as serum creatinine concentrations greater than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease

Impaired liver function define as elevated blood concentrations of liver transaminases to twice normal concentration

Pulmonary edema

new-onset headache unresponsive to medication and not accounted for by alternative diagnoses or visual symptoms

Sever preeclampsia: is characterized by the following:

Systolic blood pressure of 160 mm Hg or more, or diastolic blood pressure of 110 mm Hg or more on two occasions at least 4 hours apart (unless antihypertensive therapy is initiated before this time), Thrombocytopenia (platelet count less than $100,000 * 10^9/L$), Impaired liver function as indicated by abnormally elevated blood concentrations of liver enzymes (to twice the upper limit normal concentration), and severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnoses, Renal insufficiency (serum creatinine concentration more than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease), Pulmonary edema
 New-onset headache unresponsive to medication and not accounted for by alternative diagnoses, Visual disturbances

Study population:

Patient characteristics:

Participants who will be included in this study are pregnant women who are at high risk for developing pre-eclampsia as defined by the ACOG practice bulletin (for review (67)).

Inclusion criteria:

To be eligible to be included in the study, a woman has to have all of the following; Confirmed y, gestational age $< 12^{+0}$ weeks, live fetus at time of booking ultrasound scan



(between 11⁺⁰ and 13⁺⁶ weeks of gestation), and to be considered as high risk of preeclampsia. According to ACOG (67), pregnant women are considered high risk of preeclampsia if they showed at least one of the following; History of preeclampsia, especially when accompanied by an adverse outcome, Multifetal gestation, chronic hypertension, Type 1 or 2 diabetes, Renal disease, Autoimmune disease (ie, systemic lupus erythematosus, the antiphospholipid syndrome), OR more than one of the following: Nulliparity, Obesity (body mass index greater than 30), Family history of preeclampsia (mother or sister), Sociodemographic characteristics (African American race, low socioeconomic status), Age 35 years or older, Personal history factors (eg, low birth weight or small for gestational age, previous adverse pregnancy outcome, more than 10-year pregnancy interval)

Exclusion criteria:

Age under 18 years, hyperemesis gravidarum, unable to sign the consent form, type 1 or 2 diabetes mellitus, early gestational diabetes, auto-immune disease, fetal abnormality identified at time of scanning (between 11⁺⁰ and 13⁺⁶ weeks of gestation), bleeding disorder, peptic ulcer, hypersensitivity to aspirin or metformin, long use of NSAIDS before initiation of intervention, contraindication to metformin or aspirin and participation in another concurrent trial.

Study sites:

The study will be conducted at the Women Wellness and Research Center (WWRC), Doha, Qatar. Patient will be identified from three Primary Health Centers. There will, however, will be very few volunteers who will have all their visits and tests done at the WWRC with the health center facilitating their identification only.

Study methods:

Patient identification and randomization

If the primary physician in the Primary Health Center or the main center identifies a potential candidate for the study based on the inclusion and exclusion criteria, he/she will inform the patient about the study and document her willingness to participate in the study in the electronic medical record to avoid cold calling and according to HIPAA recommendations. The investigator of the respective site will then be contacted through email by the primary physician.

the patient will be contacted by the study investigator and arrange an appointment in the WWRC to inform her about the study and if she accepted to participate, give her the patient information leaflet (PIL) and then obtain informed consent once all her questions have been answered.



Each center has one coordinator, who will be responsible in conjunction with the investigator for recruitment, clinical interviews, determining eligibility and obtaining signed informed consent forms. After successful screening, eligible pregnant women will be randomly assigned to either intervention or control group. Random sized blocks of 4 or 6 will be computer generated with 1:2 ratio.

Sample isolation and laboratory studies (appendix 2):

Blood samples, 5 ml maternal venous whole blood will be collected by a laboratory phlebotomist or a nurse at each study visit (one at baseline, and one at each following trimester/visit and admission/s, see appendix 2) for all included subjects. The blood will be collected in vacutainer tubes without additives and will be allowed to clot for 30 min at room temperature before centrifugation for 15 min at 1000 x g. The supernatant (serum) will be labelled with research mark and the patient study number and stored at -20C until ELISA is performed. Serum levels of sFlt-1, free VEGF and free PIGF will be tested using ELISA. The study coordinators will collect and transfer the samples daily to the research laboratory at Sidra Medicine were further analysis to be carried out. Kits for ELISA will be purchased from R&D. Patient confidentiality will be maintained during this process. **Placenta samples**: Two random 2x2cm whole thickness placenta biopsies will be taken from 2cm from the cord insertion (from opposite sites) will be collected immediately after the delivery on the labor room or in the operating theatre by the nurse. These samples will be washed and flash-frozen and transported to Sidra Medicine for storage for future analysis to study the association between maternal and fetal DNA mutations (targeted genome sequencing Flt-1 region) and mRNA expression level (targeted) and development of PET.

To further our understanding of gene regulation of metformin effects in pre-eclampsia we will use a high frequency immune monitoring targeted transcriptome finger printing assay (TFA) including a high-throughput qPCR platform (68,69). This assay relies on gene sets, so called transcriptional modules, which had previously been identified by comprehensive network analysis on the basis of gene co-expression in whole blood samples of nearly 1000 subjects across 16 immunological conditions. Of these transcriptional modules, 273 target genes were selected, representing 66 functionally annotated modules, with 4 representative genes per module. This allows high frequency monitoring of the entire modular transcriptional repertoire in a cost effective way. In brief, small volumes of blood (around 50 µl) from each blood sample will be transferred into a microfuge tube containing twice the blood volume of tempus solution from the following groups of pregnant women: A) PET high risk, B) PET receiving standard treatment and C) PET receiving metformin. The sample will be mixed to disrupt cells and release the RNA. Samples will be stored at -20 C. Once all samples for the study are collected, RNA will be isolated (using the Tempus Spin RNA isolation kit from Invitrogen) and cDNA synthesized using standard protocol. The following qPCR, using primer pairs to amplify 4 representative genes per module, will be performed on the Fluidigm platform (Sidra core ~~facilities~~ ^{appendix 3} for number of patient in this analysis)



We will collect and store samples for future determination of the association between maternal and fetal DNA mutations (targeted genome sequencing Flt-1 region) and mRNA expression level (targeted) and development of PET.

Risk minimization strategy:

Loss of confidentiality:

There is a minimal risk of confidentiality loss. To minimize this risk, data will be entered in to a clinical trial management software. The software will be secured with user name and password for study investigators and only accessible by them. Data extraction will only be accessible to study PIs and co-PIs. Paper based data collection sheet will be secured in locked cabinets accessible only by study PIs and co-PIs. Only de-identified data will be shared amongst investigators and Data and Safety Monitoring Committee (DSMC). If a breach in confidentiality occurs, the IRB will be notified immediately.

Linking information between collected data and patient's identifiable information will be stored in a locked cabinet and/or PI password locked computer. Information on the password protected computer database will only be accessed by the investigators. Furthermore, the PI's office will remain locked. All study documentation will be stored according to the MRC and GCP guideline.

Data and Safety Monitoring Committee (DSMC)

A DSMC of this trial consisting of Dr. Victor Chilaka (senior consultant, WWRC), Dr. Ayman Alnaqa (Sr. Consultant Ob/Gyn department), Dr. Akinbolu Babarinsa (Senior Consultant at WWRC), Dr Ibrahim Ibrahim (Senior consultant at Sidra Medicine), Dr. Anas Ahmad Hamad (Director of Pharmacy, NCCCR) and Professor Erick Kilpatrick (Senior Consultant Clinical Biochemist at Sidra) will be set up. These are all very skilled clinical research and ethical matters and independent of the research. The committee will meet every three months to review (a) the conduct of the trial, (b) recruitment and progress, (c) randomization and (d) data collection.

Any adverse event (SAE) will be reviewed by the DSMC. Unanticipated SAEs will be reported to the DSMB within 48 hours (expedited reporting); the study Co-PIs will be responsible for reporting any unanticipated SAEs to the DSMC and the MRC.

A SAEs will be deemed Unanticipated if it is related to the study intervention. Other SAEs will be reported to the DSMC in the regular safety update. Accordingly, if required the DSMB may request follow up information and recommend continuation, modification (study protocol or consent), or termination of the study.

Study procedure:

Screening and enrollment:



ian - Revised On May 2017

Page 11 of 22

Initial screening: The study will take place at the Women Hospital. All clinical care providers at participating centers will be briefed about the study. Pregnant women presenting at their first antenatal visit (< 12⁺ weeks of gestation) will be screened by their primary physician for risk of pre-eclampsia. To avoid cold calling, a pregnant woman deemed high risk for pre-eclampsia will be informed about the study by her primary physician. If she expressed an interest in study participation, that will be documented by the primary physician in her medical record. She will be instructed to contact the study investigators directly or permit to share her information with study team to be contacted by a trained study team member to explain the study in detail, and then obtain a written informed consent (appendix 4).

Randomization and initiation of the study:

Once recruited, the patient data will be entered onto a web-based randomization software to determine study group and to the clinical trial management software, where a study ID number will be assigned. If the patient agrees to enter the trial, the site coordinator will contact the study physician to initiate study protocol (appendix 2) and then the patient will continue to be followed-up by her clinical team. In cases where patient assigned to control group and diagnosed with GDM and started on metformin, she will be moved to intervention group and analyzed as late started. If patient declined to participate initially, or dropped out from the study, all her data will be removed.

A clinical trial management software will be utilized to enter patient data. Each study Co-PI (physician) will lead a study site and supported by study coordinator. They will be responsible for reviewing patient eligibility, initiating the study protocol and patient counselling. They will meet regularly to share study updates. Study coordinators will be responsible for patient counselling, obtaining written informed consent, data collection and entry, and blood samples and placenta shipment from the study sites to study research laboratory at Sidra Medicine. The LPI will meet with the research team monthly to review various aspects of the trial to ensure conformation to the protocol and principles of GCP.

Collected data (appendix 5):

Variables to be collected include patient demographic characteristics, past medical and surgical history, past medication history, allergies, vital signs, CBC as routinely done and LFT, serum creatinine and biomarker serum level (sFlt-1, VEGF, PIGF). Blood samples will be collected by the phlebotomist or the responsible nurse. The blood investigations, 5 ml blood sample will be collected at baseline, second and third trimester and after delivery. The blood sample will be used for CBC, LFT, serum creatinine, and study serum biomarker in aim 2 (sFLT-1, VEGF, PIGF), and well as for storage for future DNA/RNA analysis for aim 3. Further, placenta samples will be collected and stored for future DNA/RNA analysis.

Medication adherence and adverse drug related events (ADRs):



Medication adherence is crucial to achieve the study objectives. Patient medication adherence is measured using the pill count method on each visit and Morisky scale (appendix 6) in

1st - Revised On May 2017

Page 12 of 22

each visit or telephone call. Furthermore, to increase patient medication adherence, a reminder SMS will be send as well as three telephone calls to individual patient on regular basis. Adverse drug reaction (ADRs) will be reported using Hamad Medical Corporation ADR form (Appendix 7) to both DSMC and MRC when they occur, however, serious ADRs will be reported within 48 hours by the study PI.

6. Sample size and calculation

Our sample size calculation was based on Syngelaki A. et al. (2016) findings. In this study, obese patient where either randomized to metformin or placebo to see the effect on neonatal birth weight. As a secondary outcome, metformin use was associated with 76% reduction in PET (3% versus 11.3% respectively, OR 0.24, 95% CI 0.10 – 0.61, $P = 0.001$).

We expect that for a study power of 80% and to detect a reduction at a level of 5% significance, with considering above outcome estimates it is approximately estimated to be 172 patients in each arm, for a total of 344 in both group based on 1:1 ratio. Allowing for a maximum 20% dropout, we estimate that 414 patients would need to undergo randomization, to give 207 patients in each group.

7. Data collection methods and procedure

These will be at baseline, in the second and again in the third trimesters. The data to be collected include patient clinical information, blood sample and placental tissue (appendix 5). At each study visit 5 ml maternal venous whole blood will be collected by a laboratory phlebotomist or a nurse (one at baseline, and one at each following trimester/visit and admission/s, see appendix 2) for all included subjects. The blood sample will be used for measuring blood markers for aim 2 and stored for future DNA/RNA analysis for aim 3. In addition, placental tissue will be collected and stored for future DNA/RNA analysis. In brief:

- (a) Baseline Data (Week 11⁺⁰ to 13⁺⁶): Patient sociodemographic data including age and ethnicity, Obstetrical, medical, surgical and medication history, current pregnancy information, CBC, as routinely done and study biomarkers of LFT, serum creatinine, sFlt-1, VEGF and PIGF.
- (b) Second (week 19⁺⁰ to 24⁺⁶) and third trimester visits (week 32⁺⁰ to 34⁺⁶): At these visits the same data collected at baseline will be obtained. If the patient is, however, admitted for pre-eclampsia before the third visit, the collection will follow the pre-eclampsia diagnosis at admission including treatment offered, length of hospitalization and management.
- (c) At delivery data to be collected will include gestational age at admission, vital signs, indication for delivery, type of delivery, compliance with protocol, blood for CBC, LFT, and the biomarkers of interest. Post-delivery, discharge vitals, antihypertensive treatment on discharge will be collected.



(d) Exit interview: patient will be called to check for any relevant ADR.

8. Data Management and Analysis plan

Data management:

Upon randomization, research coordinators will be responsible for data collection using approved CRFs. To maintain patient confidentiality, study document such as signed informed consent, and CRFs will be stored in locked cabinets, excel file containing patient identifiable information will be password protected, and all data including patient clinical information and research laboratory results will be entered into the clinical trial management software using patient generated study ID number.

Data analysis:

Descriptive data: Numerical data will be presented as mean and standard deviation where normally distributed or median and range where it's not normally distributed. Categorical data will be presented as frequency and percentages. The percentage of women developed PET will be compared between the intervention and control group using chi square test.

Quantitative data between two arms will be analyzed using either "Unpaired t test or Mann Whitney U test depending on normality of the data distribution". Also, outcomes measured at different time points should be statistically analyzed both within and between groups using repeated measure ANOVA statistical method followed by appropriate multiple comparison tests. All P values presented will be two-tailed. A multivariate logistic regression will be employed to assess the association between metformin use and PET adjusting for confounding factors such as patient demographics (age, GA at delivery, history of risk factors of PET\mentioned above) and co-morbidities such as DM, the dose of metformin, the need to use of antihypertensive drugs, including duration of treatment and any other medications. P values <0.05 will be considered as statistically significant. All Statistical analyses will be done using statistical packages SPSS 22.0 (SPSS Inc. Chicago, IL) and Epi-info (Centers for Disease Control and Prevention, Atlanta, GA) software.

9. Anticipated results and evaluation criteria

As an open label study, we hope the results of this study will generate robust data that will be used to inform the design equipped to design of a larger national study. Moreover, based on the findings of previous published literature, we predict that expression of sFlt-1, VEGF and PIGF will be significantly altered in pre-eclamptic patients. The design of this study will help determine and validate the clinical utility of the aforementioned biomarker in routine practice for the prediction of PET and disease severity. The use of metformin is expected to reduce the anticipated effects of sFlt-1 as an antiangiogenic factor, therefore, reducing the incidence of



PET and indeed improving maternal and neonatal outcomes and quality of life, reducing hospital admissions and cost of care and better utilization of health care system resources.

This collaborative study involves investigators (scientists and clinicians) from the PHCC, HGH, Women's Hospital Hamad and Sidra. Such a cross-disciplinary approach will provide an opportunity for training future scientist in the field of women's health. The more junior members of the team will have the opportunity to be trained on translational research design, and clinical trial management and thereby enhancing the research capacity which both are aligned with Qatar national health strategy. The women hospital will be better catered with enhanced research skills. Pregnant women are usually excluded from clinical trial; however, an advantage of this trial is the safety the intervention (metformin) and thereby increasing public engagement with research and awareness of its importance and benefits.

Evaluation criteria for this study are patient recruitment, quality and consistency of the data and safety of the intervention. Further, the percentage women who developed PET in each arm as well as changes in the studied biomarkers will be quantified and accepting the null hypothesis.

10. Plans for disseminating research results

Once the project is funded, the study team will initiate monthly meetings to discuss study plans and patient recruitment. Team leaders will also meet monthly to share study updates. Furthermore, the study results will be shared among peers in the department through presentation of study findings. Finally, once the study is completed we aim to present our finding at the Hamad Medical Corporation Annual Research Day, local and international conferences and in peer-reviewed journals.

11. Future plans

This an open label RCT study will generate date for a larger QNRF randomized control trial.



12. Bibliography and References

1. Draper ES, Kurinczuk JJ, Kenyon S. Maternal, Newborn and Infant Clinical Outcome Review Programme MBRRACE-UK Perinatal Confidential Enquiry Term, singleton, intrapartum stillbirth and intrapartum-related neonatal death. 2017 [cited 2017 Dec 1]; Available from: www.hqip.org.uk/national-programmes
2. Bartsch E, Medcalf KE, Park AL, Ray JG, High Risk of Pre-eclampsia Identification Group. Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies. *BMJ* [Internet]. 2016 Apr 19 [cited 2017 Nov 30];353:i1753. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27094586>
3. Duckitt K. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. *Bmj*. 2005;330(7491):565–0.
4. Mostello D, Kallogjeri D, Tungsiripat R, Leet T. Recurrence of preeclampsia: effects of gestational age at delivery of the first pregnancy, body mass index, paternity, and interval between births. *Am J Obstet Gynecol*. 2008;199(1):1–7.
5. Chappell LC, Enye S, Seed P, Briley AL, Poston L, Shennan AH. Adverse perinatal outcomes and risk factors for preeclampsia in women with chronic hypertension: A prospective study. *Hypertension*. 2008;51(4 PART 2 SUPPL.):1002–9.
6. Sibai BM, Caritis S, Hauth J, Lindheimer M, VanDorsten JP, MacPherson C, et al. Risks of preeclampsia and adverse neonatal outcomes among women with pregestational diabetes mellitus. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *Am J Obstet Gynecol* [Internet]. 2000 Feb [cited 2017 Nov 25];182(2):364–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10694338>
7. Qiu C, Williams MA, Leisenring WM, Sorensen TK, Frederick IO, Dempsey JC, et al. Family history of hypertension and type 2 diabetes in relation to preeclampsia risk. *Hypertension*. 2003;41(3 I):408–13.
8. BIANCO A, STONE J, LYNCH L, LAPINSKI R, BERKOWITZ G, BERKOWITZ R. [15] Pregnancy Outcome at Age 40 and Older. *Obstet Gynecol*. 1996 Jun;87(6):917–22.
9. Skjaerven R, Wilcox A, Lie T. The interval between pregnancies and preeclampsia. *N Engl J Med*. 2002;346(23):1831–2; author reply 1831–1832.
10. Wang Z, Wang P, Liu H, He X, Zhang J, Yan H, et al. Maternal adiposity as an independent risk factor for pre-eclampsia: A meta-analysis of prospective cohort studies. *Obes Rev*. 2013;14(6):508–21.
11. Skupski DW, Nelson S, Kowalik A, Polaneczky M, Smith-Levitin M, Hutson JM, et al. Multiple gestations from in vitro fertilization: Successful implantation alone is not associated with subsequent preeclampsia. In: *American Journal of Obstetrics and Gynecology*. Mosby; 1996. p. 1029–32.
12. Chaiworapongsa T, Chaemsathong P, Korzeniewski SJ, Yeo L, Romero R. Pre-eclampsia part 2: prediction, prevention and management. *Nat Rev Nephrol* [Internet]. 4 Jul 8 [cited 2017 Nov 27];10(9):531–40. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28711111>



<http://www.nature.com/doifinder/10.1038/nrneph.2014.103>

13. Walsh SW. What causes endothelial cell activation in preeclamptic women? *Am J Pathol* [Internet]. 2006 Oct [cited 2017 Nov 27];169(4):1104–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17003470>
14. Goldman-Wohl D, Yagel S. Regulation of trophoblast invasion: from normal implantation to pre-eclampsia. *Mol Cell Endocrinol* [Internet]. 2002 Feb 22 [cited 2017 Nov 15];187(1–2):233–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11988332>
15. Gathiram P, Moodley J. Pre-eclampsia: its pathogenesis and pathophysiology. *Cardiovasc J Afr* [Internet]. 2016 [cited 2017 Nov 27];27(2):71–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27213853>
16. Nagar T, Sharma D, Choudhary M, Khoiwal S, Nagar RP, Pandita A. The Role of Uterine and Umbilical Arterial Doppler in High-risk Pregnancy: A Prospective Observational Study from India. *Clin Med insights Reprod Heal* [Internet]. 2015 [cited 2017 Dec 1];9:1–5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25922590>
17. Mone F, McAuliffe FM, Ong S. The clinical application of Doppler ultrasound in obstetrics. *Obstet Gynaecol* [Internet]. 2015 Jan 1 [cited 2017 Dec 1];17(1):13–9. Available from: <http://doi.wiley.com/10.1111/tog.12152>
18. Bates DO. An unexpected tail of VEGF and PIGF in pre-eclampsia. *Biochem Soc Trans* [Internet]. 2011 Dec 1 [cited 2017 Nov 27];39(6):1576–82. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22103490>
19. Herraiz I, Simón E, Gómez-Arriaga PI, Martínez-Moratalla JM, García-Burguillo A, López Jiménez EA, et al. Angiogenesis-related biomarkers (sFlt-1/PLGF) in the prediction and diagnosis of placental dysfunction: An approach for clinical integration [Internet]. Vol. 16, *International Journal of Molecular Sciences*. 2015 [cited 2017 Nov 27]. p. 19009–26. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26287164>
20. Shibuya M. Vegf-vegfr signals in health and disease [Internet]. Vol. 22, *Biomolecules and Therapeutics*. The Korean Society of Applied Pharmacology; 2014 [cited 2017 Oct 30]. p. 1–9. Available from: <http://www.biomolther.org/journal/DOIx.php?id=10.4062/biomolther.2013.113>
21. Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. *Nat Rev Mol Cell Biol* [Internet]. 2016 Jul 27 [cited 2017 Oct 30];17(10):611–25. Available from: <http://www.nature.com/doifinder/10.1038/nrm.2016.87>
22. Shibuya M, Yamaguchi S, Yamane A, Ikeda T, Tojo A, Matsushime H, et al. Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (flt) closely related to the fms family. *Oncogene* [Internet]. 1990 Apr [cited 2017 Nov 13];5(4):519–24. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2158038>
23. Parker MW, Xu P, Li X, Vander Kooi CW. Structural basis for selective vascular endothelial growth factor-A (VEGF-A) binding to neuropilin-1. *J Biol Chem*. 2003;278(14):11082–9.



24. Jin Z-G, Ueba H, Tanimoto T, Lungu A, Frame M, Berk B. Ligand-Independent Activation of Vascular Endothelial Growth Factor Receptor 2 by Fluid Shear Stress Regulates Activation of Endothelial Nitric Oxide Synthase. *Circ Res* [Internet]. 2003;93(4):354–63. Available from: <http://circres.ahajournals.org/cgi/doi/10.1161/01.RES.0000089257.94002.96>
25. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, et al. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature* [Internet]. 2005;437(7057):426–31. Available from: <http://www.nature.com/doifinder/10.1038/nature03952>
26. Gu Y, Lewis DF, Wang Y. Placental productions and expressions of soluble endoglin, soluble fms-like tyrosine kinase receptor-1, and placental growth factor in normal and preeclamptic pregnancies. *J Clin Endocrinol Metab* [Internet]. 2008 Jan [cited 2017 Nov 23];93(1):260–6. Available from: <https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2007-1550>
27. Li H, Gu B, Zhang Y, Lewis DF, Wang Y. Hypoxia-induced increase in soluble Flt-1 production correlates with enhanced oxidative stress in trophoblast cells from the human placenta. *Placenta* [Internet]. 2005 Feb [cited 2017 Nov 23];26(2–3):210–7. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0143400404001468>
28. Kaufman MR, Brown TL. AMPK and Placental Progenitor Cells. In Springer, Cham; 2016 [cited 2017 Nov 23]. p. 73–9. Available from: http://link.springer.com/10.1007/978-3-319-43589-3_4
29. Koga K, Osuga Y, Yoshino O, Hirota Y, Ruimeng X, Hirata T, et al. Elevated serum soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) levels in women with preeclampsia. *J Clin Endocrinol Metab* [Internet]. 2003;88(5):2348–51. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12727995>
30. Brownfoot FC, Hastie R, Hannan NJ, Cannon P, Tuohey L, Parry LJ, et al. Metformin as a prevention and treatment for preeclampsia: effects on soluble fms-like tyrosine kinase 1 and soluble endoglin secretion and endothelial dysfunction. *Am J Obstet Gynecol* [Internet]. 2016 Mar [cited 2017 Oct 16];214(3):356.e1–356.e15. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26721779>
31. Rajakumar A, Chu T, Handley DE, Bunce KD, Burke B, Hubel CA, et al. Maternal gene expression profiling during pregnancy and preeclampsia in human peripheral blood mononuclear cells. *Placenta* [Internet]. 2011 Jan [cited 2017 Dec 6];32(1):70–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21075447>
32. Wang Y, Zhao S. Vascular Biology of the Placenta [Internet]. Morgan & Claypool Life Sciences; 2010 [cited 2017 Nov 23]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK53255/#s7.1>
33. Achen MG, Gad JM, Stacker SA, Wilks AF. Placenta growth factor and vascular endothelial growth factor are co-expressed during early embryonic development. *Growth Factors* [Internet]. 1997 [cited 2017 Nov 23];15(1):69–80. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9401819>



34. Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, De Mol M, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med* [Internet]. 2001 May 1 [cited 2017 Nov 23];7(5):575–83. Available from: <http://www.nature.com/doifinder/10.1038/87904>
35. Ribatti D. The discovery of the placental growth factor and its role in angiogenesis: a historical review. *Angiogenesis* [Internet]. 2008 Sep 21 [cited 2017 Nov 23];11(3):215–21. Available from: <http://link.springer.com/10.1007/s10456-008-9114-4>
36. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* [Internet]. 1996 Apr 4 [cited 2017 Nov 13];380(6573):435–9. Available from: <http://www.nature.com/doifinder/10.1038/380435a0>
37. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene [Internet]. Vol. 380, *Nature*. 1996. p. 439–42. Available from: <http://www.nature.com/doifinder/10.1038/380439a0>
38. Luttun A, Carmeliet P. Soluble VEGF receptor Flt1: The elusive preeclampsia factor discovered? [Internet]. Vol. 111, *Journal of Clinical Investigation*. American Society for Clinical Investigation; 2003 [cited 2017 Nov 15]. p. 600–2. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12618513>
39. King BF. Ultrastructural differentiation of stromal and vascular components in early macaque placental villi. *Am J Anat* [Internet]. 1987 Jan [cited 2017 Nov 15];178(1):30–44. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/2435141>
40. Zhou Y, McMaster M, Woo K, Janatpour M, Perry J, Karpanen T, et al. Vascular endothelial growth factor ligands and receptors that regulate human cytotrophoblast survival are dysregulated in severe preeclampsia and hemolysis, elevated liver enzymes, and low platelets syndrome. *Am J Pathol* [Internet]. 2002 Apr [cited 2017 Nov 15];160(4):1405–23. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11943725>
41. Levine RJ, Maynard SE, Qian C, Lim K-H, England LJ, Yu KF, et al. Circulating Angiogenic Factors and the Risk of Preeclampsia. *N Engl J Med* [Internet]. 2004;350(7):672–83. Available from: <http://www.nejm.org/doi/abs/10.1056/NEJMoa031884>
42. Maynard SE, Min J, Merchan J, Lim K-H, Li J, Mondal S, et al. Excess Placental Soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest*. 2003;111(5):649–58.
43. Yusuf AM, Kahane A, Ray JG. First and Second Trimester Serum sFlt-1/PIGF Ratio and Subsequent Preeclampsia: A Systematic Review. *J Obstet Gynaecol Can* [Internet]. 2017 Sep 15 [cited 2017 Dec 1];0(0). Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28927814>

ang Y-S, Chen C-N, Jeng S-F, Su Y-N, Chen C-Y, Chou H-C, et al. The sFlt-1/PIGF ratio as a predictor for poor pregnancy and neonatal outcomes. *Pediatr Neonatol*



[Internet]. 2017 Dec 10 [cited 2017 Dec 1];58(6):529–33. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28571908>

45. Zhao M, Zhu Z, Liu C, Zhang Z. Dual-cutoff of sFlt-1/PIGF ratio in the stratification of preeclampsia: a systematic review and meta-analysis. *Arch Gynecol Obstet.* 2017;295(5):1079–87.
46. Stepan H, Herraiz I, Schlembach D, Verlohren S, Brennecke S, Chantraine F, et al. Implementation of the sFlt-1/PIGF ratio for prediction and diagnosis of pre-eclampsia in singleton pregnancy: Implications for clinical practice [Internet]. Vol. 45, *Ultrasound in Obstetrics and Gynecology*. Wiley-Blackwell; 2015 [cited 2017 Nov 24]. p. 241–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25736847>
47. Syngelaki A, Nicolaides KH, Balani J, Hyer S, Akolekar R, Kotecha R, et al. Metformin versus Placebo in Obese Pregnant Women without Diabetes Mellitus. *N Engl J Med* [Internet]. 2016 Feb 4 [cited 2017 Nov 24];374(5):434–43. Available from: <http://www.nejm.org/doi/10.1056/NEJMoa1509819>
48. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP. Metformin versus Insulin for the Treatment of Gestational Diabetes. *N Engl J Med* [Internet]. 2008 May 8 [cited 2017 Nov 24];358(19):2003–15. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18463376>
49. Zheng J, Shan PF, Gu W. The efficacy of metformin in pregnant women with polycystic ovary syndrome: a meta-analysis of clinical trials. *J Endocrinol Invest* [Internet]. 2013 Nov [cited 2017 Nov 24];36(10):797–802. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23580001>
50. Butalia S, Gutierrez L, Lodha A, Aitken E, Zakariasen A, Donovan L. Short- and long-term outcomes of metformin compared with insulin alone in pregnancy: a systematic review and meta-analysis. *Diabet Med* [Internet]. 2017 Jan [cited 2017 Nov 28];34(1):27–36. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27150509>
51. Zhao L-P, Sheng X-Y, Zhou S, Yang T, Ma L-Y, Zhou Y, et al. Metformin versus insulin for gestational diabetes mellitus: a meta-analysis. *Br J Clin Pharmacol* [Internet]. 2015 Nov [cited 2017 Nov 28];80(5):1224–34. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25925501>
52. Reece SW, Parihar HS, LoBello C. Metformin in Gestational Diabetes Mellitus. *Diabetes Spectr* [Internet]. 2014 Nov 17 [cited 2017 Nov 27];27(4):289–95. Available from: <http://spectrum.diabetesjournals.org/lookup/doi/10.2337/diaspect.27.4.289>
53. Gilbert C, Valois M, Koren G. Pregnancy outcome after first-trimester exposure to metformin: a meta-analysis. *Fertil Steril* [Internet]. 2006 Sep [cited 2017 Nov 24];86(3):658–63. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16879826>
54. De Leo V, Musacchio MC, Piomboni P, Di Sabatino A, Morgante G. The administration of metformin during pregnancy reduces polycystic ovary syndrome related gestational complications. *Eur J Obstet Gynecol Reprod Biol* [Internet]. 2011 Jul [cited 2017 Nov 24];157(1):63–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21530058>

X, Li S, Chang Y, Fang C, Liu H, Zhang X, et al. Effect of metformin treatment



during pregnancy on women with PCOS: A systematic review and meta-analysis. *Clin Investig Med [Internet]*. 2016 Sep 11 [cited 2017 Oct 16];39(4):E120–31. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27619399>

56. Zhu B, Zhang L, Fan YY, Wang L, Li XG, Liu T, et al. Metformin versus insulin in gestational diabetes mellitus: a meta-analysis of randomized clinical trials. *Ir J Med Sci [Internet]*. 2016 May 9 [cited 2017 Oct 16];185(2):371–81. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26860118>
57. Lexicomp online. MetFORMIN [Internet]. [cited 2018 Jul 16]. Available from: http://online.lexi.com/lco/action/doc/retrieve/docid/hamad_f/3069840#adr
58. Lautatzis M-E, Goulis DG, Vrontakis M. Efficacy and safety of metformin during pregnancy in women with gestational diabetes mellitus or polycystic ovary syndrome: A systematic review. *Metabolism [Internet]*. 2013 Nov [cited 2017 Nov 27];62(11):1522–34. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23886298>
59. Alqudah A, McKinley MC, McNally R, Graham U, Watson CJ, Lyons TJ, et al. Risk of pre-eclampsia in women taking metformin: a systematic review and meta-analysis. *Diabet Med [Internet]*. 2017 Oct 16 [cited 2017 Nov 24]; Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29044702>
60. Chasan-Taber L, Silveira M, Waring ME, Pekow P, Braun B, Manson JE, et al. Gestational Weight Gain, Body Mass Index, and Risk of Hypertensive Disorders of Pregnancy in a Predominantly Puerto Rican Population. *Matern Child Health J [Internet]*. 2016 Sep 22 [cited 2017 Dec 1];20(9):1804–13. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27003150>
61. Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, et al. Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol [Internet]*. 2015 Oct [cited 2017 Dec 1];3(10):778–86. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26165398>
62. Dallaglio K, Bruno A, Cantelmo AR, Esposito AI, Ruggiero L, Orecchioni S, et al. Paradoxical effects of metformin on endothelial cells and angiogenesis. *Carcinogenesis [Internet]*. 2014 May [cited 2017 Oct 30];35(5):1055–66. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24419232>
63. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-melody J, et al. Role of AMP-Activated Protein Kinase in Mechanism of Metformin Action Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*. 2001;108(8):1167–74.
64. Viollet B, Guigas B, Garcia NS, Leclerc J, Foretz M, Andreelli F. Cellular and molecular mechanisms of metformin: an overview. *Clin Sci [Internet]*. 2012 Mar 1 [cited 2017 Dec 6];122(6):253–70. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22117616>
65. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia [Internet]*. 2017 Sep 3 [cited 2017 Dec 6];60(9):1577–85. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28776086>



66. Romero R, Erez O, Hüttemann M, Maymon E, Panaitescu B, Conde-Agudelo A, et al. Metformin, the aspirin of the 21st century: its role in gestational diabetes mellitus, prevention of preeclampsia and cancer, and the promotion of longevity. *Am J Obstet Gynecol* [Internet]. 2017 Sep [cited 2017 Oct 16];217(3):282–302. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28619690>
67. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 202: Gestational Hypertension and Preeclampsia. *Obstet Gynecol* [Internet]. 2019 Jan [cited 2019 Jul 4];133(1):e1–25. Available from: <http://insights.ovid.com/crossref?an=00006250-201901000-00049>
68. Chaussabel D, Quinn C, Shen J, Patel P, Glaser C, Baldwin N, et al. A Modular Analysis Framework for Blood Genomics Studies: Application to Systemic Lupus Erythematosus. *Immunity* [Internet]. 2008 Jul 18 [cited 2017 Dec 6];29(1):150–64. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S1074761308002835>
69. Chaussabel D, Baldwin N. Democratizing systems immunology with modular transcriptional repertoire analyses. *Nat Rev Immunol* [Internet]. 2014 Mar 25 [cited 2017 Dec 6];14(4):271–80. Available from: <http://www.nature.com/doifinder/10.1038/nri3642>