First trimester screening for late-onset preeclampsia:

Evaluation of novel predictive biomarker MMP-7 in combination with MAP and uterine artery pulsatility index in a Danish population

An undergraduate research project protocol by

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Introduction

The Fetal Medicine Foundation (FMF) algorithm is developed with the objective of identifying women at high risk of pre-eclampsia (PE) in the first trimester of pregnancy, allowing for effective prophylactic use of low-dose acetylsalicylic acid (ASA) started before 16 weeks of pregnancy (1). The algorithm utilizes Bayes' theorem by combining maternal characteristics and medical history with uterine artery pulsatility index (PI), mean arterial pressure (MAP), maternal serum pregnancy-associated plasma protein-A (PAPP-A), and placental growth factor (PlGF) (2) and has been validated in several populations (3–5). It has been shown to identify up to 90% of early-onset PE (EO-PE) cases, however, the detection rate of late-onset PE (LO-PE) remains low and the biomarkers PAPP-A and PlGF do not appear to significantly improve performance of screening for these cases compared to screening by maternal characteristics and medical history alone (6).

An abundance of biomarkers have been suggested and researched for the prediction of LO-PE (7–9) and of these, matrix metalloproteinase 7 (MMP-7) has shown to be a potentially useful predictor with a sensitivity of 69% at a false positive rate (FPR) 20% at 8-16 weeks of pregnancy (8). Additionally, MAP appears to be a relatively good predictor for LO-PE at the same gestational age (GA) (6). Ultimately, the search for more accurate biomarkers and prediction models for PE and especially for LO-PE remains relevant and necessary, both with regards to improving antenatal care and taking the necessary precautions for PE mothers, researching potential treatments, and gaining a better understanding of PE pathophysiology altogether.

Background

Preeclampsia affects 2-8% of pregnancies and is a leading cause for mortality and morbidity in both mother and child. Potential complications of PE include eclampsia, stroke, renal necrosis, pulmonary oedema and HELLP syndrome in the mother as well as intrauterine growth restriction (IUGR) and death of the child (10). Additionally, several long-term consequences are known to affect both parties, notably a much increased risk of cardiovascular disease in the child later in life (7,10).

PE has traditionally been divided into several subtypes depending on GA at time of delivery. Although this view has in some contexts been deserted in favor of the idea of PE as a disease spectrum (2), it is worth noting that there appear to be distinct differences between the PE types both in terms of incidence, severity, and etiology, making the subdivision clinically useful (8). In this article, the following classification will be used: early-onset PE (EO-PE) with delivery <34 weeks and late-onset PE (LO-PE) with delivery ≥34 weeks, a subcategory of which is term PE (tPE) with delivery ≥37 weeks (2,9). EO-PE and LO-PE have previously been referred to as "severe" and "mild" PE, respectively, however these terms are no longer recommended as references to GA at delivery, as all cases of PE are potentially threatening (11).

The pathophysiology of PE is complex and not yet fully understood. Especially for EO-PE, it is believed that a lack of deep cytotrophoblast migration toward the uterine spiral arterioles leads to reduced placental perfusion. This then results in placental ischemia as the pregnancy progresses, releasing various anti-angiogenic factors into maternal circulation and eventually leading to maternal vasoconstriction and potentially organ failure (7). Multiple pathways have been implied as contributing to these events, a likely explanation being that the condition is a result of several synergistic factors (7,12). However, LO-PE appears to be more closely related to a mismatch between fetal metabolic demands and maternal supply

close to term, coupled with maternal predisposition to inflammation, a high BMI, and/or preexisting hypertension (12). Findings supporting this idea include increased maternal cardiac output and relatively unchanged total vascular resistance, paired with a general lack of the findings usually signifying placental hypoperfusion and vascular malfunction in these pregnancies. In accordance with this, neonates born from pregnancies with LO-PE are usually not small for gestational age (8). Despite being associated with a lower incidence of adverse outcome than is EO-PE (2), LO-PE accounts for 75-90% of PE cases (2,13) and hence forms a significant burden of disease in any obstetric healthcare setting.

PE is a syndrome rather than a distinct disease, its diagnosis depending on the simultaneous occurrence of a group of in isolation non-specific symptoms (12). Currently, this diagnosis requires a combination of gestational hypertension and either proteinuria or other signs of organ dysfunction (including utero-placental dysfunction) (14). The only curative treatment is delivery of the placenta, however, the condition can be palliated with the objective of gaining fetal maturation, although doing so increases the risk of intrauterine death (12). Treatment of manifest PE is primarily symptomatic, consisting of antihypertensives and the anticonvulsant magnesium sulfate (10).

Several preventative treatments for PE are currently available or in the process of being reviewed. It has been shown that a daily intake of at least 1000 mg of calcium lowers the risk of PE in women with low dietary intake (15). Additionally, prophylactic intervention consisting of 150 mg ASA daily initiated before 16 weeks of pregnancy is recommended for women at risk of PE, reducing the risk of development of PE <37 weeks by more than half in this group (1,14). It is worth noting that ASA intervention has not been shown to significantly reduce the risk of tPE (1). New studies suggest that metformin administered from first or second trimester could be effective for the prevention of LO-PE, however, accurate screening markers are needed to allow for optimal prophylaxis trials (16).

Hypothesis and aim

The aim of this study is to improve the current first trimester screening regimes for early detection of late-onset PE using maternal serum biomarkers in combination with maternal history, uterine artery flow, and mean arterial blood pressure.

Hypothesis: Matrix metalloproteinase 7 (MMP-7) in combination with maternal history, uterine artery flow and mean arterial blood pressure can improve the detection rate of late-onset PE.

Study design

This study will be a nested case-control study carried out as a sub-study of the larger multicenter study "Preeclampsia Screening in Denmark" (PRESIDE) comprising six major hospitals in Denmark, including Odense University Hospital (OUH). Throughout the time period September 2019 to March 2020, 1400 participants are expected to enroll in PRESIDE through OUH with the goal of validating the FMF screening algorithm in a Danish population. These women will be giving birth from March to June 2020. In our sub-study, the case group will consist of women diagnosed with PE after 34 weeks of gestation. With an expected late-onset PE incidence of 2,5%, the sample size of the case group will be n=35. The control group will include 165 participants who were not diagnosed with PE in their pregnancies.

Methods and materials

All maternal characteristics and medical history needed for this study (except for family history of PE) are collected as part of standard procedure in connection with first trimester screening for chromosomal abnormalities. During enrollment into the study, participants are asked to verbally confirm aforementioned information as well as indicate any family history of PE, all of which is entered into the OUH fetal medicine database Astraia.

Bilateral uterine artery pulsatility indices are measured by transabdominal color Doppler ultrasound as a supplement to the regular first trimester scan and the average value is calculated and stored in Astraia.

Blood pressure is measured by an automated system in triplicate after a minimum rest period of 5 minutes with patients seated and cuffs placed around both arms simultaneously and raised to the level of the heart by a supported chair. These measurements are automatically transferred to a REDCap database where all study data, including consent forms and outcome data, is stored.

All information regarding pregnancy and neonatal outcome as well as subsequent use of aspirin during pregnancy will be collected from registers and individual patient files.

A total of 10 mL of venous blood will be sampled, and aliquots will be stored as part of a research biobank and a biobank for future research at the Department of Clinical Biochemistry and Pharmacology (KBF), OUH. The samples for measurement of MMP-7 will be taken from the research biobank and targeted quantitation of candidate protein markers for LO-PE will be performed by ELISA (Human Total MMP-7 Quantikine ELISA Kit) and by targeted multiplex multiple-reaction-monitoring mass spectrometry (MRM-MS) essentially as described previously (17).

The case group will consist of women diagnosed with PE after 34 weeks of pregnancy. With an expected incidence of 2.5%, the sample size of the case group will be n=35 in the OUH cohort (n=1400). The control group will consist of 165 women who were not diagnosed with PE in their pregnancies.

We expect biomarkers to show at least a 15% difference in the expression level between groups and with an expected standard deviation of 25%, a statistical power of 80% ($1-\beta = 0.80$) and a risk of type 1 error of 5% ($\alpha = 0.05$) at 95% confidence interval, we calculate that a sample size of at least 26 cases and 156 controls be necessary for sufficient power. Thus, inclusion of 35 cases and 165 controls is adequate for the proposed study.

Participants and recruitment

The cohort consists of pregnant women assigned to the Department of Obstetrics at Odense University Hospital for their first trimester nuchal translucency between September 2019 and March 2020; who are included/excluded according to the criteria below; and who agree to enroll in the study. The potential participants will receive information about the study by e-boks (online communication platform) at least two days before their appointment for the first trimester scan. Upon arrival to the department, the women will be approached by project staff and those who agree to receive more information will be orally informed about the project and its implications. Each participant will provide written consent followed by blood sampling, MAP measurements, and ultrasound procedures in any order, depending on time and convenience for participants and staff.

Inclusion criteria:

- A planned first trimester nuchal translucency scan
- Informed consent
- Crown-rump length 45-84 mm at the first trimester scan

Exclusion criteria:

- Age < 18 years
- ≥ 2 fetuses
- Participants must read and understand Danish or English

Perspective

PE accounts for at least 63,000 maternal deaths annually, 99% of these occurring in low-and-middle-income countries (LMIC) (10,12). And although LO-PE is typically considered to be a mild-moderate condition with favorable outcome, those that lack accessibility to adequate healthcare, as if often the case, may have a different story to tell (18,19). In some populations, LO-PE is shown to present with severe disease in more than half of cases and complications such as eclampsia and HELLP syndrome are common (18). However, ascertainment of the true extent and consequences of PE is uncertain especially in LMIC due to inaccurate diagnostic criteria and incomplete reporting (12,18).

Ultimately, achieving diagnostic accuracy of PE could contribute to our knowledge not only of its true epidemiology, but also of the pathophysiological pathways which may open the door to new and improved treatment options. Furthermore, early prediction could allow for interventions to be made with the potential of substantially improving maternal health in some of the most vulnerable populations.

Safety, side effects, and ethics

The study will be performed in accordance with the applicable regulatory legislation. The PRESIDE study has been approved by the Committee on Health Research Ethics (Videnskabsetisk Komité) of Region Hovedstaden and has been reported to the Danish Data Protection Agency (Datatilsynet). The proposed sub-study was filed as an addendum and final approval was obtained on September 10 2019.

There are no health risks associated with participation in the study. All blood samples will be double-blinded, and the data analyzed retrospectively. Results will therefore not be available until after the pregnancy is completed. Women who during the study meet the criteria for gestational hypertension, PE, and/or have suspected abnormal fetal growth will be followed and offered treatment according to Danish Obstetric Guidelines.

Practical feasibility

Assoc. prof. Martin Overgaard will be supervising all aspects of study design, blood sampling, biobanking, and statistical analyses, as he leads a biomarker research group using state-of-the art mass spectrometry equipment for targeted proteomics available to support the project.

Consultant, assoc. prof. Lene Sperling is head of the OUH clinic for ultrasound and will be supervising all aspects relating to inclusion of participant and biophysical measurements.

All aspects of the PRESIDE study including approvals, financing, recruitment protocols, and standard operating procedure for biophysical measurements are provided by the PRESIDE steering group, headed by Prof. Ann Tabor and Assoc. professors Charlotte Ekelund and Line Rode, Rigshospitalet.

Time schedule

Activity	Q1 Sep-Nov 2019	Q2 Dec-Feb 2019/20	Q3 Mar-May 2020	Q4 June-Aug 2020
Enrollment of study participants				
Apply ethics committee for biomarker sub-study				
Formation of study groups				
Protein analysis of blood samples				
Outcome data processing				
Thesis and manuscript preparation				

Financing

The PRESIDE study has received grants for a total of 3.201 mio DKK, including 0.5 mio DKK from the RH-OUH research foundation. The expenses for a 6-month pregraduate stipend is covered by the current budget. We plan to seek external funding to cover expenses for the suggested biochemical measurements, which we estimate to be covered by a budget of 30.000 DKK.

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