First trimester screening, forecasting adverse pregnancy outcomes

PhD thesis

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Budapest
2023

1. Introduction

Several pregnancy pathologies can be caused by placental insufficiency: preeclampsia, intrauterine growth restriction (IUGR), gestational diabetes mellitus (GDM). Early screening would be important - to reduce and avoid complications. It is only prevalent in preeclampsia.

At the end of the first trimester, the measurement of the uterine artery pulsatility index (UtAPI) is becoming more and more common - it is reliable when supplemented with placental hormones in preeclampsia. Less in IUGR (additional examination required).

GDM is one of the most common pregnancy pathologies, the most common metabolic/endocrine pregnancy pathology. There are known risk factors: BMI>30, positive anamnesis, higher fasting blood sugar, high maternal age, history of a high birth weight newborn, polycystic ovary syndrome. There is no early screening or prediction. GDM promotes the occurrence of complications during pregnancy and increases the long-term cardiometabolic risk of both the mother and the unborn fetus.

Oxidative and nitrative stress is important in physiological pregnancy, its level increases continuously, at the end of the first trimester, during trophoblast invasion, there is a big jump. It has a role in placentation. However, the level is significantly higher in manifest pregnancy pathologies: GDM, preecalmpsia.

Soluble urokinase plasminogen activator receptor (SuPAR): The receptor is also expressed on immune cells and the syncytiotrophoblast membrane. It plays a role in inflammatory processes and conditions associated with hyperglycemia. However, it was not investigated before in GDM.

During pregnancy, steroid production is a strictly regulated process not only in the mother's body, but also in the fetal placenta. In placental disorders, this balance is disrupted.

2. Objectives

The aims of our study were:
1. to examine the relationship of systemic oxidative-nitrative stress and uterine artery pulsatility index in the first trimester and their correlation to pregnancy outcomes.
2. in the first trimester we collected patient baseline data, we measured routine laboratory parameters, oxidative-nitrative stress markers and steroid metabolites to examine their correlation to the development of GDM later during pregnancy, to identify possible novel early markers for GDM prediction.

3. Methods

3.1. Measuring oxidative stress and pulsatility of the uterine artery in early phase pregnancies

3.1.1. Study protocol

Between 27. May 2016. and 12. December 2017., a prospective type of observational study was conducted at the Department of Obstetrics and Gynecology together with the Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary. Methods of inclusion: during the early phase of pregnancy – namely at the 12^{th} week ultrasound (gestation week 12-13) – otherwise healthy patients (between the ages of 18-40) were randomly chosed and offered the opportunity to participate in the study. Women suffering from hypertension, obesity (BMI over 30), malignant tumors, diabetes mellitus, chronic inflammatory diseases or having twin pregnancies were excluded. Transabdominal ultrasonography was performed for obtaining flowmetric data regarding the uterine artery pulsatility index. Two groups were formed; one was the low UtAPI group, (UtAPI <2.3), (n=31) and the other group of pregnant women was the high UtAPI group, (n=30).
Patient history and blood samples were taken. We checked rutin lab parameters, and the following things were isolated from

blood samples: serum, plasma, and mononuclear leukocyte fractions. Pregnancies were followed until birth to track pregnancy complications, labor circumstances, and anthropometric data of the newborns.

3.1.2. Measuring oxidative-nitrative stress markers

Colorimetry method was used to measure the total plasma peroxide (PRX) concentration of the serum samples; this is a marker of systemic oxidative stress. Total Antioxidant Capacity (TAC) Assay Kit was used to determine plasma total antioxidant capacity levels of the samples. To measure nitrative stress Nitrotyrosine Elisa Kit was used by competitive ELISA method. Intracellular nitrative stress was measured on the leukocyte smears using anti-nitrotyrosine rabbit polyclonal antibodies.
The ratio of positive-stained area (cellular only) was compared to the total cellular area. To analyze the potential additive value of measuring PRX regarding the correlation between birthweight and UtAPI, a new, previously unused parameter was defined and calculated: the ratio of UtAPI to plasma PRX; PIPX= (UtAPI/PRX) * 100.

3.1.3. Statistical analysis

Two-tailed unpaired Student's t-test was implemeted to determine the statistical significance the two patient groups. Chi-square test was used to determine Data regarding normal distribution are presented as mean ± SEM. Non-Gaussian distribution was presented as median [IQR] following logarithmic transformation (TAC). Significancy level was determined at $p<0.05$.

3.2. Prediction of gestational diabetes mellitus in the first trimester

3.2.1. Study protocol

A prospective cohort study was performed between 2010 and 2012 at the Department of Obstetrics and Gynecology at the University of Debrecen Medical and Health Science Centre, Debrecen, Hungary and at the Department of Obstetrics and Gynecology at the Andras Josa County and Teaching Hospital, Nyíregyháza, Hungary. In total 2545 pregnant women (between 11 (+0 days) and 13 (+6 days) weeks of gestation) were recruited. Maternal characteristics, data from the screening ultrasound of the first trimester, medical history, blood (serum and plasma) and urine samples were collected from them, and these were stored at –80°C in an accredited biobank to be available for further study. Pregnancies were followed-up until birth, regarding the following complications of pregnancy: small for gestational age newborns, preeclampsia, gestational diabetes mellitus and macrosomia.

As a result of the collaboration of the Biobank of Debrecen University and Semmelweis University, the GIPS (GDM and IUGR Prediction Study), a retrospective observational study was created. Our aim was to identify novel early risk assessment factors that may be measured and used for screening of GDM and IUGR. Debrecen University provided samples (serum and plasma), clinical data, routine laboratory parameters (CRP, hepatic function, glucose, fructosamine, and creatine kinase), pregnancy outcomes, labor circumstances and newborn parameters of 55 healthy controls and 55-55 patients who subsequently developed GDM or IUGR. As part of the GIPS study, we introduce the results of both control and the GDM groups regarding early prediction of GDM.

3.2.2. Determination of oxidative-nitrative stress related parameters

Total serum peroxide (PRX) concentration, reflecting systemic oxidative stress, was determined from serum samples using colorimetric method - OxyStat assay. Serum total antioxidant capacity was measured from serum samples by commercially available assay kit. Oxidative index (OI) was calculated from the ratio of oxidative stress and TAC. Sandwich ELISA technique was used to determine Soluble Plasminogen Activator Urokinase Receptor (SuPAR) levels. Nitrative stress was determined by measuring plasma levels of 3-nitrotyrosine (NT) using competitive ELISA.

3.2.3. Measuring steroid levels

Steroid levels were measured at the Department of Laboratory Medicine, Semmelweis University. Using reversed-phase liquid chromatography-tandem mass spectrometry (LC-MS/MS) that following parameters were assayed: androstenedione, aldosterone, dehydroepiandrosterone, 11-deoxycorticosterone, dehydroepiandrosterone sulfate, 11-deoxycortisol, dihydrotestosterone, 21-deoxycortisol, 17alpha-hydroxypregnenolone, corticosterone, 17alpha-hydroxyprogesterone, cortisol, pregnenolone, cortisone, testosterone, and progesterone.

3.2.4. Statistical analysis

Either the two-tailed unpaired Student's t-test or - in case of non-normal distribution - the Mann-Whitney test was used to analyze statistical significance between the groups. Pearson's test was used to determine correlation between the parameters. Chi-square test was used to determine nominal variables. To analyze the predictive power of both previously known and potentially novel risk factors regarding prediction of GDM, multivariate logistic regression models were used. $p<0.05$ was considered

significant. Normal distribution data are presented as mean ± SD.

4. Results

4.1. Oxidative stress and uterine artery pulsatility index (UtAPI) in early pregnancy

As a grouping variable UtAPI in the high resistance group was measured to be significantly higher **(Figure 1/A.)**. Systemic oxidative stress, characterized by plasma total peroxide level was significantly lower in the high UtAPI group **(Figure 1/B)**, while TAC was significantly higher in this group **(Figure 1/C)**. PIPX is calculated using UtAPI and PRX values as follows: PIPX=(UtAPI/PRX) *100. These values were found to be significantly higher in the high UtAPI group **(Figure 1/D)**.

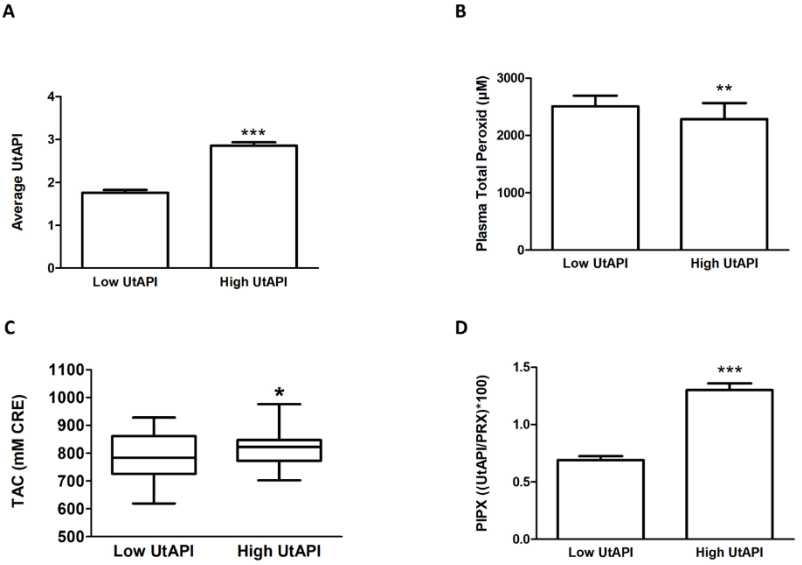

Figure 2. Pulsatility index and oxidative stress markers.
Two-tailed unpaired Student's t-test. Data are presented as mean±SEM, or Median [IQR] in case of TAC. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

The rate of Cesarean section was similar in the two groups. All newborns were in the normal body parameter range; however, newborns of the high UtAPI group had significantly lower birthweight and chest circumference than ones of the low UtAPI group. There was no difference in gestational weeks at labour, therefore these differences were not due to earlier delivery **(Table 1.)**.

Table 1. Pregnancy outcomes and anthropometric data of newborns in low and high UtAPI groups.

Two-tailed unpaired Student's t-test. Values are the means±SEM

Variable	*low UtAPI (n = 31)*	*high UtAPI (n = 30)*	*Significance*
Pregnancies outcome			
Cesarean section (n)	13	11	ns
GDM (n)	3	3	
Bodyweight (g)	3517.41 ± 77.02	3316.79 ± 63.76	$p<0.05$
Gestational week	39.80 ± 0.19	39.30 ± 0.20	ns
Chest circumference (cm)	34.41 ± 0.29	33.57 ± 0.26	$p<0.05$
Head circumference (cm)	35.00 ± 0.26	34.39 ± 0.30	ns
Apgar 1'	9.44 ± 0.11	9.61 ± 0.09	ns
Apgar 5'	10.00 ± 0.00	10.00 ± 0.00	ns

4.2. Gestational diabetes mellitus – prediction in the first trimester

4.2.1. Patient characteristics in early pregnancy - at the end of the first trimester

Patients who developed GDM in our cohort were 3.2 years older on average, they also demonstrated both higher body weight measurements (by 9.04 kg on average) and BMI values (by 3.2 kg/m^2 on average)

4.2.2. Glucose, CRP, fructosamine, liver function and creatin kinase

The two study groups did not differ significantly regarding CRP, liver function and creatine kinase levels and neither did fasting glucose measurements. Levels of fructosamine were found in a normal range, however, these values were markedly higher in the GDM group compared to controls.

4.2.3. Oxidative – nitrative stress parameters and SuPAR

Serum total peroxide levels did not demonstrate differences between the groups, TAC was measured to be significantly higher in the GDM group, however oxidative stress index (calculated from serum total peroxide levels and TAC) did not demonstrate differences between the groups **(Figure 2. A, B, C)**. Plasma 3-nitrotyrosin levels did not differ between the two study groups in our series **(Figure 2. D)**. The later GDM women had significantly lower serum SuPAR levels compared to the controls **(Figure 2. E)**.

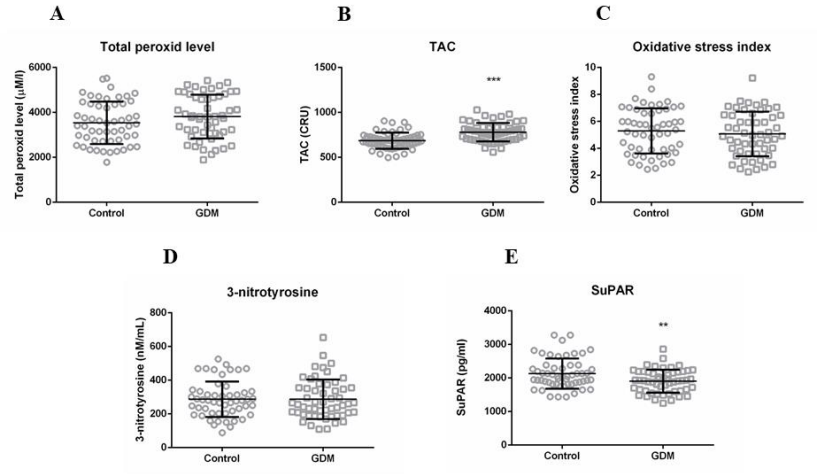

Figure 2. Oxidative – nitrative stress parameters and SuPAR.
Data are shown as mean±SD; two-tailed unpaired Student's t-test and Mann-Whitney test for Oxystat and SuPAR. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ Control vs. GDM

4.2.4. Steroid metabolites

Androgens

Serum levels of DHEA-S (which is the most key adrenal androgen metabolite) were significantly lower in the GDM group. The key ovarian metabolite – testosterone. was found to be significantly higher in the GDM group. The physiologically active metabolite of ovarian testosterone, namely dihydrotestosterone, was decreased in the GDM group leading to a demonstrably decreased DHT/T ratio **(Figure 3. A, B, C, D)**.

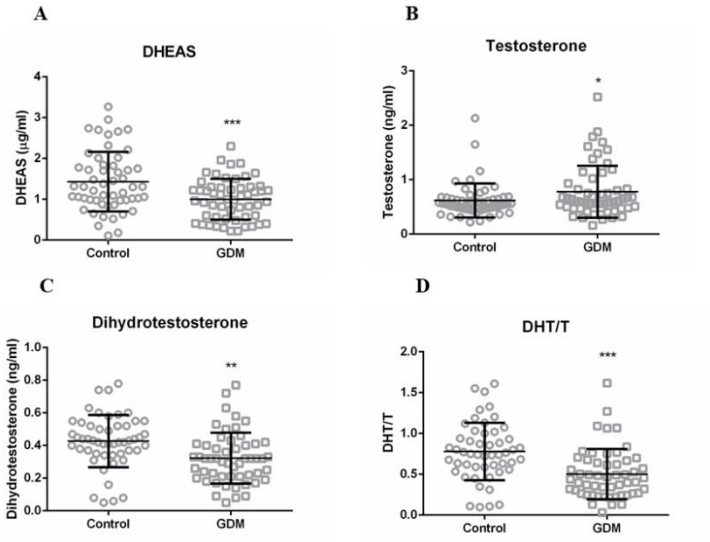

Figure 3. Androgens

Data are shown as mean±SD; two-tailed unpaired Student's t-test. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ Control vs. GDM

Further adrenal steroids: mineralo-and glucocorticoid metabolites

Women in the GDM group were found to have lower cortisol and elevated cortisone levels compared to the control group. The GDM group also demonstrated elevated 21-deoxycortisol levels (a metabolite derived from 17α-hydroxyprogesterone). 11-deoxycorticosterone (a mineralocorticoid and an aldosterone precursor) was significantly lower in the GDM group **(Figure 4. A, B, C, D)**.

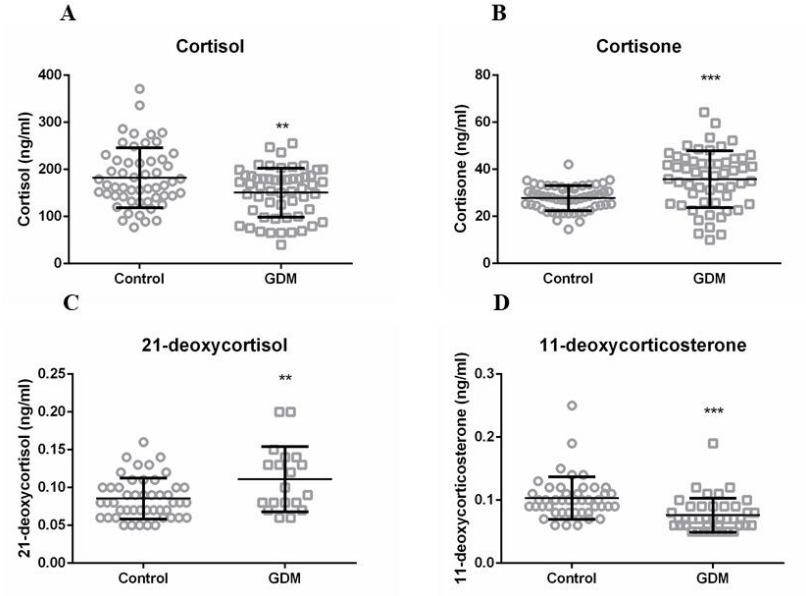

Figure 4. Further steroids

Data are shown as mean±SD; two-tailed unpaired Student's t-test. **: $p < 0.01$, ***: $p < 0.001$ Control vs. GDM

4.2.5. Classic risk factors vs proposed new candidates for assesment of risk – findings regarding potential additional predictive value

We used a stepwise forward multivariate logistic regression model analysis to create a GDM prediction algorithm
that included not only the traditional but also all the novel markers. The highest predictive power that could be achieved was R^2=0.943 ($p<0.001$). This model included the following risk factor candidates: fructosamine, cortison, cortisol, 11-deoxycorticosterone and SuPAR, this model was shown to have a specificity of 96.6% and a sensitivity of 97.5%, respectively.

4.2.6. Maternal and neonatal pregnancy outcomes

Higher body weight, height, head, and-chest circumferences, were found in the GDM newborn group.
A higher number of cesarean sections were performed in the GDM group ($p<0.001$) – the calculated relative risk from the date was 2.067 (Confidence Interval was: 1.266 to 3.375). GDM newborns were also found to demonstrate hypoglycemia ($p<0.01$) more frequently – the calculated relative risk from the data was 1.146 (Confidence Interval was 1.036 to 1.268).

5. Conclusions

After analyzing the results from our study, we propose the following conclusions:
1. Measuring oxidative stress and pulsatility of the uterine artery in early phase pregnancies
-In our cohort high UtAPI alone (with no additional risk factors) resulted in normal range but lower weight newborns.
-LDH and plasma oxidative stress (associated with abnormalities of placentation) were however decreased in our high UtAPI group.

-In further support of this hypothesis, we also found that a combined parameter (PIPX: including both UtAPI and PRX- a marker of oxidative stress) strengthens correlation to birthweight. Higher uterine artery pulsatility index and lower oxidative stress in the first trimester – during the placentation process, are predict lower birthweight.

2. Regarding later onset GDM the following novel risk prediction markers – measured at the end of the first triemster- were identified: SuPAR, TAC, DHEA S, testosterone DHT, cortisone, cortisol, 21-deoxycortisol and 11-deoxycorticosterone.
- When measured at the end of the first trimester women who had GDM pregnancies had significantly higher serum TAC levels and decreased serum SuPAR levels.
- In the later onset GDM group cortisol levels were decreased, while both cortisone and 21-deoxycortisol levels were increased.
- The GDM group demonstrated decreased DHEA-S and dihydrotestosterone levels, and DHT/T ratios, while serum testosterone levels were increased.
- In the GDM group 11-deoxycorticosterone levels were observed to be lower.
-By including both previously known "classical" risk factors and the novel ones, we were successful in building an effective logistic regression model for early prediction of GDM. This enhanced GDM prediction model is superior to any previously published one with a specificity index of 96.6% and a sensitivity of 97.5%. This model included the following risk factor candidates: fructosamine, cortison, cortisol, 11-deoxycorticosterone and SuPAR.

6. Bibliography of the candidate's publications

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