


Risk Estimation of Gestational Diabetes Mellitus in the First Trimester

Dóra Gerszi,^{1,2}  Gergő Orosz,³ Marianna Török,^{1,4} Balázs Szalay,⁵ Gellért Karvaly,⁶ László Orosz,³ Judit Hetthéssy,⁴ Barna Vásárhelyi,⁵ Olga Török,³ Eszter M. Horváth,^{2,*} and Szabolcs Várbíró^{1,4,*}

¹Department of Obstetrics and Gynecology, Faculty of Medicine, Semmelweis University, Budapest H-1082, Hungary

²Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest H-1094, Hungary

³Department of Obstetrics and Gynecology, Faculty of Medicine, University of Debrecen Medical and Health Science Centre, Debrecen H-4032, Hungary

⁴Workgroup for Science Management, Doctoral School, Semmelweis University, Budapest H-1085, Hungary

⁵Department of Laboratory Medicine, Semmelweis University, Budapest H-1083, Hungary

⁶Laboratory of Mass Spectrometry and Separation Technology, Department of Laboratory Medicine, Semmelweis University, Budapest H-1089, Hungary

Correspondence: Dóra Gerszi, MD, Department of Obstetrics and Gynecology, Faculty of Medicine, Semmelweis University, Budapest, Üllői út 78/A, H-1082, Hungary. Email: gerszi.dora@med.semmelweis-univ.hu.

*These authors contributed equally to this work.

Abstract

Context: There is no early, first-trimester risk estimation available to predict later (gestational week 24–28) gestational diabetes mellitus (GDM); however, it would be beneficial to start an early treatment to prevent the development of complications.

Objective: We aimed to identify early, first-trimester prediction markers for GDM.

Methods: The present case–control study is based on the study cohort of a Hungarian biobank containing biological samples and follow-up data from 2545 pregnant women. Oxidative–nitrate stress-related parameters, steroid hormone, and metabolite levels were measured in the serum/plasma samples collected at the end of the first trimester from 55 randomly selected control and 55 women who developed GDM later.

Results: Pregnant women who developed GDM later during the pregnancy were older and had higher body mass index. The following parameters showed higher concentration in their serum/plasma samples: fructosamine, total antioxidant capacity, testosterone, cortisol, 21-deoxycortisol; soluble urokinase plasminogen activator receptor, dehydroepiandrosterone sulfate, dihydrotestosterone, cortisol, and 11-deoxycorticosterone levels were lower. Analyzing these variables using a forward stepwise multivariate logistic regression model, we established a GDM prediction model with a specificity of 96.6% and sensitivity of 97.5% (included variables: fructosamine, cortisol, cortisone, 11-deoxycorticosterone, SuPAR).

Conclusion: Based on these measurements, we accurately predict the development of later-onset GDM (24th–28th weeks of pregnancy). Early risk estimation provides the opportunity for targeted prevention and the timely treatment of GDM. Prevention and slowing the progression of GDM result in a lower lifelong metabolic risk for both mother and offspring.

Key Words: GDM, first trimester, early risk estimation, oxidative-nitrate stress, steroid metabolites

Abbreviations: BMI, body mass index; CRP, C-reactive protein; DHEAS, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; DHT/T, dihydrotestosterone/testosterone ratio; DM, diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; GDM, gestational diabetes mellitus; PXR, total serum peroxide; SuPAR, soluble urokinase plasminogen activator receptor; TAC, total antioxidant capacity; uPAR, urokinase plasminogen activator receptor.

According to the International Association of Diabetes and Pregnancy Study Groups screening criteria, gestational diabetes mellitus (GDM) is the most common metabolic disorder during pregnancy (1–3), with a prevalence of 17% (2–4). GDM increases the lifelong cardiometabolic risk for both the mother and the offspring (5). There is a direct need for an effective clinical risk assessment tool because current risk estimation tools are neither timely nor reliable. Twenty percent of pregnant women diagnosed with GDM present without acknowledged risk factors (6, 7). However, there are meta-analytic data available suggesting that improving the detection rate of GDM in the early phase of pregnancy would

allow for early intervention, in some cases prevention, and in others a more effective treatment: in other words, moderate physical exercise reduced the risk of GDM by 30% in high-risk populations (8, 9). Preeclampsia risk assessment is widely accepted to be performed during the 12th week (10). However, there is no similar risk estimation tool for gestational diabetes in the early phases of pregnancy.

Assessment markers such as fasting glucose, and HbA1c have previously been measured in the first trimester. High levels of HbA1c were only found to correlate with GDM independently between gestational weeks 24 and 28 in diabetes; even though HbA1c is not an effective tool for early

prediction, it does mark elevated risk (11). Serum uric acid and certain DNA methylation patterns were also found to be associated with higher GDM risk (12, 13), but these methods were not sensitive or specific enough to be considered reliable tools regarding risk estimation. A more effective and reliable method is needed to improve outcomes.

Physiological pregnancy increases oxidative stress (14); however, oxidative stress plays a pivotal role in the development of inflammation, hyperglycemia, and diabetic complications (15, 16). Manifest GDM (15, 17) is characterized by increased oxidative stress (defined here as higher than in physiological pregnancy). Both oxidative damage and dysfunction of antioxidant defense mechanisms appear in type 2 diabetes mellitus and GDM (15). Altered oxidative stress and total antioxidant capacity (TAC) have been proposed to be a contributing factors to the development of GDM, but currently these are not examined in the first trimester.

Urokinase plasminogen activator receptor (uPAR) is a membrane-bound receptor of the immunologically active cell membranes; it plays a role in inflammation and immune responses (18). Syncytiotrophoblast cells also express uPAR during implantation (19); its soluble form (SuPAR) is derived from membrane-bound uPAR. Increased plasma or serum levels are associated with inflammation, diabetes mellitus (DM), diabetic complications (20), and preeclampsia (21). The correlation between SuPAR and GDM has not been investigated yet, but GDM is a type of diabetes and inflammation plays a role in the development of GDM (22).

During pregnancy, the interaction between the fetal and maternal adrenal cortex (Fig. 1) and the placenta provides the optimal production of placental steroid hormones. Dehydroepiandrosterone (DHEA) and the sulfated form (DHEAS) are the major precursors of estrogen, and they are produced by the adrenal glands, although the fetal liver may modify them via 16- α hydroxylase enzyme resulting in 16-hydroxy-DHEA and DHEAS. The placenta in turn synthesizes estrone, estradiol, and estriol from these precursors, resulting in androstenedione and testosterone as intermediate metabolites. Alterations of androgen hormone production are linked to insulin resistance in nonpregnant women (23); however, possible roles and predictive values regarding the development of GDM have rarely been investigated until now. Higher total testosterone level was described in the first trimester in women who developed GDM later during the pregnancy (24). Corticotropin-releasing hormone and 11- β -hydroxysteroid dehydrogenases 1 and 2 are also expressed by the placenta: these regulate the interaction between the maternal and fetal cortisol homeostasis (25). Higher cortisol levels have been demonstrated in the second trimester in GDM (25, 26); however, the alterations of cortisol and its metabolites have not yet been studied in the first trimester.

In the present case-control study, we examined oxidative-nitrative stress markers, SuPAR, and steroid metabolites in the first trimester to identify possible novel early markers of later-onset GDM.

Materials and Methods

Patient History, Sampling

The Department of Obstetrics and Gynecology at the University of Debrecen Medical and Health Science Centre, Debrecen, Hungary, and the Department of Obstetrics and Gynecology at the Andras Josa County and Teaching

Hospital, Nyiregyhaza, Hungary, performed a prospective cohort study between 2010 and 2012. A total of 2545 pregnant women (Caucasian population) were recruited to the study between 11 (+0 days) and 13 (+6 days) weeks of gestation. In the study, maternal characteristics, medical history, and the first trimester screening ultrasound were recorded. At the same time, blood (serum and plasma) and urine samples were collected and stored at -80°C in an accredited biobank for further study. Pregnancies were followed up to screen for major adverse pregnancy complications. The study protocol was approved by the ethics committee (identification number: DEOEC RKEB/IKEB 3092-2010), and written informed consent was obtained from all participants.

Current Study

The present case-control study, the GIPS (GDM and IUGR Prediction Study) was based on a collaboration between Debrecen University (DE) Biobank and Semmelweis University to identify measurable novel early risk assessment factors regarding GDM and IUGR (ethics approval: ETT TUKEB 4/4414-4/2020/EKU). DE provided samples (serum and plasma), clinical data, routine laboratory parameters (C-reactive protein [CRP], hepatic function, glucose, fructosamine, and creatine kinase), pregnancy outcomes, labor circumstances, and newborn parameters of 55 healthy controls and 55 patients who subsequently developed GDM or intrauterine growth restriction (IUGR). GDM patients were selected based on NICE criteria: fasting glucose level ≥ 5.6 mmol/L and/or 120 minutes glucose level ≥ 7.8 mmol/L. Exclusion criteria were age below 18 and over 40 years, class II obesity (body mass index [BMI] over 35 kg/m^2), malignant tumors, hypertension, DM, twin pregnancy, and chronic inflammatory diseases. In the first publication of the GIPS study, we introduce the results of the control and GDM groups for the prediction of GDM.

Determination of Oxidative-Nitrative Stress-Related Parameters

Total serum peroxide (PRX) concentration, reflecting systemic oxidative stress, was determined from serum samples using a colorimetric method (OxyStat assay; Biomedica, Wien, Austria, Cat# BI-5007). Serum TAC was measured from serum samples using an OxiSelect TAC Assay Kit, (Cell Biolabs Inc., San Diego, CA, USA, Cat# STA-360). The oxidative index was calculated from the ratio of oxidative stress and TAC. SuPAR was measured from serum samples using the enzyme-linked immunosorbent assay (ELISA) (SuPAR human ELISA kit, BioVendor, Brno, Czech Republic), (RRID: AB_2927796). Nitrative stress was determined by measuring plasma levels of 3-nitrotyrosine with competitive ELISA using horseradish peroxidase conjugated anti-3-nitrotyrosine antibodies (OxiSelect Nitrotyrosine Elisa Kit, Cell Biolabs Inc., San Diego, CA) (RRID:AB_2927798).

Determination of Steroid Levels

This was performed by the Department of Laboratory Medicine, Semmelweis University. Aldosterone, androstenedione, DHEA, DHEAS, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisol, dihydrotestosterone (DHT), 17- α -hydroxypregnenolone, 17- α -hydroxyprogesterone, corticosterone, cortisol, cortisone, pregnenolone, progesterone, and testosterone were assayed using reversed phase liquid

no differences between the control and GDM groups in terms of height, weight gain during pregnancy, gravidity, and parity (Table 1). No one admitted to drinking alcohol or smoking.

Glucose, Fructosamine, CRP, Creatine Kinase, and Liver Function

Fasting glucose, CRP, creatine kinase, and liver function did not differ significantly between the groups. Fructosamine levels were in the normal range; however, the GDM group values were significantly higher than controls (Table 2).

Oxidative—Nitrate Stress Parameters and SuPAR

Although serum total PXR levels were similar in the 2 groups; TAC was significantly increased in the GDM group, whereas the oxidative stress index calculated from the previous 2 parameters was not significantly different between the 2 groups (Fig. 2A-2C). There were no differences regarding plasma 3-nitrotyrosine levels between the groups (Fig. 2D). Serum SuPAR was significantly decreased in the GDM group compared with controls (Fig. 2E).

Steroid Metabolites

Androgens

DHEAS, the main adrenal androgen metabolite, was significantly decreased in the GDM group. Testosterone, which is the main ovarian androgen metabolite, was significantly elevated in the GDM group, in contrast with DHT, the active metabolite of ovarian testosterone, which was decreased, resulting in a markedly decreased DHT/T ratio (Fig. 3A-3D).

Mineralocorticoid and Glucocorticoid Metabolites

The cortisol level was lower; however, the level of cortisone was higher in the GDM group than controls. 21-Deoxycortisol (which is formed as a metabolite from 17- α -hydroxyprogesterone) was elevated in the GDM group. 11-Deoxycorticosterone (has mineralocorticoid

activity and acts as a precursor to aldosterone) was significantly decreased in the GDM group compared with the control group (Fig. 4A-4D).

Correlations Regarding Known Risk Factors of GDM and New Candidates for Risk Factor Markers

Plasma TAC, which was elevated in the GDM group, showed a positive correlation with BMI. SuPAR, which was lower in the GDM group, did not correlate with any of the known and analyzed risk factors. DHEAS and DHT were reduced in women who later developed GDM, and they also showed a negative correlation with maternal age. DHT also showed a negative correlation with serum fructosamine levels. Regarding androgen hormones, testosterone had no significant correlation with the measured parameters. Cortisol levels were reduced in the GDM group, and they correlated negatively with gravidity and parity; cortisone levels showed no significant correlations. 11-Deoxycorticosterone levels were also lower in the GDM group, and they correlated negatively with BMI. (Table 3).

Additional Predictive Value of the New Risk Factors Compared With the Classic Ones

A multivariate logistic regression model was implemented for each candidate risk factor, including known risk parameters of GDM, that showed a significant correlation with GDM in our study cohort: age, BMI, and fructosamine levels. Including only these 3 parameters from the end of the first trimester, the baseline model showed a significant correlation with the development of GDM later in the pregnancy ($R^2 = .464$, $P < .001$), and each parameter was found to be an independent risk factor ($P < .01$ for each variable). Integrating TAC into the model (age, BMI, fructosamine, TAC) increased $R^2 = .597$ ($P < .001$)—all 4 variables were demonstrated to be significant independent determinants ($P < .05$). Similarly, to TAC, all examined novel markers—apart from 21-deoxycortisol—had significant additive values to the baseline logistic regression model and were determined to be independent determinants. The predictive power of the models by the inclusion of these variables altered as follows: SuPAR, $R^2 = .514$; testosterone, $R^2 = .526$; DHT, $R^2 = .512$; DHEAS, $R^2 = .530$; cortisone, $R^2 = .619$; 11-deoxycorticosterone, $R^2 = .495$ ($P < .001$ for all models, and $P < .05$ for all 4 variables in each model). Implementing a stepwise forward multivariate logistic regression model including all traditional and novel markers, the highest predictive power was $R^2 = .943$ ($P < .001$). This model included fructosamine, cortisol, cortisone, 11-deoxycorticosterone, and SuPAR, and demonstrated specificity and sensitivity of 96.6% and 97.5% respectively.

Maternal and Neonatal Pregnancy Outcome Parameters

The rate of cesarean section was higher in the GDM group ($P < .001$)—the relative risk was 2.067 (CI 1.266-3.375).

GDM newborns demonstrated higher body weight, height, and head and chest circumference values than controls (Table 4). Frequency of jaundice ($P < .01$) and hypoglycemia ($P < .01$) were higher in GDM newborns—the relative risks were 1.146 in both parameters (CI 0.9488-1.385 and 1.036-1.268, respectively).

Table 2. Glucose metabolism, CRP, and liver function parameters at the 12th week

Variable	Control (n = 55)	GDM (n = 55)	Significance
Glucose (mmol/L)	4.47 \pm 0.81	4.68 \pm 1.13	ns
Fructosamine (μ mol/L)	200.93 \pm 13.53	214.02 \pm 22.98	$P < 0.001$
Creatine kinase (U/L)	53.25 \pm 21.94	52.48 \pm 18.67	ns
CRP (mg/L)	6.38 \pm 6.26	8.62 \pm 8.40	ns
ALP (U/L)	59.20 \pm 14.20	58.91 \pm 14.20	ns
LDH (U/L)	146.22 \pm 27.20	139.94 \pm 33.80	ns
AST (U/L)	15.66 \pm 3.65	15.20 \pm 3.51	ns
ALT (U/L)	12.58 \pm 5.00	13.49 \pm 5.49	ns
GGT (U/L)	12.89 \pm 6.85	14.50 \pm 9.05	ns

GDM patients demonstrated significantly higher fructosamine levels. Other labor parameters did not differ between the 2 groups. Two-tailed unpaired Student's t-test. Values are the means \pm SD.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, c-reactive protein; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase.

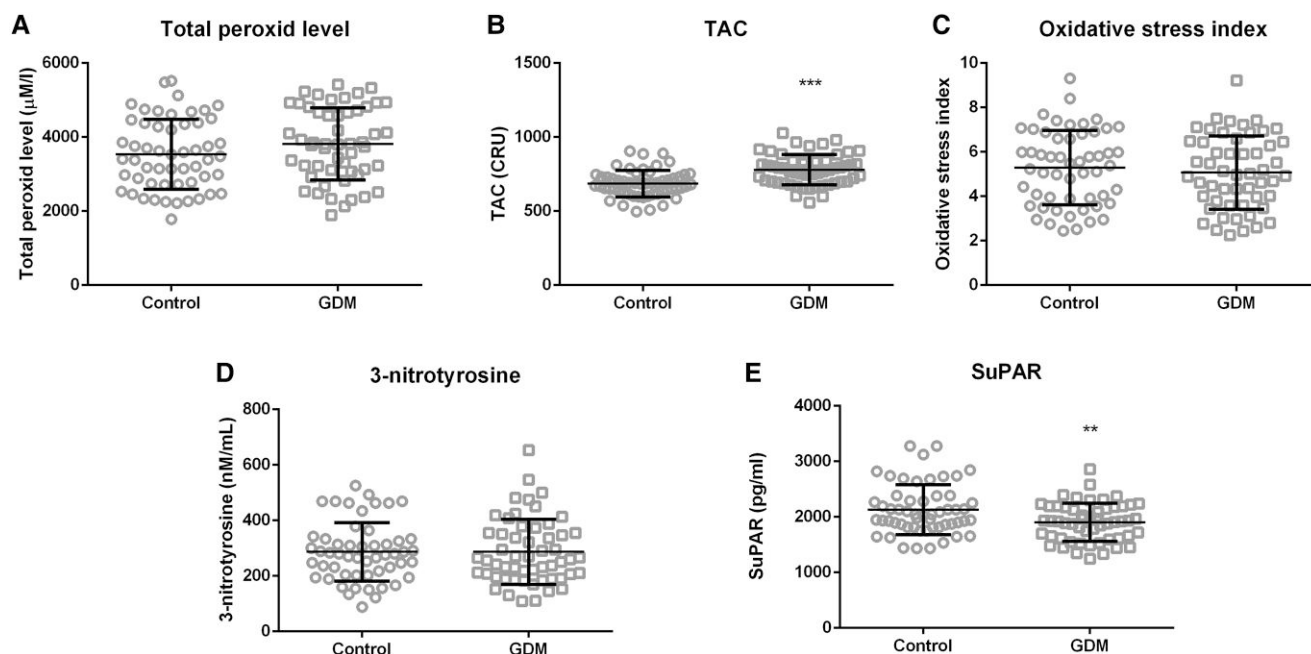


Figure 2. Oxidative–nitrosative stress parameters and SuPAR. (A) Serum total peroxide level. The oxidative stress in the serum was similar in the 2 groups ($n = 54$ and $n = 53$). (B) Total antioxidant capacity (TAC). GDM was associated with more pronounced TAC ($n = 55$ and $n = 55$). (C) Oxidative stress index. The oxidative stress index did not differ between the control and the GDM groups ($n = 54$ and $n = 53$). (D) Plasma levels of 3-nitrotyrosine (NT). The nitrosative stress did not differ between the control and GDM groups ($n = 53$ and $n = 55$). (E) Soluble urokinase plasminogen activator receptor (SuPAR). The GDM group showed significantly reduced serum SuPAR levels ($n = 55$ and $n = 54$). Data are shown as mean \pm SD; 2-tailed unpaired Student's *t*-test and Mann–Whitney test for Oxystat and SuPAR. ** $P < .01$, *** $P < .001$ control vs GDM.

Discussion

In this case–control study, the serum level of new oxidative stress markers and steroid metabolites used for the prediction of GDM were measured at the end of the first trimester. These parameters when pooled with the classical risk factors allow a significantly more precise, and therefore clinically relevant, risk estimation for GDM at the end of the first trimester regarding the development of GDM later during the pregnancy. This novel stepwise forward multivariate logistic regression model resulted in specificity and sensitivity values of over 95% at the end of the first trimester, making it an effective clinical tool regarding early risk estimation for later-onset GDM.

In this study, from the classic risk factors, GDM patients were older, and had higher body weight, BMI, and serum fructosamine levels, even in the first trimester. There is no useful and widely accepted risk assessment for GDM available for the first trimester. Maternal age, BMI, parity, history of previous GDM or large for gestational age fetus, fasting and post-load glucose, and HbA1c have a moderate predictive value regarding GDM risk assessment (28–32). Inflammatory biomarkers (eg, CRP, tumor necrosis factor alpha), liver function (alanine aminotransferase, gamma-glutamyl transferase) (33), serum vitamin and trace element concentrations (vitamin D, vitamin A, selenium) (34–36), platelet characteristics (37), specific miRNAs (38), or adipokines (adiponectin, leptin) (33) and their combination have been examined but until now have failed to be sufficiently effective regarding the early prediction of GDM (the best combination demonstrated 60–77% specificity and sensitivity at the 12th or 16th week) (39, 40).

Our current analysis revealed that serum TAC was significantly higher and serum SuPAR was decreased in the GDM

group when measured at the end of the first trimester. 21-Deoxycortisol and cortisone levels were increased, while cortisol levels were decreased in women who developed GDM later during their pregnancy. DHEAS, DHT, and DHT/T ratios also decreased, and testosterone levels were increased in the GDM group. 11-Deoxycorticosterone levels were decreased. Our proposed risk estimation model—based on these parameters—with its specificity and sensitivity values of over 95% is currently the most effective first-trimester GDM assessment algorithm available.

Oxidative-nitrosative Stress Markers and SuPAR in GDM Risk Estimation

Our results showed that serum TAC was significantly increased at the end of the first trimester in women who developed GDM later during the pregnancy, without a significant change in plasma total PRX levels and oxidative stress index calculated by PRX/TAC, suggesting an elevated level of reactive free radical formation that is compensated by the increased production of endogenous antioxidants. PRX and TAC showed a positive correlation with BMI, which underlines the role of obesity in the development of oxidative stress. However, according to the multiple logistic linear regression model, increased TAC at the end of the first trimester was independently associated with the later development of GDM. This is the first report on oxidative stress in the first trimester.

Oxidative–nitrosative stress is elevated due to the elevated blood glucose levels in both type 1 and type 2 DM (41). Decreased antioxidant levels have also been found in patients with type 2 DM and complications (42). On the other hand, increased oxidative–nitrosative stress plays a role in the

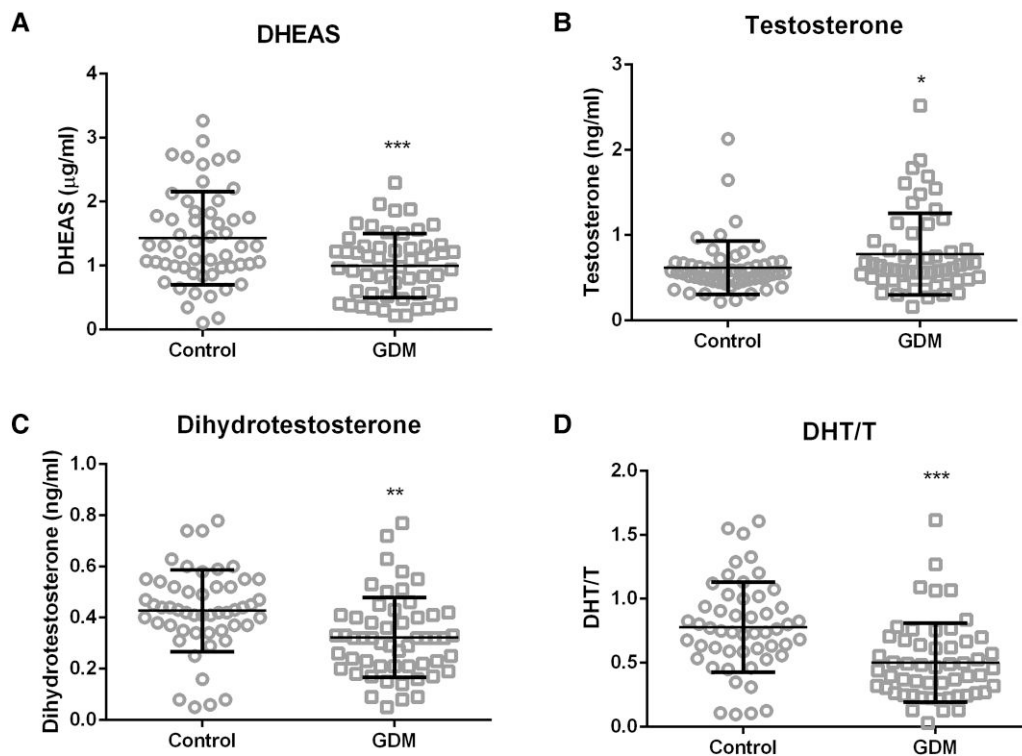


Figure 3. Androgens. (A) Dehydroepiandrosterone sulfate (DHEAS). The level of DHEAS was significantly lower in the gestational diabetes mellitus (GDM) group (n = 54 and n = 55). (B) Testosterone (T). GDM was associated with an elevated level of testosterone (n = 54 and n = 55). (C) Dihydrotestosterone (DHT). The level of DHT was significantly decreased in the GDM group (n = 51 and n = 54). (D) DHT/T ratio. The DHT/T ratio was significantly lower in the GDM group (n = 51 and n = 54). Data are shown as mean \pm SD; 2-tailed unpaired Student's t-test. * P < .05, ** P < .01, *** P < .001 control vs GDM.

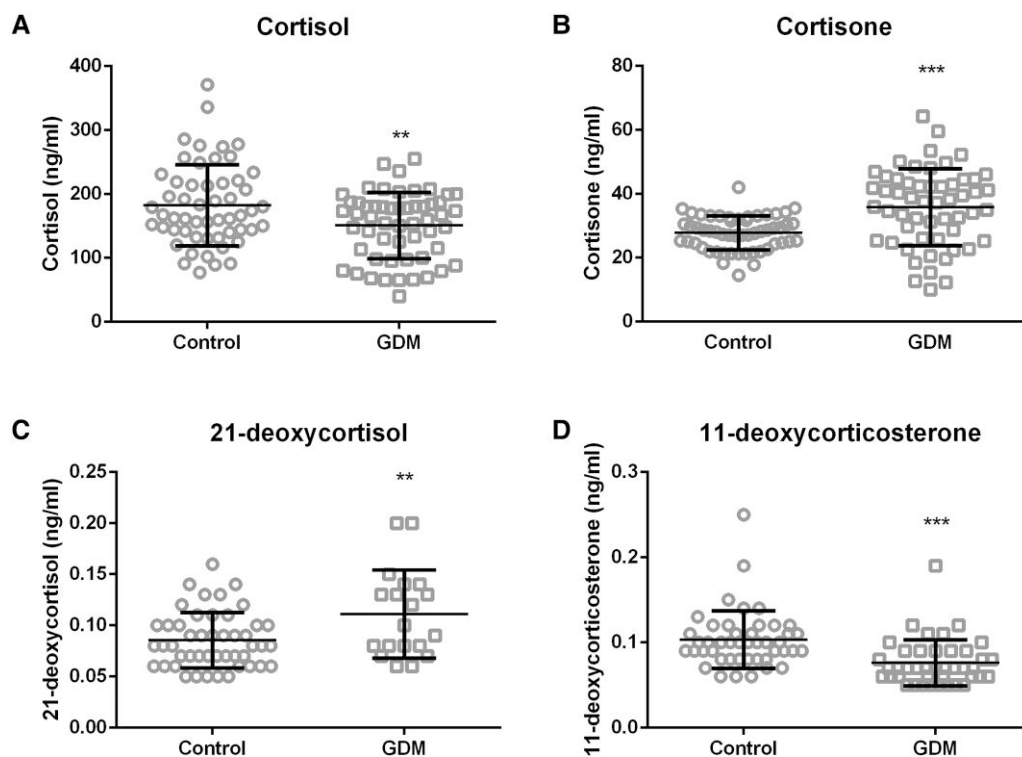


Figure 4. Other steroids. (A) The level of cortisol was significantly lower in the GDM group (n = 54 and n = 55). (B) The level of cortisone was elevated in the GDM group (n = 54 and n = 55). (C) The 21-deoxycortisol was significantly higher in the GDM group than controls (n = 47 and n = 19). (D) The level of 11-deoxycorticosterone was significantly lower in the GDM group (n = 45 and n = 41). Data are shown as mean \pm SD; 2-tailed unpaired Student's t-test. ** P < 0.01, *** P < 0.001 control vs GDM.

Table 3. Correlation of known risk factors of GDM to TAC, SuPAR and steroid hormone metabolites that were found significantly different in the GDM group compared to controls

	Age (years)	BMI (kg/m ²)	Gravidity	Parity	Fructosamine (μmol/L)
TAC (CRU)	<i>P</i> = .24 <i>r</i> = .11	<i>P</i> = .01 <i>r</i> = .32	<i>P</i> = .23 <i>r</i> = .12	<i>P</i> = .46 <i>r</i> = .076	<i>P</i> = .94 <i>r</i> = -.007
SuPAR (pg/mL)	<i>P</i> = .13 <i>r</i> = -.15	<i>P</i> = .81 <i>r</i> = -.02	<i>P</i> = .17 <i>r</i> = -.13	<i>P</i> = .18 <i>r</i> = -.13	<i>P</i> = .07 <i>r</i> = -.17
DHEAS (ng/mL)	<i>P</i> = .01 <i>r</i> = -.25	<i>P</i> = .75 <i>r</i> = -.03	<i>P</i> = .12 <i>r</i> = -.15	<i>P</i> = .07 <i>r</i> = -.17	<i>P</i> = .88 <i>r</i> = -.02
T (ng/mL)	<i>P</i> = .44 <i>r</i> = -.08.	<i>P</i> = .06 <i>r</i> = .18	<i>P</i> = .35 <i>r</i> = -.09	<i>P</i> = .41 <i>r</i> = -.08	<i>P</i> = .09 <i>r</i> = -.16
DHT (ng/mL)	<i>P</i> = .035 <i>r</i> = -.24	<i>P</i> = .58 <i>r</i> = -.05.	<i>P</i> = .23 <i>r</i> = -.12.	<i>P</i> = .25 <i>r</i> = -.11	<i>P</i> = .015 <i>r</i> = -.24
21-Deoxycortisol (ng/mL)	<i>P</i> = .76 <i>r</i> = .04	<i>P</i> = .08 <i>r</i> = .22	<i>P</i> = .38 <i>r</i> = -.11	<i>P</i> = .36 <i>r</i> = -.11	<i>P</i> = .12 <i>r</i> = .19
Cortisol (ng/mL)	<i>P</i> = .11 <i>r</i> = -.15	<i>P</i> = .11 <i>r</i> = -.16	<i>P</i> = .024 <i>r</i> = -.22	<i>P</i> = .01 <i>r</i> = -.24	<i>P</i> = .72 <i>r</i> = -.04
Cortisone (ng/mL)	<i>P</i> = .74 <i>r</i> = .03	<i>P</i> = .37 <i>r</i> = .09	<i>P</i> = .08 <i>r</i> = -.17	<i>P</i> = .08 <i>r</i> = -.17	<i>P</i> = .51 <i>r</i> = .07
11-deoxycorticosterone (ng/mL)	<i>P</i> = .07 <i>r</i> = -.2	<i>P</i> = .001 <i>r</i> = -.35	<i>P</i> = .49 <i>r</i> = -.08	<i>P</i> = .26 <i>r</i> = -.12	<i>P</i> = .82 <i>r</i> = .03

TAC showed a positive correlation with BMI. DHEAS correlated negatively with maternal age. DHT correlated negatively with maternal age and with serum fructosamine levels. Serum cortisol correlated negatively with gravidity and with parity also. 11-Deoxycorticosterone showed a negative correlation with BMI. Statistical analysis: Pearson correlations. Level of significance *P* < .05.

Abbreviations: DHEAS, dehydroepiandrosterone sulfate; SuPAR, soluble urokinase activator receptor; T, total testosterone; DHT, dihydrotestosterone; TAC, total antioxidant capacity.

Table 4. Neonatal pregnancy outcome parameters

Variable	Control (n = 55)	GDM (n = 55)	Significance
Birth weight (g)	3266.90 ± 236.1	4587.10 ± 630.80	<i>P</i> < .001
Birth height (cm)	51.24 ± 1.72	52.84 ± 2.29	<i>P</i> < .001
Head circumference (cm)	34.42 ± 1.46	35.61 ± 1.46	<i>P</i> < .001
Chest circumference (cm)	33.42 ± 1.38	35.58 ± 1.85	<i>P</i> < .001

GDM newborns were found to have higher body weight, height, and head-and chest circumference values. Two-tailed unpaired Student's t-test. Values are the means ± SD.

Abbreviations: GDM, gestational diabetes mellitus.

development of insulin resistance and beta cell dysfunction (43) independently from pregnancy.

Oxidative-nitrative stress increases in a healthy pregnancy, whereas GDM was accompanied by an even more pronounced elevation (15). These investigations, however, were carried out following the diagnosis of GDM, in the third trimester or in the second trimester, at the 16th week, in the high-risk population. Li et al found elevated levels of protein carbonyl, 8-iso-prostaglandin F_{2a} and advanced oxidative protein products at 16-20 weeks and 32-36 weeks of gestation in GDM (44). Murthy et al examined oxidative stress and proinflammatory cytokines in GDM pregnancies (24-28 weeks and 12-16 weeks for high-risk patients). The proinflammatory cytokines interleukin-6 and interleukin-8 were also significantly elevated in the GDM group (45). Several studies found that TAC levels (46, 47) were significantly decreased in manifest GDM and the diet and antioxidant intake may reduce abnormal glucose levels and parallel oxidative stress in pregnant women (48). In contrast, Zamani-Ahari et al found elevated TAC levels in the saliva of pregnant women with manifest GDM (49).

We found lower levels of serum SuPAR in pregnant women at the end of the first trimester who developed GDM later. In our model, SuPAR was also an independent risk factor regarding the development of GDM—this is the first observation, no other studies have focused on SuPAR in GDM or GDM prediction. We suppose a biphasic change of SuPAR in GDM pregnancy; lower levels at the first trimester and higher in

the third because SuPAR levels were measured only in type 2 DM where it was elevated (50).

Steroid Results

Glucocorticoids

In our current study at the end of the first trimester, we found that 21-deoxycortisol and cortisone were increased in the GDM group, while cortisol levels were decreased. One of the reasons behind impaired glucose tolerance in pregnancy is an increase in cortisol and derivatives, as contra-insular hormones. Previous studies have focused on measuring metabolites of steroid hormone biosynthesis and tryptophan metabolism in maternal urine samples in gestational diabetes (51). Until now, changes in steroid metabolites were studied in the third trimester—not for early risk assessment, but to diagnose manifest GDM, and increased clearance of tetrahydroaldosterone-3-glucuronide, 11-oxo-androsterone-glucuronide, 5-androstene-3b,16b,17a-triol, cortolone-3-glucuronide, 21-deoxycortisol was observed (51). 11-Beta-hydroxysteroid dehydrogenase (11β-HSD) is responsible for transforming active cortisol into inactive cortisone. At term, maternal serum cortisol levels were significantly higher while cortisone levels did not differ in GDM (52). In GDM, 11β-HSD1 levels were decreased while 11β-HSD2 were increased in the placenta (52). In our current study, decreased cortisol and increased cortisone levels were measured in the plasma at the end of the first trimester in pregnant women who developed GDM later during pregnancy.

Increased cortisone was an independent risk factor for GDM. Changing cortisol and cortisone levels from early to late pregnancy in GDM might sign altered placental function from the time of placentation.

Androgens

In our current study, the DHEAS level was significantly decreased at the end of the first trimester, while the testosterone level was significantly increased in GDM compared with the controls. This indicates that patients who develop GDM later during their pregnancy may have altered sulfatase or sulfotransferase enzyme functions at this stage of the pregnancy.

Also, in our current study elevated testosterone levels were associated with decreased DHT levels and reduced DHT/T ratio, suggesting saturation or reduced capacity of the 5-alpha reductase enzyme. This hypothesis is supported by the finding that urine 5-alpha-tetrahydrocortisol/tetrahydrocortisol ratio also was decreased in GDM (53). Our results suggest that these changes in maternal androgenic metabolism in the first trimester have a significant predictive role in the development of GDM later.

Mineralocorticoids

In this study, 11-deoxycorticosterone levels were significantly decreased at the end of the first trimester in the patients who developed GDM later. This is the first study that examines alterations of 11-deoxycorticosterone levels in the prediction of GDM until now. Previous studies in prediabetes and type 2 DM have found elevated levels of aldosterone and 11-deoxycorticosterone (however, the latter was not statistically significant following matching for the known risk factors) (54).

GDM Prediction in the Study Cohort

According to our data TAC, SuPAR, cortisone, cortisol, testosterone, DHT, DHEAS, and 11-deoxycorticosterone measured at the end of the first trimester may all significantly increase the predictive power of GDM risk estimation over the classic risk factors as independent determinants. Our model has significantly higher predictive power than any previously published one: with a specificity of 96.6% and sensitivity of 97.5% (included variables: fructosamine, cortisol, cortisone, 11-deoxycorticosterone, and SuPAR) at the 12th week of pregnancy.

Conclusions

In this study, the following novel risk estimation markers were identified regarding GDM at the end of the first trimester: SuPAR, cortisol, cortisone, DHEAS, testosterone DHT, TAC, 11-deoxycorticosterone, and 21-deoxycortisol. Using the well-established risk factors and these new predictors, we were able to build a logistic regression model for clinically relevant early prediction of later-onset GDM. This novel model provides the opportunity for early intervention, prevention, and improving pregnancy outcomes and decreasing complications with early screening.

Strengths and Limitations

In the present case-control study, we measured various possible early markers of GDM. We focused on 2 promising fields: oxidative stress and steroid hormone metabolites. We

were able to identify 9 possible new markers and build a prediction model. This study is based on the big population GIPS study from Hungary.

Funding

This project was supported by grants from the Hungarian Hypertension Society (M.T., S.V.), the Dean of the Medical Faculty, Semmelweis University (S.V.), and the Semmelweis Science and Innovation Fund (STIA-OTKA-2021, S.V. and OTKA-PD 113022 NKFIH-FK 129206).

Author Contributions

D.G.: study design, measurements, statistics, preparing the manuscript. G.O.: sample recruitment, study design, revising the manuscript. M.T.: preparing the manuscript. B.Sz.: SuPar measurements. G.K.: steroid measurements, revising the manuscript. L.O.: sample recruitment. J.H.: preparing and revising the manuscript. B.V.: measurements. O.T.: study design, revising the manuscript. E.M.H.: study design, measurements, statistics, preparing the manuscript. Sz.V.: study design, statistics, preparing the manuscript.

Disclosures

The authors declare no conflict of interest.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

- Ding Q, Hu Y, Fu Y, Qian L. Systematic review and meta-analysis of the correlation between intestinal flora and gestational diabetes mellitus. *Ann Palliat Med.* 2021;10(9):9752-9764.
- Guariguata L, Linnenkamp U, Beagley J, Whiting DR, Cho NH. Global estimates of the prevalence of hyperglycaemia in pregnancy. *Diabetes Res Clin Pract.* 2014;103(2):176-185.
- International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care.* 2010;33(7):676-682.
- Shah NS, Wang MC, Freaney PM, et al. Trends in gestational diabetes at first live birth by race and ethnicity in the US, 2011-2019. *JAMA.* 2021;326(7):660-669.
- Feng Y, Zhao Z, Fu D, Gao W, Zhang F. Maternal and neonatal outcomes after energy-restricted diet for women with gestational diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. *Medicine (Baltimore).* 2021;100(14):e25279.
- Lorenzo-Almorós A, Hang T, Peiró C, et al. Predictive and diagnostic biomarkers for gestational diabetes and its associated metabolic and cardiovascular diseases. *Cardiovasc Diabetol.* 2019;18(1):140.
- Farrar D, Simmonds M, Bryant M, et al. Risk factor screening to identify women requiring oral glucose tolerance testing to diagnose gestational diabetes: a systematic review and meta-analysis and analysis of two pregnancy cohorts. *PLoS One.* 2017;12(4):e0175288.
- Russo LM, Nobles C, Ertel KA, Chasan-Taber L, Whitcomb BW. Physical activity interventions in pregnancy and risk of gestational diabetes mellitus: a systematic review and meta-analysis. *Obstet Gynecol.* 2015;125(3):576-582.

9. Sanabria-Martínez G, García-Hermoso A, Poyatos-León R, Álvarez-Bueno C, Sánchez-López M, Martínez-Vizcaino V. Effectiveness of physical activity interventions on preventing gestational diabetes mellitus and excessive maternal weight gain: a meta-analysis. *BJOG*. 2015;122(9):1167-1174.
10. Choi YJ, Shin S. Aspirin prophylaxis during pregnancy: a systematic review and meta-analysis. *Am J Prev Med*. 2021;61(1):e31-e45.
11. Benaiges D, Flores-Le Roux JA, Marcelo I, et al. Is first-trimester HbA1c useful in the diagnosis of gestational diabetes? *Diabetes Res Clin Pract*. 2017;133:85-91.
12. Zhao Y, Zhao Y, Fan K, Jin L. Serum uric acid in early pregnancy and risk of gestational diabetes mellitus: a cohort study of 85,609 pregnant women. *Diabetes Metab*. 2021;48(3):101293.
13. Wang X, Huang J, Zheng Y, et al. Study on the relationship between DNA methylation of target CpG sites in peripheral blood and gestational diabetes during early pregnancy. *Sci Rep*. 2021;11(1):20455.
14. Chiarello DI, Abad C, Rojas D, et al. Oxidative stress: normal pregnancy versus preeclampsia. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866(2):165354.
15. Chen X, Scholl TO. Oxidative stress: changes in pregnancy and with gestational diabetes mellitus. *Curr Diab Rep*. 2005;5(4):282-288.
16. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. 2010;107(9):1058-1070.
17. Horváth EM, Magenheimer R, Kugler E, et al. Nitrate stress and poly(ADP-ribose) polymerase activation in healthy and gestational diabetic pregnancies. *Diabetologia*. 2009;52(9):1935-1943.
18. Zhang Q, Li L, Chen H, et al. Soluble urokinase plasminogen activator receptor associates with higher risk, advanced disease severity as well as inflammation, and might serve as a prognostic biomarker of severe acute pancreatitis. *J Clin Lab Anal*. 2020;34(3):e23097.
19. Liu S, Zheng Q, Cui XY, et al. Expression of uPAR in human trophoblast and its role in trophoblast invasion. *Int J Clin Exp Pathol*. 2015;8(11):14325-14334.
20. Rasmussen LJH, Petersen JEV, Eugen-Olsen J. Soluble urokinase plasminogen activator receptor (suPAR) as a biomarker of systemic chronic inflammation. *Front Immunol*. 2021;12:780641.
21. Odden N, Roland MC, Lorentzen B, Mørkrid L, Henriksen T. PP071. suPAR levels in normal- and preeclamptic pregnancies. *Pregnancy Hypertens*. 2013;3(2):93.
22. Pantham P, Aye IL, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta*. 2015;36(7):709-715.
23. Azziz R, Carmina E, Chen Z, et al. Polycystic ovary syndrome. *Nat Rev Dis Primers*. 2016;2(1):16057.
24. Gözükarar YM, Aytan H, Ertunc D, et al. Role of first trimester total testosterone in prediction of subsequent gestational diabetes mellitus. *J Obstet Gynaecol Res*. 2015;41(2):193-198.
25. Ma R, Liu J, Wu L, et al. Differential expression of placental 11 β -hydroxysteroid dehydrogenases in pregnant women with diet-treated gestational diabetes mellitus. *Steroids*. 2012;77(7):798-805.
26. Feng Y, Feng Q, Qu H, et al. Stress adaptation is associated with insulin resistance in women with gestational diabetes mellitus. *Nutr Diabetes*. 2020;10(1):4.
27. Karvaly G, Kovacs K, Meszaros K, Kocsis I, Patocs A, Vasarhelyi B. The comprehensive characterization of adrenocortical steroidogenesis using two-dimensional ultra-performance liquid chromatography—electrospray ionization tandem mass spectrometry. *J Pharm Biomed Anal*. 2018;153:274-283.
28. Sun Y, Shen Z, Zhan Y, et al. Effects of pre-pregnancy body mass index and gestational weight gain on maternal and infant complications. *BMC Pregnancy Childbirth*. 2020;20(1):390.
29. Lee KW, Ching SM, Ramachandran V, et al. Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis. *BMC Pregnancy Childbirth*. 2018;18(1):494.
30. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. *Nat Rev Dis Primers*. 2019;5(1):47.
31. Saeedi M, Hanson U, Simmons D, Fadl H. Characteristics of different risk factors and fasting plasma glucose for identifying GDM when using IADPSG criteria: a cross-sectional study. *BMC Pregnancy Childbirth*. 2018;18(1):225.
32. Fong A, Serra AE, Gabby L, Wing DA, Berkowitz KM. Use of hemoglobin A1c as an early predictor of gestational diabetes mellitus. *Am J Obstet Gynecol*. 2014;211(6):641.e1.
33. Powe CE. Early pregnancy biochemical predictors of gestational diabetes mellitus. *Curr Diab Rep*. 2017;17(2):12.
34. Mahendra A, Fall CHD. Maternal vitamin D deficiency and GDM risk: evidence for the case of investing more attention in antenatal clinics. *Proc Nutr Soc*. 2021:1-7.
35. Liang JW, Chen MX, Hu XA, Zhou M, Zhang Y, Wang LL. Potential biomarkers in early pregnancy for predicting gestational diabetes mellitus and adverse pregnancy outcomes. *Clin Lab*. 2021;67(8).
36. Xu W, Tang Y, Ji Y, et al. The association between serum selenium level and gestational diabetes mellitus: a systematic review and meta-analysis. *Diabetes Metab Res Rev*. 2022;38(4):e3522.
37. Huang Y, Chen X, You ZS, et al. The value of first-trimester platelet parameters in predicting gestational diabetes mellitus. *J Matern Fetal Neonatal Med*. 2022;35(11):2031-2035.
38. Dias S, Pheiffer C, Abrahams Y, Rheeder P, Adam S. Molecular biomarkers for gestational diabetes mellitus. *Int J Mol Sci*. 2018;19(10):2926.
39. Kotzaeridi G, Blatter J, Eppel D, et al. Performance of early risk assessment tools to predict the later development of gestational diabetes. *Eur J Clin Invest*. 2021;51(12):e13630.
40. Francis EC, Li M, Hinkle SN, et al. Adipokines in early and mid-pregnancy and subsequent risk of gestational diabetes: a longitudinal study in a multiracial cohort. *BMJ Open Diabetes Res Care*. 2020;8(1):e001333.
41. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. *J Physiol Pharmacol*. 2019;70(6).
42. Najafi A, Pourfarzam M, Zadhoush F. Oxidant/antioxidant status in type-2 diabetes mellitus patients with metabolic syndrome. *J Res Med Sci*. 2021;26(1):6.
43. Keane KN, Cruzat VF, Carlessi R, de Bittencourt PI Jr, Newsholme P. Molecular events linking oxidative stress and inflammation to insulin resistance and beta-cell dysfunction. *Oxid Med Cell Longev*. 2015;2015:181643.
44. Li H, Yin Q, Li N, Ouyang Z, Zhong M. Plasma markers of oxidative stress in patients with gestational diabetes mellitus in the second and third trimester. *Obstet Gynecol Int*. 2016;2016:3865454.
45. Murthy KAS, Bhandiwada A, Chandan SL, Gowda SL, Sindhusree G. Evaluation of oxidative stress and proinflammatory cytokines in gestational diabetes mellitus and their correlation with pregnancy outcome. *Indian J Endocrinol Metab*. 2018;22(1):79-84.
46. Parast VM, Paknahad Z. Antioxidant status and risk of gestational diabetes mellitus: a case-control study. *Clin Nutr Res*. 2017;6(2):81-88.
47. Ma H, Qiao Z, Li N, Zhao Y, Zhang S. The relationship between changes in vitamin A, vitamin E, and oxidative stress levels, and pregnancy outcomes in patients with gestational diabetes mellitus. *Ann Palliat Med*. 2021;10(6):6630-6636.
48. Daneshzad E, Tehrani H, Bellissimo N, Azadbakht L. Dietary total antioxidant capacity and gestational diabetes mellitus: a case-control study. *Oxid Med Cell Longev*. 2020;2020:5471316.
49. Zamani-Ahari U, Zamani-Ahari S, Fardi-Azar Z, Falsafi P, Ghanizadeh M. Comparison of total antioxidant capacity of saliva in women with gestational diabetes mellitus and non-diabetic pregnant women. *J Clin Exp Dent*. 2017;9(11):e1282-e1286.
50. Hayek SS, Divers J, Raad M, et al. Predicting mortality in African Americans with type 2 diabetes mellitus: soluble urokinase plasminogen activator receptor, coronary artery calcium, and high-

- sensitivity C-reactive protein. *J Am Heart Assoc.* 2018;7(9):e008194.
51. Lopez-Hernandez Y, Herrera-Van Oostdam AS, Toro-Ortiz JC, *et al.* Urinary metabolites altered during the third trimester in pregnancies complicated by gestational diabetes mellitus: relationship with potential upcoming metabolic disorders. *Int J Mol Sci.* 2019;20(5):1186.
52. Ma R, Liu J, Wu L, *et al.* Differential expression of placental 11beta-hydroxysteroid dehydrogenases in pregnant women with diet-treated gestational diabetes mellitus. *Steroids.* 2012;77(7):798-805.
53. Manjunath-Gowda S, Charles C, Muneyyirci-Delale O, Nacharaju V. Cortisol metabolism in normal pregnancy and pregnancy associated with gestational diabetes. *Fertil Steril Home.* 2013;100(3):S333.
54. Wei D, Liu X, Jiang J, *et al.* Mineralocorticoids, glucose homeostasis and type 2 diabetes mellitus: the Henan rural cohort study. *J Diabetes Complicat.* 2020;34(5):107558.