Biochemical Markers Predicting the Risk of Gestational Diabetes Mellitus

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Gestational diabetes mellitus (GDM), defined by the occurrence or discovery of glucose intolerance during pregnancy is associated with higher risk of perinatal complications and long-term development of chronic diseases both in the mother and her child. Recent data suggest that women diagnosed earlier in pregnancy, even having more risk factors, develop fewer complications. The aim of the current study is to analyse biochemical markers that play a role in the pathophysiology of GDM and could lead to an early diagnosis. The authors performed a case-control study on 50 pregnant women that finally developed GDM and 50 pregnant women with risk factors for GDM which did non develop the disease. In all cases there were monitored a series of biochemical markers like glycated haemoglobin (HbA1c), sex hormone binding globulin (SHBG), magnesium (Mg), C-reactive protein (CRP), plasma insulin level, and pregnancy-associated plasma protein A (PAPP-A). All these factors were statistically analysed using univariate and multivariate tests in order to evaluate their predicting value. The combination of traditional risk factors with HBA1c, SHBG, PAPP-A and CRP proved significant prognosis value (75% sensitivity rate, 9% false positive rate) for GDM. In conclusion, these four biochemical markers available in early pregnancy have improved the performance of predicting models concerning the development of severe GDM needing insulin treatment and predisposing to maternal and foetal complications.

Keywords: gestational diabetes mellitus, biochemical markers, glycated haemoglobin, sex hormone binding globhulin

Gestational diabetes mellitus (GDM), defined by the occurrence or discovery of glucose intolerance during pregnancy [1], is an important cause of gestation associated complications [2]. Using the traditional diagnostic criteria, GDM prevalence generally varies between 2 and 6% in developed countries [3]. Increasing maternal age and overweight/obesity in the population, combined with the increase in immigration from high-risk populations, resulted in a growing number of GDM cases. GDM is associated with higher risk of perinatal complications and long-term development of chronic diseases (diabetes, hypertension, cardiovascular disease) both in the mother and her child [4-7]. Studies have found that women diagnosed earlier in pregnancy, even having more risk factors, develop fewer complications like polyhydramnios, prematurity and fetal macrosomia [8-10].

In addition to traditional risk factors, several biochemical markers (glycated haemoglobin, sex hormone binding globulin, C-reactive protein, cytokines, pregnancy-associated plasma protein A, etc.) that may play a role in the pathophysiology of GDM, mostly involved in the mechanisms related to insulin resistance or chronic inflammation have been studied to early predict the risk of developing GDM.

The glycated hemoglobin (HbA1c) is produced by the non-enzymatic glycation of hemoglobin and reflects medium-term glucose concentrations (period of about 120 days) [11]. Several studies have proved that HbA1c is increased in first trimester in women who develop GDM [12, 13]. HbA1c was also suggested as a predictor of the need for insulin therapy in women with GDM.

Sex hormone binding globulin (SHBG) (a homodimer each monomer being composed of 402 aminoacids with a molecular weight of 43.7 kDa, synthesised by the liver) has been associated with insulin resistance and the development of type 2 diabetes mellitus [14]. SHBG is considered a marker of hyperinsulinism and lower levels of SHBG were observed in the first trimester in women who develop GDM [15, 16], especially those that require insulin treatment [17].

More and more data support the hypothesis that inflammation is causally related with insulin resistance and the deterioration of pancreatic beta cells [18]. Higher concentrations of C-reactive protein (CRP) (an acute-phase protein composed of 224 amino acids with a homo-pentameric structure and Ca-binding specificity for phosphocholine, has a monomer molecular mass of 25106 Da), as measured by a highly sensitive test (hsCRP), have also been observed in women with GDM in the first quarter, but only a few studies have reported increases independent of fat mass and maternal body mass index (BMI) [18].

Bardicef et al. indicated that presence pregnancy itself is associated to a magnesium (Mg) depletion and to a greater extent in gestational diabetes. Excessive Mg depletion may predispose to vascular complications and could be an early marker of GDM [19].

Other markers, including pregnancy-associated plasma protein A (PAPP-A), visfatin, resistin, insulin and interleukin-6 were investigated in women with GDM, but few data are available and the results are conflicting [21, 22].

The aim of the current study is to analyse biochemical markers that play a role in the pathophysiology of GDM and to combine them with traditional risk factors in a predicting model that could lead to an early diagnosis.

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Experimental part

Material and methods

The authors performed a case-control study on 50 pregnant women that finally developed GDM and 50 pregnant women with risk factors for GDM which did non develop the disease. In all cases there were monitored a series of biochemical markers like glycated haemoglobin (HbA1c), sex hormone binding globulin (SHBG), magnesium (Mg), C-reactive protein (CRP), plasma insulin level, and pregnancy-associated plasma protein A (PAPP-A).

All women were recruited in the first trimester of pregnancy, were followed and gave birth at the Obstetrics & Gynecology Hospital Cuza Voda from Iasi, Romania, between January 2010-september 2014.

GDM diagnosis was established using the World Health Organisation (WHO) criteria [23]:

- fasting plasma glucose 5.1-6.9 mmol/L (92 -125 mg/dl);
- 1h plasma glucose ≥10.0 mmol/L (180 mg/dL) following a 75g oral glucose load;
- 2h plasma glucose 8.5-11.0 mmol/L (153 -199 mg/dL) following a 75g oral glucose load.

In order to develop models combining clinical factors and biochemical markers, only patients who had their first visit to the institution between 14 and 17 weeks of pregnancy were selected. At their first prenatal visit, all eligible women were invited to sign a consent form and provide blood samples. The samples were immediately placed at 4°C, centrifuged within 2 hours after collection and serum was divided into aliquots, labelled and stored at -80°C. At the time of the screening test for gestational diabetes (between 24 and 28 weeks of pregnancy), participants completed a self-administered questionnaire to collect socio-demographic and medical information on the GDM risk factors and were divided according to the presence of GDM risk factors and occurrence of GDM. As 50 participants developed GDM there were randomly selected other 50 participants with risk factors that did not develop GDM for statistical analysis of the prognosis value of biochemical factors. None of the participants presented active infection or other disease that could bias the results.

Statistical analysis included both univariate and multivariate tests (multivariate regression) and was performed using SPSS 21.0 for Mac. The model performance was evaluated in terms of sensitivity, specificity, positive predictive value (PPV) and negative (NPV) and area under the ROC curve (AUC).

Results and discussions

Main participants' characteristics at the first prenatal visit are summarized in table 1.

Women who developed GDM were significantly older and had higher BMI at the first prenatal visit as confirmed by univariate statistical tests (Mann-Whitney U-test).

The percentage of participants with familial history of diabetes, prior GDM and history of macrosomic infants and nulliparity were significantly higher in the GDM group (Pearson chi-square test).

Addictive behaviour (smoking) did not influence the development of GDM.

Concerning the biochemical markers, the registered values are detailed in table 2.

Participants who developed GDM had significantly higher serum CRP, lower extracellular and intracellular Mg, lower serum PAPP-A, lower serum SHBG and higher whole blood HbA1c measured between 14 and 17 weeks of gestation (Mann-Whitney U-test).

All traditional markers and biochemical markers that proved to be significantly associated with GDM at univariate tests were introduced in a multivariate model.

Using logistic regression and multiple regression with stepwise approach and backward elimination, the following variables were retained for the prediction of GDM: BMI, past history of GDM or macrosomia, family history, HbA1c, SHBG, CRP, and PAPP-A. The most predictive variables were past history of GDM or macrosomia, HbA1c, family history of diabetes and BMI. Women with missing results were excluded from the analysis.

The selected model yielded an AUC of 0.90 (0.87-0.94) and a sensitivity of 75% at a false-positive rate (FPR) of 9% confirming the robustness of the model and the strong predictive value of included variables.

In the model, the variables selected were retained in the bootstrap samples thus confirming selection's strength. The calibration of the selected model was good as evaluated by goodness-of-fit (χ^2=582.73, P=0.417).

	Group 1 (developed GDM)	Group 2 (risk factors, did not develop GDM)	p
Age	31.4 ± 5.11 years	28.1 ± 3.22 years	0.0032
Weight at first visit	65.7 ± 9.1 kg (BMI 24.6 kg/m^2)	62.4 ± 7.7 kg (BMI 23.1 kg/m^2)	0.0014
Height	163.48 ± 10.72 cm	164.35 ± 9.95 cm	n.s.
Smoking	11 (22%)	7 (14%)	n.s.
Family history	23 (46%)	13 (26%)	0.0001
Nulliparity	30 (60%)	12 (24%)	0.0001
Prior GDM or macrosomic infant	17 (85%)	0%	0.0001

Table 1
STUDY GROUPS CHARACTERISTICS

	Group 1 (developed GDM)	Group 2 (risk factors, did not develop GDM)	p
CRP	9.89 ± 14,32 mg/L	7.86 ± 2,31 mg/L L	0.017
Extracellular Mg	1.6 ± 0,76 mg/dL	2.02 ± 1,54 mg/dL	0.020
Intracellular Mg	3.4 ± 0,73 mEq/L	4.29 ± 0,74 mEq/L	0.011
PAPP-A	2.53 ± 2,46 mIU/mL	3.67 ± 2,78 mIU/mL	0.014
SHBG	78.57 ± 45,58 mmol/L	88.9 ± 53,11 mmol/L	0.002
Serum insulin	8.08 ± 4,66 µU/mL	3.08 µU/mL	biased
HbA1c	5.89%	4.72%	0.001

Table 2
BIOCHEMICAL MARKERS

Despite the fact that maternal age was higher among women who developed GDM, the variable was not retained in the multivariate models because there was more difficult to observe a significant difference as the cohort included a small number of young women in whom the risk is lower.

Measurement of four biochemical markers readily available between 14 and 17 weeks of pregnancy has improved the performance of the model. The usefulness of a combination of different markers can be explained by the multifactorial origin of the GDM, which involves both environmental and genetic factors. Obesity, by mechanisms involving, among other inflammation and insulin resistance, is a recognized risk factor for type 2 diabetes mellitus, which shares a similar pathophysiology with GDM. A woman with GDM in a previous pregnancy is at high risk of developing the disease in a subsequent pregnancy, in the presence of a similar stress-inducing insulin resistance.

Before 17 weeks of pregnancy, insulin resistance induced by placental hormones is not yet installed [24, 25]. Thus, the differences observed for the four biochemical markers are more suggestive of an existing insulin resistance. HbA1c is a marker of blood glucose levels during the first trimester, which was slightly increased in women who finally developed GDM in our study group, even in the absence of pre-existing diabetes. Similar results were obtained by Hanas et al. and plead for it's value as a reasonably sensitive screening measure in high risk population. Another biochemical marker, SHBG is considered to be directly implicated in the pathophysiology of type 2 diabetes, as shown by Mendelian randomization studies [26]. Data suggest that the protein could influence the hepatic glucose production [27]. In our study, SHBG registered lower values in GDM group a condition generally considered to be associated with serologic hyperandrogenism a recognized risk factor for type 2 diabetes in women independent to gestational status. Normally, during pregnancy SHBG has levels have to increase as induced by estradiol rise, failure to do so being a indicator of pregnancy hormone milieu alteration [15]. Lower concentrations were observed consistently in the first trimester in women who develop GDM [16].

C-Reactive Protein, a common marker for assessing the level of inflammation, may also be involved in the development of insulin resistance and the GDM. In group 1, CRP registered significantly higher values than in group 2, in the absence of coexistent acute or chronic inflammatory state. It's association to GDM could be explained by the hypothesis raised by Denison et al. who stated that maternal obesity, a recognized risk factor for GDM, is a state of chronic low-grade inflammation [18]. Due to this circumstance, CRP could be regarded as a risk factor for any condition related to obesity like metabolic and cardiovascular diseases.

A less well studied biochemical factor, PAPP-A is a zinc-binding matrix metalloproteinase synthesised by the trophoblast and detectable in maternal blood from the 28th day of conception. PAPP-A is traditionally considered a marker for aneuploid foetuses and placental issues but recently it was proved that it also mediates dissociation of insulin growth factor (IGF) (a small peptide consisting of 70 amino acids with a molecular weight of 7649 Da) from insulin growth factor binding proteins (IGFBP-s), thus increasing IGF-1 availability [28]. In this context, PAPP-A could be considered indicator of glycaemic control particularly associated to HbA1c. The hypothesis is confirmed by the current research which proves lower PAPP-A levels in participants that further developed GDM.

Conventional screening methods involve clinical risk factors, glycaemia and oral glucose tolerance test (OGTT) but the positivity of the later two occurs late when the disease is already established. Using these methods, GDM is currently diagnosed late in pregnancy, usually in the third trimester, limiting the possibilities of early interventions.

In this context, a model to identify women at risk of developing severe GDM requiring insulin therapy early in pregnancy course would allow more targeted interventions and a better glycaemic control. The interval between 14 and 17 weeks of pregnancy offers the opportunity to take advantage of other screening programs already in place and partially using the same markers, such as in screening for trisomy 21 that also involves PAPP-A.

The women identified as being at high risk of developing GDM based on proposed biochemical markers could then be referred early in the second trimester to a specialized clinic for personalized monitoring.

Women who develop GDM have a particularly high risk of adverse outcomes of pregnancy, including macrosomia, caesarean delivery and neonatal hypoglycemia, particularly higher in women needing insulin therapy [29].

The four selected biochemical markers do not require fasting prior to sampling, are stable during transport to the laboratory and easily measured with automated instruments.

Conclusions

In conclusion, these four biochemical markers available in early pregnancy have improved the performance of predicting models concerning the development of severe GDM needing insulin treatment and predisposing to maternal and fetal complications. These markers could be included in screening algorithms to allow an early diagnosis and treatment.

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