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ORIGINAL ARTICLE

Early second trimester maternal serum markers in the prediction of gestational diabetes mellitus

Baihui Zhao, Xiujun Han, Qing Meng, Qiong Luo*

Department of Obstetrics, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China

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***Correspondence**
Qiong Luo
Tel.: +86-571-8706-1501
Fax: +86-571-8706-1878
E-mail address:
luoq@zju.edu.cn

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ABSTRACT

Aims/Introduction: To determine whether maternal serum markers in the early second trimester are useful for prediction of gestational diabetes mellitus (GDM).

Materials and methods: A total of 876 singleton pregnancies were recruited in the present study. Blood samples were collected during 16–20 gestational weeks. GDM women were diagnosed by an oral glucose tolerance test during 24–28 gestational weeks. A total of 56 women with GDM and 73 healthy pregnant women were selected. Maternal serum concentrations of placental protein 13 (PP13), pentraxin 3 (PTX3), soluble fms-like tyrosine kinase-1 (sFlt-1), myostatin and follistatin (FST) were detected at 16–20 weeks' gestation. All of these markers concentrations were expressed as multiples of the medians. The Mann–Whitney *U*-test was used for comparison of the multiples of the medians of different concentrations of these five serum markers between the GDM group and the control group. Receiver operating characteristic curve analysis was applied to assess the sensitivity and specificity of significant serum markers from a Mann–Whitney *U*-test comparison.

Results: Compared with healthy pregnancies, the serum levels of PP13, PTX3, sFlt-1, myostatin and FST in the early second trimester were significantly increased in patients who had developed GDM late. In screening for GDM by PP13, PTX3, sFlt-1, myostatin and FST, the detection rates were 92.3, 94.9, 94.9, 92.5 and 92.3%, respectively at 80% specificity. PTX3 and sFlt-1 were the most sensitive markers.

Conclusions: Maternal serum markers including PP13, PTX3, sFlt-1, myostatin and FST increase in the early second trimester of women with GDM. These five markers, especially PTX3 and sFlt-1, could have the value of prediction for those patients who would develop GDM in the late second trimester.

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance during pregnancy that is developed or recognized for the first time[1,2]. The prevalence of GDM is increasing steadily[3]. The relationship between maternal hyperglycemia and fetal macrosomia together with other complications has been confirmed[4]. Women with GDM have a higher long-term risk of type 2 diabetes mellitus or cardiovascular diseases[5]. Therefore, we sometimes describe GDM as "a disease across two generations." Although the exact mechanisms responsible for developing GDM are not known, some theories postulate insufficient pancreatic function against an increased insulin resistance, which is triggered primarily by diabetogenic hormones secreted from the placenta during pregnancy, diminished insulin sensitivity and decreased insulin response[6].

Placental protein 13 (PP13) was reported to have a homologous structure to the galectin family, known in various human tissues, such as cells of the immune system, endometrium, placenta and endothelial tissue[7]. Previously, a study reported a relationship between serum PP13 and GDM in the third trimester of pregnancy[8]. Pentraxin 3 (PTX3) is an essential component of innate immunity and is independently associated with the risk of developing vascular events[9]. Soluble fms-like tyrosine kinase-1 (sFlt-1) is a circulating antagonist to vascular

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endothelial growth factor and is an important anti-angiogenic factor in placentation, which has been associated with placentation disorders, including pre-eclampsia and intrauterine growth restriction[10,11]. Myostatin, a member of the transforming growth factors, is a hormone secreted by muscles. Specific inhibition of myostatin in muscle has been identified to be most effective in improving glucose metabolism and insulin sensitivity[12]. Follistatin (FST) promotes adipogenic differentiation of progenitor cells[13], which influences visceral fat mass, glucose tolerance and insulin sensitivity. However, few reports in the literature have reported the value of serum PP13, PTX3, sFlt-1, myostatin and FST in predicting GDM in the early second trimester. In the present study, maternal serum PP13, PTX3, sFlt-1, myostatin and FST were detected in pregnant women in the early second trimester to analyze whether these markers could be applied for the prediction of GDM, which could be diagnosed later by the oral glucose tolerance test (OGTT) during 24–28 weeks of gestation.

METHODS

Participants

From July 2014 to July 2015, a total of 876 singleton normal pregnancies without a family history of diabetes mellitus were recruited. All the pregnant women took part in an antenatal examination at the Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China. All of the women included in the study understood the purpose of drawing blood, and they all agreed to cooperate and signed the informed consent. Blood samples were collected during 16–20 gestational weeks. There were 65 women diagnosed with GDM by OGTT during 24–28 gestational weeks. At the same time, for cost-effectiveness, we selected 130 women as the control group matched for age, gravidity, parity and gestational weeks. Exclusion criteria at term included any known fetal chromosomal or structural anomaly, preterm ruptured membranes, preterm delivery, pre-eclampsia or eclampsia, placenta previa, or any infections (Figure 1). A total of 56 cases of GDM and 73 cases without any pregnancy complications by term were selected for the present study. Fasting blood samples were centrifuged with a rotational speed of 2,500 *g* at room temperature within 20 min. We extracted serum to store at −80°C and the concentration of serum markers were detected after OGTT during 24–28 gestational weeks. Gestational weeks at birth and infant birthweight would be recorded when the baby was delivered. In the present study, we detected the concentrations of five serum markers including PP13, PTX3, sFlt-1, myostatin and FST to evaluate the predictive value of these markers in GDM.

Determination of serum concentration of five markers

Serum concentration of PP13, PTX3, sFlt-1, myostatin and FST were detected with competitive enzyme-linked immunosorbent assay. The reagents used for the serum PP13, PTX3, sFlt-1, myostatin and FST markers were all from Hermes Criterion Biotechnology (Vancouver, Canada). The sensitivity of this assay for human recombinant PP13, PTX3, sFlt-1, myostatin and FST was 1.0 pg/mL, 0.1, 0.1, 0.1 and 0.1 ng/mL, respectively. The assays were measured according to the manufacturer's instructions.

Statistical analysis

We compared the pregnant women's characteristics between the two groups using Student's *t*-test and presented as mean ± standard deviation. The serum concentrations of PP13, PTX3, sFlt-1, myostatin and FST were adjusted for gestational age by using multiples of the median (MoM). The concentrations of these five markers were expressed by medians of MoM and interquartile range. Data analysis was carried out using the Mann–Whitney *U*-test, and $P < 0.05$ was considered to be statistically significant. The receiver operating characteristic curve was used to visualize detection specificity and sensitivity values of the serum PP13, PTX3, sFlt-1, myostatin and FST from Mann–Whitney *U*-test analysis for GDM. SPSS 22.0 (IBM, Armonk, NY, USA) was used for data analysis.

RESULTS

Clinic characteristics of the GDM group and the control group

The study included 56 pregnant women with GDM and 73 healthy pregnant women. The clinical characteristics of these two groups are compared in Table 1. We can see that the groups were similar regarding maternal age, gravidity, parity and gestational age of sampling. Gestational age at delivery in the GDM group was significantly lower than that in the healthy pregnancies ($P < 0.05$). This difference could be attributed to the practice bulletin for GDM of the American College of Obstetricians and Gynecologists (2013); we induced labor after 39 weeks of gestation for women with GDM with good glycemic control who were receiving medical therapy. Compared with the control group, the birthweight of neonates had no statistical significant in the GDM group, but the mean of birthweight of neonates was lower in the GDM group.

Serum concentration of these five maternal markers in the GDM group and the control group

A comparison of the concentrations of these five markers between the GDM group and the control group is shown in Figure 2, including five box plots, which showed the distribution of the concentration MoM of these five serum markers. Their median MoM and interquartile ranges are shown in Table 2. Compared with the control group, the levels of serum PP13, PTX3, sFlt-1, myostatin and FST MoM were strikingly significantly elevated in the GDM group.

Value of GDM prediction by these five biomarkers

From Table 2, we concluded that PP13, PTX3, sFlt-1, myostatin and FST have the value to predict GDM. Hence, we used the receiver operating characteristic curve to estimate the

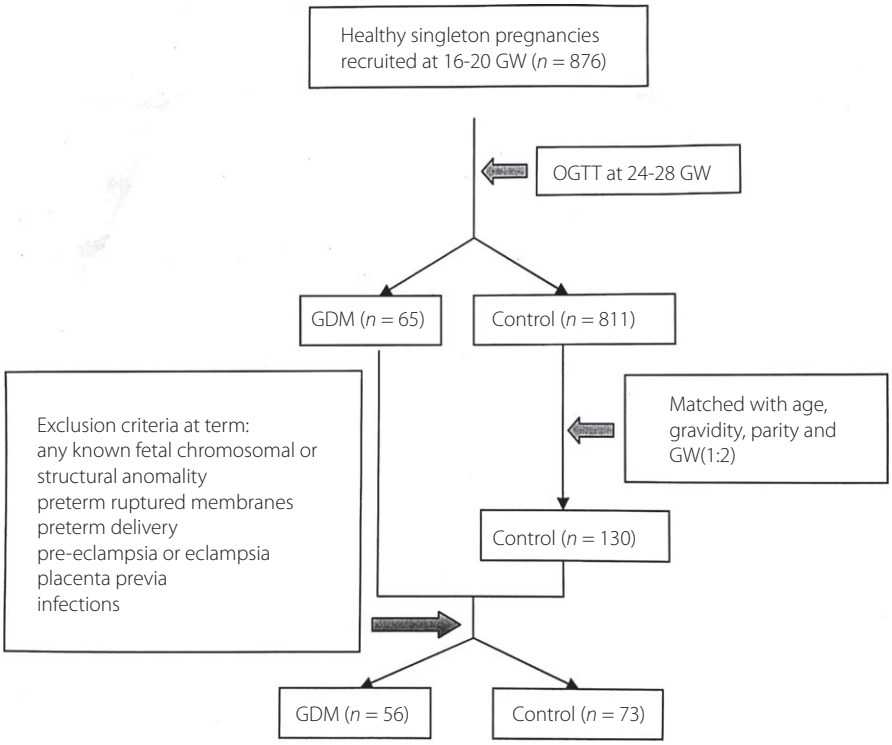

Figure 1 | The process of cohort selection. A total of 876 singleton normal pregnancies without family history of diabetes mellitus were recruited. Blood samples were collected during 16–20 gestational weeks (GW). There were 65 women diagnosed with gestational diabetes mellitus (GDM) by an oral glucose tolerance test (OGTT) during 24–28 GW. We selected 130 pregnant women as the control group at the same time matched for age, gravidity, parity and GW. The exclusion criteria at term are listed in the frame. A total of 56 women with GDM and 73 women without any pregnancy complications by term were selected for the present study. Blood samples were detected after an oral glucose tolerance test during 24–28 GW.

Table 1 | Clinical characteristics of the gestational diabetes group and the control group

	GDM (n = 56)	Healthy pregnancy (n = 73)	P-value
Age at sampling (years)	30.27 ± 4.15	29.03 ± 3.35	0.77
Gravidity	2.40 ± 1.60	2.20 ± 1.20	0.75
Parity	0.38 ± 0.50	0.25 ± 0.50	0.16
GA at sampling (weeks)	26.46 ± 1.26	25.93 ± 2.88	0.36
GA at delivery (weeks)	37.90 ± 2.00*	39.00 ± 1.30	0.00
Birthweight (g)	3,061.10 ± 783.50	3,345.90 ± 449.40	0.07

Data are expressed as mean ± standard deviation. *P < 0.05. GA, gestational age; GDM, gestational diabetes mellitus.

diagnosis rate of screening for GDM and estimated the area under the curve of each marker. When the specificity was fixed at 80%, the sensitivity of PP13 was 92.30% for predicting GDM, PTX3 94.90%, sFlt-1 94.90%, myostatin 92.50%, FST 92.30% and PTX3 + sFlt-1 98.20%. When the sensitivity was fixed at 80%, the specificity of PP13, PTX3, sFlt-1, myostatin, FST and PTX3 + sFlt-1 was 62.30, 32.80, 42.60, 65.60, 57.40 and 41.10% separately for predicting GDM. The area under the curve of PP13, PTX3, sFlt-1, myostatin, FST and PTX3 + sFlt-1 was 0.63 (0.52–0.74), 0.72 (0.62–0.83), 0.70 (0.59–0.80), 0.84 (0.75–0.93), 0.67 (0.56–0.78) and 0.751 (0.67–0.84; Figure 3; Table 3).

DISCUSSION

In the present study, we represent a case–control analysis of maternal serum concentrations of PP13, PTX3, sFlt-1, myostatin and FST in the early second trimester of pregnancy. We found that serum PP13, PTX3, sFlt-1, myostatin and FST could

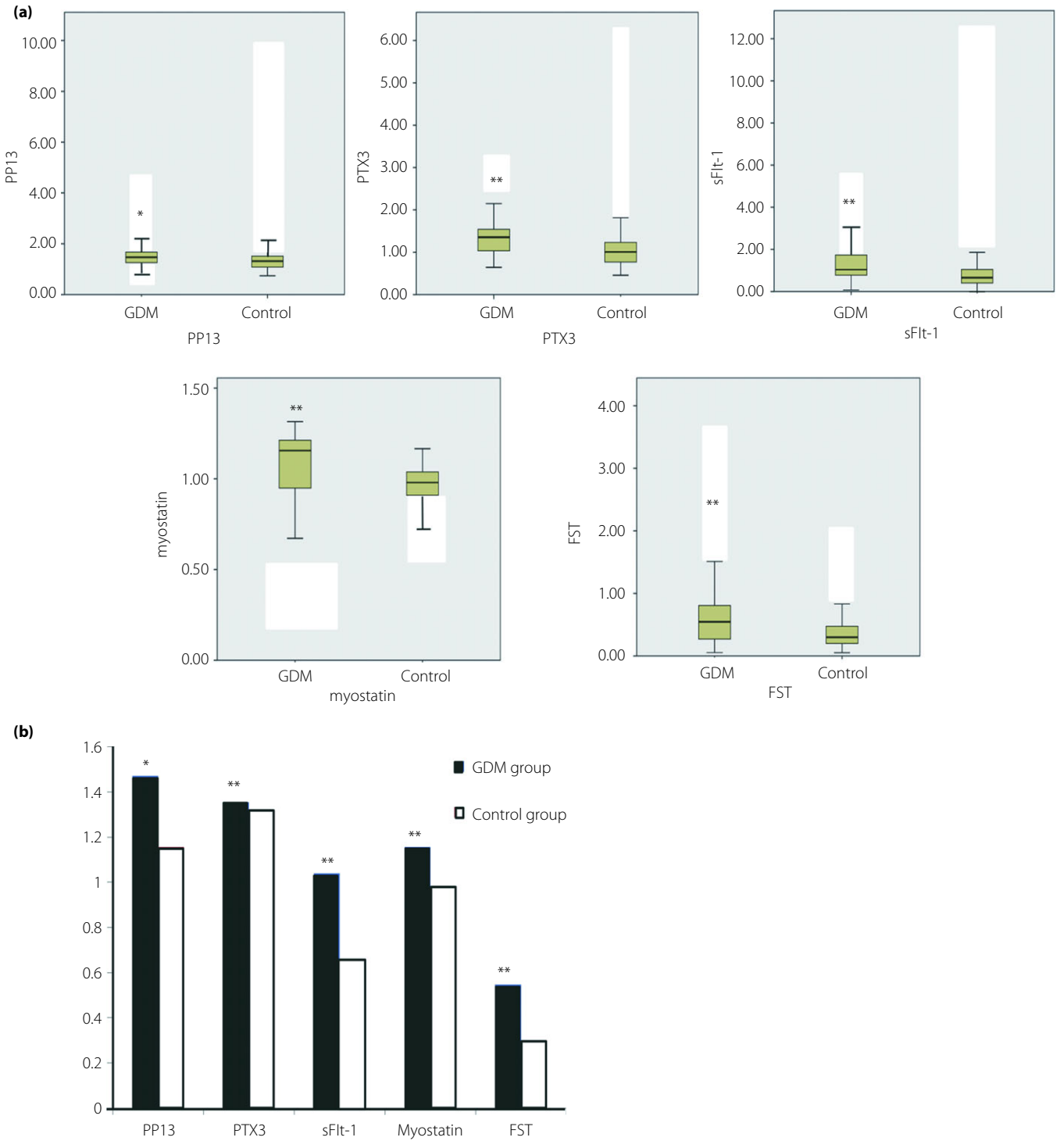

Figure 2 | Maternal serum levels of placental protein 13 (PP13), pentraxin 3 (PTX3), soluble fms-like tyrosine kinase-1 (sFlt-1), myostatin and follistatin (FST) in women who developed gestational diabetes (GDM) and those in the control group. These five markers were significantly higher in women who developed GDM than those in the control group in the second trimester. (a) Five different box plots show the five markers by the median, the 25th percentile and the 75th percentile, respectively. Outliers were defined as lying greater than three interquartile ranges outside the 25th or 75th percentile. (b) Comparison of these five markers in the GDM group and the control group with column. The detailed data and comparison between the two groups are shown in Table 2. *$P < 0.05$, **$P < 0.01$ compared with the value in the control group.

Table 2 | Multiples of the median of five maternal serum markers between the diabetes mellitus group and the control group

	GDM (n = 56)	Healthy pregnancy (n = 73)	P-value
PP13	1.47 (1.25–1.68)*	1.16 (0.94–1.21)	0.01
PTX3	1.35 (1.03–1.54)**	1.32 (1.08–1.51)	0.00
sFlt-1	1.04 (0.77–1.75)**	0.66 (0.37–1.07)	0.00
Myostatin	1.16 (0.94–1.21)**	0.98 (0.91–1.04)	0.00
FST	0.55 (0.27–0.91)**	0.30 (0.20–0.49)	0.00

Data are expressed by median of multiples of the median (interquartile range). *P < 0.05, **P < 0.01 compared with the value in the control group. FST, follistatin; PP13, placental protein 13; PTX3, pentraxin 3; sFlt-1, soluble fms-like tyrosine kinase-1.

have the value of predicting the patients who subsequently develop GDM in the late second trimester. Although PP13, PTX3, sFlt-1, myostatin and FST can be determined as general markers for an abnormal pregnancy in early pregnancy[14–20], there are also some studies that reported that these five markers had great significance in women with GDM[21–26].

PP13, a member of the galectin family, is produced predominantly by the syncytiotrophoblast[27]. According to previous studies, PP13 levels in maternal serum were dependent on gestational age[28]. During pregnancy, serum PP13 levels are higher in the third trimester than in the first trimester. In pregnancies with complications, such as pre-eclampsia, intrauterine growth restriction and preterm delivery, the levels of maternal serum PP13 are lower in the first trimester. However, in the third trimester, the levels of PP13 are significantly higher in women with pregnancy complications than in normal pregnancies[16]. In the present study, we found significantly higher PP13 levels in the sera of women with GDM in the early second trimester, and PP13 holds the value of the prediction of subsequent GDM, which is consistent with previous studies. Unverdorben *et al.*[8] found lower PP13 levels in term placentas of women with GDM in comparison with healthy controls, and the inconsistency of the present study might be caused by the difference of the time and organs of detection (placentas vs sera). PP13 was reported to play a role in the regulation of the maternal immune system; disorders of serum PP13 might contribute to an imbalance in inflammation processes during pregnancy and therefore possibly lead to GDM.

PTX3, as an interleukin-1-β-inducible gene in vascular endothelial cells and a tumor necrosis factor (TNF-α)-stimulated gene in monocytes[29], produced by a number of cells, such

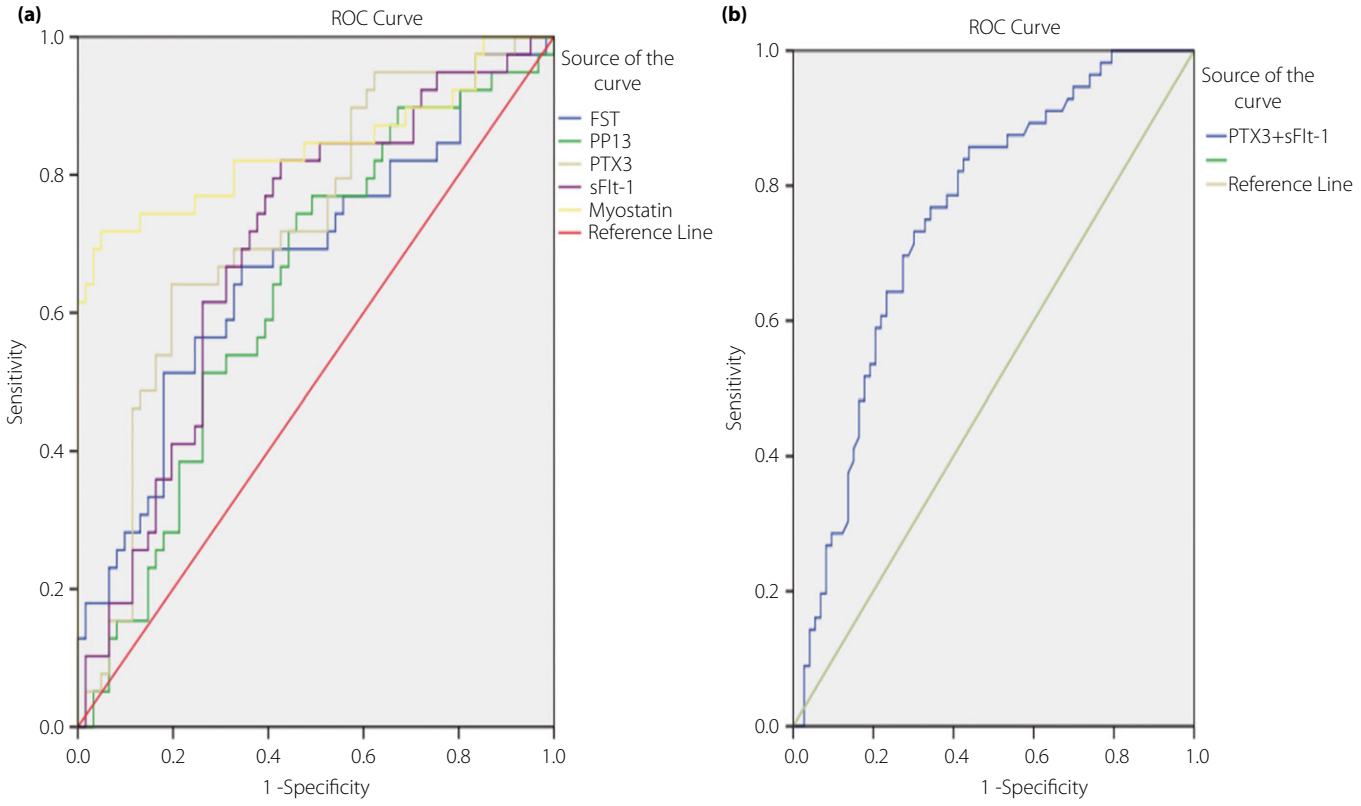

Figure 3 | Receiver operating characteristic (ROC) curves for the five markers concentration multiples of the median and a combination model. (a) Placental protein 13 (PP13), pentraxin 3 (PTX3), soluble fms-like tyrosine kinase-1 (sFlt-1), myostatin and follistatin (FST) single ROC curves. (b) The markers combination model (PTX3 + sFlt-1).

Table 3 | Diagnostic indices and the area under the receiver operating characteristic curve of the significant markers multiples of the median for the subsequent development of gestational diabetes

Screening test	AUC (95% CI)	*P*-value*	Sensitivity at 80% specificity	Specificity at 80% sensitivity
PP13	0.63 (0.52–0.74)	0.03	92.30%	62.30%
PTX3	0.72 (0.62–0.83)	0.00	94.90%	57.40%
sFlt-1	0.70 (0.59–0.80)	0.00	94.90%	42.60%
Myostatin	0.84 (0.75–0.93)	0.00	92.50%	32.80%
FST	0.67 (0.56–0.78)	0.00	92.30%	65.60%
PTX3 + sFlt-1	0.751 (0.67–0.84)	0.00	98.20%	41.10%

**P* of the area under the receiver operating characteristic curve (AUC) were calculated, compared with AUC = 0.50. CI, confidence interval, FST, follistatin; PP13, placental protein 13; PTX3, pentraxin 3; sFlt-1, soluble fms-like tyrosine kinase-1.

as adipocytes, monocytes, endothelial cells, fibroblasts, dendritic cells, smooth muscle cells and so on[27], directly representing the tissue inflammatory response, especially the one involving the vascular bed[30]. Aydogdu *et al.*[31] investigated the association between PTX3 levels and insulin resistance in patients with polycystic ovarian syndrome, and they found increased plasma PTX3 levels in women with polycystic ovarian syndrome compared with healthy controls. Furthermore, high serum PTX3 values were observed in patients with diabetes mellitus relative to healthy non-diabetic controls[32]. In accordance with the study of Todoric *et al.*[26], we also found significantly higher PTX3 levels in the GDM group, and serum PTX3 levels had the value to predict GDM. Previous studies suggested that significantly higher PTX3 levels were detected in high blood glucose patients as polycystic ovary syndrome, diabetes mellitus and GDM. These findings were consistent with the present results that PTX3 values were higher in patients with higher blood glucose levels. Glucose and PTX3 might involve a mutual cause–effect relationship, and they create a vicious cycle by either induction or acceleration of vascular inflammation in patients with GDM[26]. But, interestingly, there is also one study that reported the PTX3 concentration was lower in women with subsequent GDM than normal pregnancies[33], the limited cases of that study might be the reason for the discrepancy.

sFlt-1 is an angiogenic marker that plays an important role in the development of endothelial dysfunction[34]. The present study also showed that in the second trimester, the level of serum sFlt-1 was higher in patients with GDM than in healthy pregnancies. There are extensive data showing a strong relationship between higher sFlt-1 levels and the risk of GDM[22]. The sFlt-1 levels are stable in healthy pregnancies in the first to second trimester, and from 33–36 weeks the levels increase tremendously[22]. Lappas[35] found that increased sFlt-1 from adipose tissue might affect angiogenesis, inflammation, and lipid and glucose metabolism in both mothers and their offspring.

Myostatin, as a transforming growth factor (TGF-β) superfamily, can be expressed in numerous different tissues, such as muscle, brain, cardiac, endometrium and human placenta[36]. It not only carries out a traditional role in the inhibition of skeletal muscle growth, but also plays an important role in metabolism, specifically modulating glucose homeostasis[37]. The correlation of myostatin and pregnancy remains largely unknown. A study showed that increased myostatin concentration plays a role in GDM[17]. Another study suggested that myostatin is active in the placenta, and could affect glucose homoeostasis and/or cytokine production, thereby altering the function of the placenta[25], although they did not find a significant difference in myostatin concentration in the plasma of presymptomatic GDM patients compared with healthy women (blood collected at 8–17 weeks' gestation). We found significant higher myostatin levels in the blood sera of women with GDM in the late second trimester, and serum myostatin had the value to predict GDM. Further investigations are required to evaluate the effects of myostatin on insulin resistance and glucose homoeostasis.

Follistatin, known as an activin-binding protein, was identified as a protein that antagonizes the function of activin[38]. FST might be involved in the regulation of glucose homeostasis during pregnancy[39], although it was found that circulating FST levels are reduced in GDM compared with healthy pregnant women in the early third trimester of pregnancy. The present study showed high FST expressed in the serum of the GDM group in the early second trimester. We found significantly higher FST levels in the blood sera of women with GDM in the early second trimester, and serum FST has the value to predict GDM.

In conclusion, elevated levels of PP13, PTX3, SFlt-1, myostatin and FST in the early second trimester maternal serum surely has great value to predict GDM subsequently detected by OGTT in the late second trimester. Receiver operating characteristic curve analysis showed that a combination of PTX3 and sFlt-1 leads to a higher area under the curve sensitivity and specificity, suggesting that they might serve as a biomarkers panel to aid in the screening of GDM in the relatively early stages of pregnancy. The present results showed that GDM is a multifactorial disorder with complicated pathophysiological changes, among which inflammatory and immune responses might play important roles in the pathogenesis of GDM. Five maternal serum markers, PP13, PTX3, SFlt-1, myostatin and FST with a high sensitivity and specificity, might provide effective early screening for GDM.

Prediction of women who are at risk of GDM, especially those who missed or refused the OGTT in the second trimester, might allow earlier intervention of those patients with potential metabolic abnormalities, and effectively avoid maternal and fetal morbidities associated with GDM. However, there were still some limitations to our study. Many confounding factors could affect the serum levels of these markers, we had excluded most factors, such as family history of diabetes mellitus, abnormal fetus and placenta, pre-eclampsia or eclampsia, infections and so on. However, some factors, such as body mass index, alcohol drinking, cigarettes smoking, diet and exercise during pregnancy, that might affect the levels of blood glucose had not been well considered. In future study, we could detect the levels of these maternal serum markers in early pregnancy, and make our predictions for GDM even more advanced and consider more confounding factors.

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DISCLOSURE

The authors declare no conflict of interest.

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