Original Article

Association between Maternal Serum Concentrations of Angiopoietin-like Protein 2 in Early Pregnancy and Subsequent Risk of Gestational Diabetes Mellitus

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Abstract

Background: A recent study reported a positive association between elevated serum levels of angiopoietin-like protein 2 (ANGPTL2) and the development of type 2 diabetes in a general population. However, the relationship of serum ANGPTL2 levels with the risk of developing gestational diabetes mellitus (GDM) has not been reported to date. The aim of this study was to investigate the change of maternal serum ANGPTL2 concentrations in the first trimester of pregnancy and to determine whether ANGPTL2 is a biomarker for subsequent GDM development.

Methods: We conducted a prospective, nested case-control study in a pregnancy cohort. First-trimester ANGPTL2 levels were measured using a high-resolution assay in 89 women who subsequently developed GDM and in a random sample of 177 women who remained euglycemic throughout the pregnancy. Median ANGPTL2 levels were compared using Mann-Whitney *U*-test. Logistic regression was used to compute unadjusted and multivariable-adjusted odds ratios for developing GDM among ANGPTL2 quartiles.

Results: The serum levels of ANGPTL2 was higher in women with GDM than that in women without GDM (3.06 [2.59, 3.65] ng/ml vs. 2.46 [2.05, 2.96] ng/ml, $P = 0.003$). Fasting blood glucose was higher in women with GDM than that in women without GDM (5.0 ± 0.9 mmol/L vs. 4.4 ± 0.6 mmol/L, $P < 0.001$). Glucose challenge test showed that the blood glucose was higher in women with GDM than that in women without GDM (9.1 ± 3.5 mmol/L vs. 6.2 ± 1.2 mmol/L, $P < 0.001$). A multivariate model adjusted for baseline characteristics, medical complications, and gestational characteristics revealed that the risk of developing GDM among women in Q4 compared with Q1 was 2.90-fold more likely to develop GDM later in pregnancy.

Conclusions: At 11–13 weeks in pregnancies that develop GDM, the serum concentration of ANGPTL2 is increased, and it can be combined with maternal factors to provide effective early screening for GDM.

Key words: Angiopoietin-like Protein 2; First-trimester Pregnancy; Gestational Diabetes Mellitus; Pregnancy

Introduction

Gestational diabetes mellitus (GDM) is defined as any glucose intolerance that occurred or is diagnosed for the first time during pregnancy.[1] The incidence of GDM has doubled over the last 6–8 years and is paralleling the obesity epidemic.[2] GDM carries long-term implications for the subsequent development of type 2 diabetes mellitus (T2DM) in the mother and increased risk for obesity and glucose intolerance in the offspring.[3-6] The usual recommendation for screening is between 24 and 28 weeks of gestation.[7] New diagnostic approaches that allow earlier assessment can facilitate a shift from the current standard-of-care practices by enabling earlier treatment. Early universal screening[7,8] means screening all pregnant women soon after the diagnosis of pregnancy irrespective of the presence of risk factors.[9] Effective early identification of the high-risk group for subsequent development of GDM might allow earlier interventions, potentially reducing either the later diagnosis of GDM or its associated morbidities.

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Angiopoietin-like proteins (ANGPTLs), which are structurally similar to angiopoietins, are characterized by a coiled-coil domain in the N-terminus and a fibrinogen-like domain in the C-terminus. Seven ANGPTLs have been identified to date;[10-12] one of them, ANGPTL2, has been shown to be expressed abundantly in adipose tissues and to be a key mediator linking obesity to adipose tissue inflammation and systemic insulin resistance in mice.[13,14] In humans, ANGPTL2 is also closely related to adiposity and inflammation.[15-17] A recent study reported a positive association of elevated serum ANGPTL2 levels with the development of T2DM in a general population, independent of other risk factors including high sensitivity C-reactive protein levels.[18] However, the relationship of serum ANGPTL2 levels with the risk of developing GDM has not been reported to date. The aim of this study was to investigate the change of maternal serum ANGPTL2 concentrations in the first trimester of pregnancy and to determine whether ANGPTL2 is a biomarker for subsequent GDM development.

Methods

Study design

In this prospective, nested case-control study, pregnant women who first attended their routine hospital visit during their first trimester at Peking University Third Hospital between December 2011 and September 2012 were invited to participate in the study. Exclusion criteria were as follows: Age <18 years or >40 years, multiple pregnancy, pregestational diabetes (type 1 or 2), drug and/or alcohol abuse, uncontrolled endocrine disease, renal failure, or other major medical conditions that would affect glucose regulation. In the first visit, participants received a series of standard obstetric examinations if they planned to deliver at our hospital. These included a combined screening for aneuploidies by measurement of the fetal crown-rump length and nuchal translucency thickness by ultrasound, and fasting glucose, hemoglobin, and ANGPTL2 were analyzed using maternal blood samples. Maternal characteristics and medical history were collected and recorded. Demographic information from each case included maternal age, parity, prepregnancy body mass index (BMI, calculated as the ratio between habitual weight [in kilograms] and height [in meters] squared), weight gain during gestation, family history of diabetes and hypertension, and previous history of GDM. Cases were then attended obstetric visits in a regular basis: Once every month in the first 27^{+6} weeks, once 2 weeks from 28 to 35^{+6} weeks, and once a week after 36 weeks till delivery. Neonatal data including week of delivery, birth weight, and birth weight percentile were collected. All participants were Chinese and provided written informed consent for participation in the study. Totally, 1198 pregnant women who met these criteria were enrolled initially in this study; however, women had early abortion were excluded ($n = 25$) from the study. Women with unknown outcome of pregnancy due to any of the following reasons were also excluded from the study: Moved, delivered elsewhere, and/or missing medical records ($n = 57$). Among the 1116 (93.16%) participants, there were 89 women who were diagnosed with GDM (7.97%) and 1027 who were not (92.03%). From this pool of eligible control cases, 178 (2 for each case) were randomly selected for the current study using random number generator software. Upon manual review of each of the potential control cases' prenatal flow sheets, one woman was excluded from the study because of a history of chronic inflammatory disease, leaving a final sample of 177 control cases. The project was approved by the Ethics Review Board of Peking University Third Hospital (No. 2014073).

Screening and diagnosis of gestational diabetes mellitus

Screening for GDM in our hospital was based on a two-step approach.[19] First, a random blood glucose test was performed during the first trimester (≤13 weeks of gestation), and second, the 50-g 1-h oral glucose challenge test (GCT) was performed in the 24–28 weeks of gestation. Those patients who failed this screening test (glucose ≥7.8 mmol/L) were then further tested within 1–2 weeks with a 75-g oral glucose tolerance test (OGTT). Thresholds of glucose levels just before and at 1 and 2 h were 5.3 mmol/L, 10.0 mmol/L, and 8.6 mmol/L, respectively. If one or more of these values for the 75-g OGTT reached or exceeded the thresholds, the participant was diagnosed with GDM. All glucose levels were measured using standard glucose oxidase assays with an intra- and inter-assay coefficient of variation (CV) of <2%.

Women who were diagnosed with GDM were given dietary and exercise advice and were instructed to self-monitor capillary blood glucose before and 2 h after each meal. If during a period of 1–2 weeks the premeal blood glucose >5.3 mmol/L or 2-h postmeal blood glucose >6.7 mmol/L, the women were to receive insulin treatment.

Quantitation of angiopoietin-like protein 2

Plasma samples to quantify ANGPTL2 levels were obtained at the time of routine blood testing in the first visit (during the first trimester, ≤13 weeks of gestation). Analyses were performed by a person who was blinded to the outcome of pregnancy. Sandwich enzyme linked immunosorbent assays (ELISAs) for human ANGPTL2 were performed in duplicate, using commercially available kits (Immuno-Biological Laboratories Co., Ltd, Japan). The detection limit was 0.05 ng/ml. Inter-assay CV and intra-assay CV were 4.4% and 5.5%, respectively.

Statistical analysis

All statistical analyses were performed using the SPSS® statistical package (version 17.0; SPSS Inc., Chicago, IL, USA) for Windows®. Statistical significance was defined as two-sided $P < 0.05$. The continuous variables were expressed as mean ± standard deviation (SD) or median (quartile) when distribution was normal or skewed. Data for categorical variables were given as frequencies or percentages. Differences between two groups were tested

using Mann-Whitney *U*-test, Student's *t*-test, or Chi-square test when appropriate. Correlation coefficients between ANGPTL2 and other clinic variables were calculated using correlation index. In total, three models were tested to assess the relationship between ANGPTL2 and GDM development. Model 1 was an unadjusted model. Model 2 was adjusted for age, parity, blood pressure, smoking, BMI, family history of diabetes or hypertension, and random blood glucose levels at recruitment (<13 weeks of gestation); Model 3 was Model 2 further adjusted for gestational characteristics and outcomes, gestational weight gain, birth weight, and placental weight. Logistic regression was used to calculate unadjusted and multivariable adjusted odds ratios (*OR*s) and 95% confidence intervals (*CI*s) for developing GDM with the lowest tertile serving as the reference group.

Results

Demographic and pregnant characteristics, angiopoietin-like protein 2 concentrations, and fasting blood glucose levels

Sociodemographic, medical, and behavioral characteristics of the study population, according to case status, are shown in Table 1. Women who developed GDM had a greater prepregnancy BMI, prior history of GDM, and family history of type 2 diabetes. They also had higher systolic blood pressure and fasting blood glucose, but there were no relevant differences in parity, smoking history, or method of conception. All ANGPTL2 concentrations were above the level required for detection (≥0.05 ng/ml). The serum levels of ANGPTL2 was higher in women with GDM than that in women without GDM (3.06 [2.59, 3.65] ng/ml vs. 2.46 [2.05, 2.96] ng/ml, $U = 3.790$, $P = 0.003$). Fasting blood glucose was higher in women with GDM than that in women without GDM (5.0 ± 0.9 mmol/L vs. 4.4 ± 0.6 mmol/L, $t = -5.881$, $P < 0.001$). GCT showed that the blood glucose was higher in women with GDM than that in women without GDM (9.1 ± 3.5 mmol/L vs. 6.2 ± 1.2 mmol/L, $t = -7.661$, $P < 0.001$). Other baseline characteristics and birth outcomes among cases with GDM and without GDM were comparable.

Distribution of gestational diabetes mellitus by angiopoietin-like protein 2 quartile and association between angiopoietin-like protein 2 concentrations during the first trimester and probability of developing gestational diabetes mellitus

Cases were divided into ANGPTL2 quartile using cut points defined by the distribution of ANGPTL2 among study cases (tertile 1: <2.05 ng/ml; tertile 2: 2.05–2.79 ng/ml; tertile 3: 2.80–3.45 ng/ml; and tertile 4: >3.45 ng/ml). Table 2 shows the results of the logistic regression analysis for the association between plasma ANGPTL2 quartiles and the probability of developing GDM. In unadjusted Model 1, the *OR* for GDM was 2.58 for Q4 compared with Q1 (Q4/Q1) (95% *CI*: 1.22–6.96, $P = 0.022$). In Model 2 (adjusted for maternal age, parity, BMI before pregnancy, previous GDM, family history of diabetes, fasting blood sugar levels during the first trimester; and MAP at recruitment), the *OR* was 2.83 for Q4 compared with Q1 (95% *CI*: 1.16–7.54, $P = 0.026$); and in Model 3 (adjusted for variables in Model 2 in addition to gestational characteristics and outcome and MAP at GCT), the association between plasma ANGPTL2 concentrations and the probability of developing GDM was statistically significant. The *OR* was 2.90 for Q4 compared with Q1 (95% *CI*: 1.17–7.25, $P = 0.029$). That is, women with higher plasma ANGPTL2 concentrations during early pregnancy were 2.90-fold more likely to develop GDM later in pregnancy.

Discussion

In the current study, we investigated the potential of predicting the development of GDM by a combination of maternal characteristics and maternal serum concentration of ANGPTL2 at the 11th–13th weeks' gestation. In this prospective, nested case-control study, we found that higher levels of ANGPTL2 in early pregnancy were significantly

Table 1: Characteristics of study participants according to GDM case-control status

Characteristics	Without GDM (n = 177)	GDM (n = 89)	Statistical values	P
Age (years)	32.2 ± 4.9	31.6 ± 2.4	0.678*	0.336
Prepregnancy BMI (kg/m^2)	20.7 ± 3.9	25.3 ± 2.4	−2.812*	0.010
Gestation at delivery (weeks)	39.3 ± 1.4	39.2 ± 1.5	0.537*	0.592
Weight gain during gestation (kg)	11.2 ± 3.8	12.2 ± 4.1	0.703*	0.360
MAP at GCT (mmHg)	74.4 ± 8.2	77.4 ± 8.8	−1.975*	0.040
Fasting glucose at the first trimester (mmol/L)	4.4 ± 0.6	5.0 ± 0.9	−5.881*	<0.001
50-g GCT (mmol/L)	6.2 ± 1.2	9.1 ± 3.5	−7.661*	<0.001
ANGPTL2 (ng/ml)	2.46 (2.05, 2.96)	3.06 (2.59, 3.65)	3.790†	0.003
Birth weight (g)	3245 ± 362	3360 ± 402	0.611*	0.430
Placenta weight (g)	532 ± 108	539 ± 103	0.359*	0.790
Previous GDM (n/N)	1/177	5/89	5.012‡	0.030
Family history of diabetes (n/N)	25/177	30/89	4.675‡	0.040

Values for continuous measurements are mean ± SD or median (quartile). *t value, †U value, ‡χ^2 value. GDM: Gestational diabetes mellitus; SD: Standard deviation; BMI: Body mass index; GCT: Glucose challenge test; MAP: Mean arterial pressure.

Table 2: Relative risk of developing GDM according to ANGPTL2 quartiles with quartile 1 serving as the reference group

Models	ANGPTL2				*P* for trend
	Quartile 1 (<2.05 ng/ml)	Quartile 2 (2.05–2.79 ng/ml)	Quartile 3 (2.80–3.45 ng/ml)	Quartile 4 (>3.45 ng/ml)	
Model 1					
OR	1.0	0.61	1.82	2.58	0.022
95% *CI*		0.25–2.38	0.75–4.39	1.22–6.96	
Model 2					
OR	1.0	0.66	1.87	2.83	0.026
95% *CI*		0.19–2.23	0.52–3.87	1.16–7.54	
Model 3					
OR	1.0	0.63	1.39	2.90	0.029
95% *CI*		1.18–2.18	0.50–3.89	1.17–7.25	

CI: Confidence interval; *OR*: Odds ratio; GDM: Gestational diabetes mellitus; ANGPTL2: Angiopoietin-like protein 2.

associated with a higher likelihood of developing GDM in a later phase of pregnancy. Specifically, women in the highest ANGPTL2 concentration quartile (Q4) were 2.90-fold more likely to develop GDM than women in the lowest quartile (Q1) in a multivariate model. This underscores the potential predictive value of ANGPTL2 concentrations during early pregnancy for developing GDM later in pregnancy. The association between plasma ANGPTL2 concentrations and the onset of GDM was independent of established or estimated risk factors of GDM as assessed by logistic regression analysis using the three models. In all three models, ANGPTL2 concentrations during the first trimester were significantly associated with subsequent risk of developing GDM. Taken together, our results suggest that plasma ANGPTL2 concentrations can predict GDM independently of possible confounders and mediators.

In normal pregnancies, there is a physiological insulin resistance from as early as the first trimester, which increases with gestational age.[20-24] In women with GDM, there is a higher level of fasting blood glucose and insulin, and the insulin sensitivity may decrease by up to 40% in late pregnancy.[25,26] Although we could not elucidate the mechanism of how ANGPTL2 contributes to GDM pathogenesis, several reports suggested an association between insulin resistance and ANGPTL2.[12,13,27] For instance, recent studies of obesity have provided new insights into the mechanisms underlying insulin resistance and metabolic dysregulation. Numerous efforts have been made to identify key regulators of obesity-linked adipose tissue inflammation and insulin resistance.[28-30] ANGPTL2 was found to be secreted by adipose tissue, and its circulating level was closely related to adiposity, systemic insulin resistance, and inflammation in both mice and humans.[12-16] These reports, in combination with our findings, suggest that elevated ANGPTL2 levels in early pregnancy may lead to the development of GDM; nevertheless, further studies would be required to reveal whether the association is truly independent of other established inflammatory markers.

This study had certain limitations. First, we only measured ANGPTL2 at one juncture in pregnancy, and although there was little short-term variation in ANGPTL2 levels, this was, however, one of preliminary studies to examine the association between ANGPTL2 and GDM and we adjusted for gestational age of ANGPTL2 sampling in the analysis. Second, because of the small sample size examined in this nested case-control study, we were unable to identify appropriate cut points to assess the positive and negative predictive values of ANGPTL2 as a marker for GDM. However, the emphasis of this study was on expanding our understanding of disease mechanisms through the identification of an association not previously reported, rather than evaluating a novel screening or diagnostic method. Larger studies would be needed to examine ANGPTL2 as a screening method for GDM. Third, we were unable to analyze anthropometric measures beyond BMI, such as waist circumference or waist-to-hip ratio.

In summary, we found that increased ANGPTL2 concentrations in the first trimester were associated with the development of GDM in a later phase of pregnancy. Further studies are required to confirm these results and to define a potential role of ANGPTL2 screening for diabetes, both during and after pregnancy.

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Conflicts of interest

There are no conflicts of interest.

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